Antimicrobial and Antioxidant Properties of Various Phytochemicals Extracted from *Cyphomandra betacea*, *Capsicum annuum* var. *cerasiforme*, *Dicentra scandens* and *Heracleum nepalense*

A Thesis Submitted

To

Sikkim University

In Partial Fulfilment of the Requirement for the Degree of Doctor of Philosophy

By

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December 2018

DEDICATION

I Dedicate this Thesis to my

Beloved Parents

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CERTIFICATE

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It is recommended this Ph.D. thesis to be placed before the Examiners for evaluation.

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Submitted by Ms. Pramila Koirala under the supervision of Dr. Bimala Singh, Assistant Professor, Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, 737102, Sikkim, India.

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DECLARATION

I declare that the present Ph.D. thesis entitled '**Antimicrobial and Antioxidant Properties of Various Phytochemicals Extracted from *Cyphomandra betacea*, *Capsicum annuum* var. *cerasiforme*, *Dicentra scandens* and *Heracleum nepalense***' submitted by me for the award of the degree of **Doctor of Philosophy in Microbiology** of Sikkim University under the supervision of **Dr. Bimala Singh**, Assistant Professor, Department of Microbiology, School of Life Sciences, Sikkim University, is my original research work solely carried out by me in the Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok. No part thereof has been submitted for any degree or diploma in any University/ Institution.

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Pramila Koirala

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHIC PLATES

LIST OF ABBREVIATIONS

- ADD- Additive
- AIRF- Advanced Instrumentation Research Facility
- ANOVA-Analysis of Variance
- BHA- Butylated Hydroxyanisole
- BHT-Butylated Hydroxytoluene
- BSA- Bovine Serum Albumin
- CFU- Colony Forming Unit
- DMSO- Dimethyl Sulphoxide
- DNA- Deoxyribonucleic Acid
- DPPH- 2, 2 Diphenyl-1- Picryl Hydrazyl
- EDTA-Ethylenediaminetetraacetic Acid
- FRAP- Ferric Reducing Antioxidant Power
- FIC- Fractional Inhibitory Concentration
- ΣFIC- Fractional Inhibitory Concentration Index
- FTIR- Fourier-Transform Infrared Spectroscopy
- GAE- Gallic Acid Equivalent
- GC-MS- Gas Chromatography and Mass Spectrometry
- HS-SPME- Headspace Solid-Phase Microextraction
- HIV- Human Immunodeficiency Virus
- HPLC- High-Performance Liquid Chromatography
- HPLC–PDA–MS/MS-High-Performance Liquid Chromatography coupled to Photodiode Array Detector and Mass Spectrometry
- IMTECH- Institute of Microbial Technology
- IND-Indifference

- IP- Inhibition Percentage
- LC-MS- Liquid Chromatography Mass-Spectrometry
- LDL- Low-Density Lipoprotein
- MBC- Minimum Bactericidal Concentration
- MDR- Multidrug-Resistant
- MDR-ESBL-Multidrug Resistant-Extended-Spectrum Beta-Lactamases
- MHA- Muller Hinton Agar
- MHB- Muller Hinton Broth
- MIC- Minimum Inhibitory Concentration
- MRSA- Methicillin Resistant *Staphylococcus aureus*
- MTCC- Microbial Type Culture Collection and Gene Bank
- NED- Naphthyl Ethylenediamine Dihydrochloride
- NIST- National Institute of Standards and Technology
- PBS- Phosphate Buffered Saline
- RE- Rutin Equivalent
- ROS- Reactive Oxygen Species
- RPM- Revolutions Per Minute
- SD- Standard Deviation
- SEM- Scanning Electron Microscope
- SHU- Scoville Heat Units
- SNP- Sodium Nitroprusside
- SYN- Synergism
- TAE-Tannic Acid Equivalent
- TCA- Trichloroacetic Acid
- TLC- Thin Layer Chromatography

- TTC- 2,3,5-Triphenyltetrazolium Chloride
- UV/Vis- Ultraviolet Visible
- VRSA- Vancomycin Resistant *Staphylococcus aureus*
- WHO- World Health Organization
- XDR-TB- Extensively Drug-Resistant Tuberculosis

LIST OF STANDARD SI UNITS

- Centimeter- cm
- Degree centigrade- ºC
- Feet- ft
- Gram- g
- Hour- h
- Meter- m
- Microgram- µg
- Microliter- µL
- Milligram- mg
- Minute- min
- Milliliter- mL
- Millimeter- mm
- Mole- mol

CONTENTS

1. INTRODUCTION

Plants are a rich source of a vast range of complex and structurally diverse compounds termed as phytochemicals. Their chemical diversity and unique mechanisms of action have played a crucial role in the prevention and cure of many diseases (Barbieri *et al*., 2017; Yuan *et al*., 2016). These chemicals are produced by plants as defense against pathogens and to avert the effect of free radicals (Barbieri *et al*., 2017). The roles of phytochemicals in the crude form represent a prominent space in traditional medicine (Khan, 2014).

India is known for its traditional medicinal systems which include Ayurveda, Siddha, and Unani. In India, the concept of the Ayurvedic system of medicine was developed between 2500 and 500 BC. Traditional medicine system has been mentioned in the ancient Vedas and other scriptures (Pandey *et al*., 2013; Subhose *et al*., 2005; Valsaraj *et al*., 1997). Ever since the prehistoric era plants have been the basis for nearly all medicinal therapy until the synthetic drugs were developed in the nineteenth century (Jones, 1996; Rajeswari and Krishnakumari, 2010). In the 1940s, a new era of medicine, "The golden age of antibiotics" was born (Cragg *et al*., 2012). With the discovery of the antibiotic Penicillin by Alexander Fleming in 1928, antibiotics have transformed modern medicine and saved millions of lives (Gould and Bal, 2013; Sengupta *et al*., 2013; Ventola, 2015).

Antimicrobials refer to the substances which act against microorganisms (Maartens *et al*., 2011). Antibiotics include antimicrobial agents or substances produced by the microorganisms which have the ability to kill or inhibit the growth of other microorganisms (Madigan *et al*., 2009). Antimicrobial agents act on microorganism by

various mechanisms. They may damage pathogens by inhibiting the cell wall synthesis, protein synthesis, nucleic acid synthesis, disrupting microbial cell membrane structure and blocking the metabolic pathways through inhibition of key enzymes (Prescott *et al*., 2005). The development of antibiotics has revolutionized modern medicine and many incurable diseases became curable. However due to the rampant use of antibiotics microorganisms have developed resistance to commonly used antibiotics (Sengupta *et al*., 2013). This led to the emergence of multi-drug resistant strains of microorganisms and has created a major challenge for the treatment of infectious diseases (Ahmad *et al*., 1998; Hancock, 2005). The use of antibiotics is also associated with adverse side effects (Cunha, 2001).

The use of natural products especially plant extracts as an alternative form of medical treatment is gaining popularity since decades (Cowan, 1999). Plant-based antimicrobials have relatively fewer side effects, are comparatively less expensive, have an acceptance due to a long history of use and have a positive impact on human health as well as on the environment (Reyes-Munguia *et al*., 2016; Vermani and Garg, 2002). The World Health Organization (WHO) have reported that approximately 80 % of the world population depends on plant-based traditional medicine (WHO, 1993).

Many researchers have focused on the investigation of antimicrobial agents from plants source and found that the plant extracts, purified secondary metabolites and essential oils exhibited potential antimicrobial property and serve as a major alternative source of new drug molecules today (Atanasov *et al*., 2015; Cowan, 1999). Recent studies have shown that the plant extracts in a combination of two or more exhibited potential antimicrobial activity against various microorganisms including drug-resistant strains of

microorganisms (Mabrouk, 2012; Suthar *et al.*, 2016). Plant extracts in combination with the antibiotics have also been studied by various researchers (Adwan *et al.*, 2010; Adwan and Mhanna, 2008; Nascimento *et al.*, 2000). The ability of phytochemicals to act synergistically with antibiotics had shown the possibility of utilization of the herbal plants in combination therapy to fight against emerging multidrug-resistance (Haroun and Al-Kayali, 2016). Natural product including herbal plants and spices are used as food additives. Many spices and herbs used today are valued for their antimicrobial property and other medicinal effects. Investigation of plant extracts and the characterization of bioactive components have gained popularity in pharmaceutical and food processing applications (Shan *et al.*, 2007).

Phytochemicals have been reported to possess many biological activities including antimicrobial activity (Fullerton *et al.*, 2011; Kacem *et al.*, 2015; Lewis and Ausubel, 2006). Phytochemicals from fruits, vegetables and many of the medicinal plants have been isolated and characterized (Doughari *et al.*, 2009). Phytochemicals can be categorized into two groups on the basis of presence or absence of nitrogen in their structures. Nitrogen-containing compounds include alkaloids, amines, nonprotein amino acids, cyanogenic glycosides and lectins. Compounds without nitrogen are terpenoids, saponins, cardiac glycosides, anthraquinones, catechol, tannins, phenolic acids, lignans and lignins (Wink, 2003). The medicinal effects of the plants are mainly due to the presence of these secondary metabolites (Dharma *et al.*, 2014). Plants contain thousands of such constituents and are a valuable source of new and biologically active molecules. Besides their roles as antimicrobial agents, these compounds exhibit a wide spectrum of

medicinal properties, such as antioxidant, anti-inflammatory, cardioprotective and anticancer properties (Adegbola *et al*., 2017).

Antioxidants are the molecules that scavenge and prevent the formation of free radical reactions thereby preventing the cellular damage (Young and Woodside, 2001). Free radicals are molecular species that contains an unpaired electron in an atomic orbital and tend to gain or donate an electron to surrounding molecules and thus behave as oxidants or reductants (Halliwell and Gutteridge, 1985; Young and Woodside, 2001). Reactive oxygen species (ROS) includes free radicals such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), nitric oxide (NO) and peroxynitrite ($ONOO^-$) that causes oxidative stress and are involved in pathogenesis of many diseases (Finkel and Holbrook, 2000; Shyur *et al*., 2005). These free radicals if not scavenged effectively, may cause damage to crucial biological molecules such as lipid, DNA, protein, carbohydrates. This may cause DNA mutation and damage target tissues leading to various disease conditions including cancer (Lobo *et al*., 2010). Hence to prevent oxidative damage, the supply of exogenous antioxidant is essential (Sen, 1995). Knowledge and application of potential antioxidant properties in reducing oxidative stress have prompted many researchers to search for potent and cost-effective dietary antioxidants from plants (Kasote *et al*., 2015; Liu and Ng, 2000; Wang *et al*., 2004). It has been reported that the majority of the antioxidant activity in plant extract is due to the presence of phytochemicals such as flavones, isoflavones, flavonoids, anthocyanin, coumarin and catechins (Aquil *et al*., 2014).

The combination of biological and chemical screening provides important information about plant constituents. Among the several plant species, only a small proportion has

been investigated for pharmacological properties (Hostettmann, 1999). Sikkim harbors a rich diversity of medicinal plants and some of which are used as food items and also play a considerable role in the rural economy (Singh *et al.*, 2002). Sikkim is a North Eastern state of India located in the Eastern Himalayas (Barik *et al.*, 2015). The small state has just 0.2 % geographical area of the country but harbors more than 26 % of flowering plants. Sikkim has been identified as one of the biodiversity hotspots in the Eastern Himalayas (Das *et al.*, 2012). The varied climatic condition, ecological zones and topographical distribution have promoted a rich floral diversity in this region. Sikkim is also a profusion of more than twenty ethnic communities. Nepalese, Bhutias, Lepchas, Limboos and Tibetans are the dominant ones (Singh *et al.*, 2002). The region has a rich culture of folklore medicine (Idrisi *et al.*, 2010). Different communities have their folk healers with healing knowledge and are named as 'Baidya' in Nepali community, 'Amji' and 'Pow' in Bhutia community, and 'Bongthing' or 'Maon-doak' in Lepcha community (Idrisi *et al.*, 2010; Pradhan and Badola, 2008). Despite having folklore medicinal practices, at present, there is possibly not sufficient literature documenting the antimicrobial and the antioxidant properties of most of the medicinal and edible plants from Sikkim. With this background, four plants were selected from this region namely *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don.

Cyphomandra betacea is a shrub growing 2 to 3 m in height (Orwa *et al.*, 2009) and belongs to the Family Solanaceae (The Wealth of India, 1950). It is commonly called as tamarillo or tree tomato and locally named as '*Rookh tamater*'. It is consumed fresh or used in culinary preparations like salad, sauces, soups, jellies, ice creams, juices, liqueurs

(Morton, 1987) and pickle (Mandal and Ghosal, 2012; Singh *et al*., 2014). The leaves of *Cyphomandra betacea* and its fruit pulp have been used in case of a sore throat and inflamed tonsils (Bohs, 1989). The fruit of *Cyphomandra betacea* is reported to reduce symptoms of respiratory diseases and anemia (Bermejo and Leon, 1994). The fruit is reported to be low in fat and calories and contains micronutrients such as vitamins, minerals and bioactive components including anthocyanins, carotenoids and flavonoids (Osorio *et al*., 2012). It is a rich source of vitamin C, B_{12} and Niacin (The Wealth of India, 1950).

Capsicum annuum var. *cerasiforme*, commonly called as Red cherry pepper and locally called as '*Dalle Khorsani*' belongs to the Family Solanaceae. It is one of the valuable cash crops grown all over Sikkim. It is used for making the pickle, chilli paste, chilli powder and also possess medicinal properties (Bhutia *et al*., 2016; Lepcha *et al*., 2014). It is grown in the Himalayan range including hilly areas of Nepal and is consumed by the local tribal people for curing gastroduodenal diseases (Sen *et al*., 2016). It is a good source of vitamin A, vitamin C and also a source of neutral and acidic phenolic compounds (Howard *et al*., 2000).

Dicentra scandens D. Don Walp. is a climbing perennial herb with tuberous rootstock (The Wealth of India, 1952) and belongs to the Family Fumariaceae. It is commonly known as the bleeding heart vine or Athens yellow and locally called as '*Jogi laharra*'/ *Kanchi laharra*'/ '*Kundley*'. It is used in traditional medicine in Sikkim. The traditional healers use the root juice to cure enteric diseases. The root juice of *Dicentra scandens* is useful in gastritis. The leaf paste or juice is applied to cut injury and wounds (Sharma and Sharma, 2010). The dried root powder is consumed by the *Limboo* tribe residing in

the South-West of Khangchendzonga biosphere reserve in West Sikkim to cure gastritis (Badola and Pradhan, 2013). This plant has also been ecologically categorized as a rare medicinal herb found in the Darjeeling Himalaya of West Bengal, India (Yonzone *et al*., 2012).

Heracleum nepalense D. Don is a small shrub found growing in Nepal, Bhutan and Sikkim (India). It belongs to the Family Umbelliferae (Dash *et al*., 2006; The Wealth of India, 1959). It is commonly known as Nepal hogweed and locally called as '*Chimphing*'. It is extensively used in folk medicine. Fruit of *Heracleum nepalense* D. Don is taken against nausea, vomiting, typhoid and roots are used in case of diarrhea (Bose *et al*., 2007; Chettri and Sharma, 2005).

In our preliminary study, antimicrobial properties were exhibited by the aqueous extract of fresh plants, methanol and acetone extracts of dried sample of *Cyphomandra betacea, Capsicum annuum, Dicentra scandens* and *Heracleum nepalense* against various test microorganisms. The methanol extracts of the plants exhibited antioxidant activity. Analysis of phytochemicals in the plant extracts revealed the presence of various phytochemicals such as flavonoid, saponin, steroid, tannin, phenol and alkaloid (Koirala, 2012). It has been well reported that flavonoid, tannin, alkaloid, phenol, saponin and steroid have antimicrobial properties (Cowan, 1999; Tiwari *et al*., 2011). Phytochemicals such as flavonoid and phenolic compounds are known to possess antioxidant activity (Adedapo *et al*., 2008; Raj and Shalini, 1999). The holistic or partially purified extracts offer advantages over the purified compound and hence form the basis of traditional medicine. It has been well documented that the therapeutic efficacy of the extracts is attributed to the synergistic interaction among the phytochemicals within single plant

extracts or in the mixture of different plants (Williamson, 2001). Therefore, the present study was designed in order to extract these phytochemicals namely alkaloid, flavonoid, saponin, steroid and tannin from *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don using specific extraction procedures and solvents.

Hence the present study aims to extract the various phytochemicals from the selected test plants and to investigate the antimicrobial and antioxidant properties of the phytochemicals namely alkaloid, flavonoid, saponin, steroid and tannin. The phytochemicals were extracted from the red skin color fruit of *Cyphomandra betacea* (Cav.) Sendth., ripened fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, tuberous roots of *Dicentra scandens* (D. Don) Walp. and the fruits of *Heracleum nepalense* D. Don collected from South district of Sikkim (India). The study also investigated the possible synergistic antimicrobial activity of the phytochemicals. In various traditional medicines, the concoction of the medicinal herb is prepared in water (Wachtel-Galor and Benzie, 2011). However in most scientific study methanol is used as the solvent due to its extraction efficiency (Swamy *et al*., 2015). Hence in the present study, the antimicrobial and antioxidant properties of the aqueous and the methanol extracts were also investigated.

1.1. OBJECTIVES

1. To extract phytochemicals (Alkaloids, Flavonoids, Saponins, Steroids, Tannins) from *Cyphomandra betacea*, *Capsicum annuum* var. *cerasiforme*, *Dicentra scandens* and *Heracleum nepalense*.

2. To evaluate antimicrobial properties of the extracted phytochemicals of *Cyphomandra betacea*, *Capsicum annuum* var. *cerasiforme*, *Dicentra scandens* and *Heracleum nepalense* against different test microorganisms.

3. To determine the Minimum Inhibitory Concentration (MIC) of extracted phytochemicals.

4. To evaluate the possible synergistic antimicrobial effect of extracted phytochemicals against different test microorganisms.

5. To evaluate the antioxidant properties of extracted phytochemicals from selected plants.

2. REVIEW OF LITERATURE

Plants have been used since ancient times and healing with the use of medicinal plants is an age-old practice (Petrovska, 2012). Ever since the prehistoric era plants have been used for all the medicinal therapy due to their multifunctional therapeutic properties (Dias *et al*., 2012; Rajeswari and Krishnakumari, 2010). After the development of synthetic drugs in the nineteenth century, antibiotics are used extensively for the treatment of various microbial infections (Cragg *et al*., 2012). However, the rampant use of antibiotics has led to the emergence of antibiotic-resistant strains (Sengupta *et al*., 2013). The development of microbial resistance to the available antibiotics has necessitated the researchers to investigate the antimicrobial activity of medicinal plants as an alternative form of health care (Allen *et al*., 2014; Umer *et al*., 2013).

Despite advancement in the treatment of infectious diseases by antibiotic therapy, development of antibiotic resistance leading to mortality and morbidity among the hospitalized patients is a serious concern. These synthetic drugs may produce several harmful side effects. It is therefore important to reduce this problem by controlling the use of antibiotics, understanding the mechanisms of resistance and developing new and alternative therapeutic strategies (Nascimento *et al*., 2000; Valle *et al*., 2015). There are many drugs derived from plants which are used today as therapeutic agents (Kinghorn *et al*., 2011; Newman and Cragg, 2012). Artemisinin with generic names artemether and lumefantrine and trade name coartem have been used against malaria. Capsaicin with generic name capsaicin and trade name qutenza have been used against postherpetic neuralgia (Kinghorn *et al*., 2011). In the past decades, there has been an increase in research on plant-based drugs. Medicinal plants have been explored for the source of

therapeutic agents against some incurable diseases such as extensively drug-resistant tuberculosis (XDR-TB) strains of *Mycobacterium tuberculosis*, Human immunodeficiency virus (HIV) infection, Hepatitis B, Hepatitis C (Chandra *et al*., 2017). It has been reported that the aqueous leaf extract of *Phyllanthus niruri* has anti-hepatitis B activity (Venkateswaran *et al*., 1987). Various medicinal plants including *Adhatoda vasica*, *Acalypha indica*, *Allium cepa*, *Allium sativum* and *Aloe vera* have been reported to possess antituberculosis activity against multidrug-resistant (MDR) *Mycobacterium tuberculosis* isolates (Gupta *et al*., 2010).

It has been reported that the plant extract exerts antimicrobial activity mostly against Gram-positive bacteria as compared to Gram-negative bacteria (Shan *et al*., 2007). This could be due to the difference in the cell wall structure. As the Gram-negative bacteria have multilayered cell structure including the presence of the periplasmic space. It contains enzymes that are capable of breaking down foreign molecules introduced from outside thereby limiting the passage of active component into the cell (Duffy and Power, 2001). Different strains of microorganisms may exhibit different sensitivities to the plant extract being tested (Zaika, 1988). However, several studies reported the antibacterial activity of the plant extracts against Gram-negative bacteria. Antibacterial activity of various medicinal plants against major urinary tract pathogens including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterococcus faecalis* has been investigated (Sharma *et al*., 2009). Most of the genus of the Family Enterobacteriaceae comprises of pathogenic microorganisms. Among the Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* are associated with many diseases.

In the present study, two Gram-positive bacteria namely *Bacillus cereus* and *Staphylococcus aureus* and seven Gram-negative bacteria namely *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica* ser. *typhi*, *Shigella flexneri* and *Vibro cholerae* O139 were taken. The resistance of pathogenic microorganisms to antibiotics are of serious concern. Fundamental mechanisms of antimicrobial resistance include enzymatic degradation of antibacterial drugs, alteration of the antimicrobial target site, changes in membrane permeability to antibiotics (Dever and Dermody, 1991).

Staphylococcus aureus is a pathogen of great concern because of its intrinsic virulence, ability to cause various life-threatening infections and development of resistance mechanism to new antibiotics. Spread of Methicillin Resistance *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) has been a great concern (Lowy, 2003; Pantosti *et al*., 2007).

Bacillus cereus is a facultative anaerobe and causes foodborne illness. Different types of toxins released by *Bacillus cereus* causes two common types of food poisoning, diarrhoeal and emetic types (Lund and Granum, 1997). This organism is a common airborne contaminant and multiplies very readily in cooked foods. The spores of *Bacillus cereus* survive short periods of cooking and reheating. Hence the spores germinate and release enterotoxins when food is stored at room temperature. Therefore, the ingestion of such contaminated food causes nausea, vomiting, diarrhea and abdominal cramps (Bottone, 2010).

Escherichia coli are the predominant facultative microorganism in the human intestine. Many strains of *Escherichia coli* are non-pathogenic (Lim *et al*., 2010). However, some strains of *Escherichia coli* have developed virulence due to plasmid transfer or as an opportunistic pathogen (Lim *et al*., 2010). Some strains can cause gastrointestinal tract infection, urinary tract infection and diarrhea (Nataro and Kaper, 1998). It is one of the most predominant pathogen causing 80-90 % of community-acquired urinary tract infections and 30-50 % of nosocomially-acquired urinary tract infections (Ejrnaes, 2011). *Escherichia coli* O157:H7 serotype of enterohemorrhagic *Escherichia coli* is a foodborne pathogen (Lim *et al*., 2010).

Klebsiella pneumoniae is the bacteria causing pneumonia. It is accountable for community-acquired as well as hospital-acquired infections. This bacteria can develop resistance to antibiotics more easily than most bacteria through the production of new enzymes that breaks down the antibiotics (Yu *et al*., 2007). The multidrug-resistant strains of *Klebsiella pneumoniae* causing urinary tract infections are a major public health problem (Rosen *et al*., 2008).

Pseudomonas is the most important genus of Family Pseudomonadaceae. It is a cause of troublesome infection in patients with extensive thermal injury (Baarlen *et al*., 2007), leukopenia from antineoplastic chemotherapy, various forms of immunosuppressive treatment and chronic pulmonary disease such as cystic fibrosis (Lahiri, 2007).

Proteus vulgaris is a member of Family Enterobacteriaceae. It can cause wound infection and urinary tract infection. This microorganism can become deadly when in sinus and respiratory tissues if left untreated (Partrick *et al*., 1998).

Salmonella typhi is a Gram-negative flagellated bacterium that causes typhoid fever (Pui *et al*., 2011). Typhoid fever caused by *Salmonella enterica* ser. *typhi* is still a major public health problem in many developing countries (Dougan and Baker, 2014).

Shigella flexneri is a Gram-negative bacterium which causes shigellosis, the most communicable of bacterial dysenteries. Shigellosis is the cause of millions of morbidity and mortality cases each year in the developing nations (Jennison and Verma, 2004).

Vibrio cholerae is a Gram-negative enteric pathogen. *Vibrio cholerae* O139 and *Vibrio cholerae* O1 causes cholera, a potentially epidemic, life-threatening secretory diarrhea characterized by watery stools accompanied by vomiting leading to hypovolemic shock and acidosis. *Vibrio cholerae* O139 caused an epidemic of cholera in India and Bangladesh in the year 1992-1993 (Faruque *et al*., 2003; Finkelstein, 1996).

Development of antibiotic resistance is a major crisis in the modern healthcare system around the globe. The increase in multidrug-resistant strains of pathogenic microorganisms is posing a serious threat to patients (Odonkor and Addo, 2011). Numerous researchers have shown interest in biologically active components from plants and the role of these components in the elimination of pathogenic microorganisms (Essawi and Srour, 2000; Tepe *et al*., 2005). Majority of the works on antimicrobial activity of plant extracts are concerned with the study of aqueous and organic solvent extracts of plant parts and testing of individual extract for antimicrobial activity (Palombo, 2011). However, studies on the synergistic activity of the phytochemicals are gaining importance. Synergistic activity implies to the effect exhibited by the combination of the different agents which is greater than the effect of the individual

agent. The interaction may have increased therapeutic effect as well as decreased side effects (Williamson, 2001). Recent studies on the synergistic effect of plant extract revealed that the plant extracts in a combination of two or more exhibited effective antimicrobial activity against a wide range of microorganisms including drug-resistant bacteria (Ahmad and Farrukh, 2007; Mabrouk, 2012). Combination of antibiotic with the plant extract exhibited synergistic antimicrobial activity against drug-resistant strains of microorganisms (Nascimento *et al*., 2000). Synergism of phytochemicals such as flavonoid with antiviral agents has also been reported (Cushnie and Lamb, 2005). Flavonoid has been reported to increase the efficacy of another component in the extract. The antimalarial activity of artemisinin was enhanced by the flavonoids artemetin and casticin (Liu *et al*., 1992). For the determination of synergistic activity, the checkerboard assay is a widely used method (Ozseven *et al*., 2012). The result of the assay is based on fractional inhibitory concentration (FIC) index values calculated on the basis of minimum inhibitory concentration (MIC) of the extracts individually and in combination. 2,3,5-Triphenyl tetrazolium chloride (TTC) is added in the wells of a microtiter plate to detect the bacterial growth (Basri and Sandra, 2016). In the presence of live bacteria, the TTC is reduced to a red colored product known as formazan. The color is directly proportional to the viable bacterial cells (Moussa *et al*., 2013).

Medicinal properties of the plants are related to the phytochemicals present in the plants (Al-Daihan *et al*., 2013). Furthermore, it has been reported that the antimicrobial activity of different plants and the plant parts also depend largely upon the extraction procedure, extraction solvent and the bacterial strains tested (Jaberian *et al*., 2013). There are numerous literature reporting the mechanism of antimicrobial actions of phytochemicals.

Some phytochemicals may exert antimicrobial action by inducing cellular membrane perturbations, by interfering with certain microbial metabolic processes, by modulation of signal transduction pathways or by modulation of gene expression pathways (Omojate *et al*., 2014). A crucial characteristic of plant extract is their hydrophobicity which makes them attack the lipids of the bacterial cell membrane thereby making the cell permeable to extracts (Oonmetta-Aree *et al*., 2006). The antibacterial action of the ethanol extracts of *Hemidesmus indicus* (L.) R. Br. ex Schult, *Leucas aspera* (Wild.), *Plumbago zeylanica* L., and *Tridax procumbens* (L.) R. Br. ex Schult has been reported. The mechanism of antibacterial action of these extracts involved the disruption of membrane potential, inner membrane permeabilization, blebbing and leakage of cellular contents of the test bacterial strains (Saritha *et al*., 2015). Various literature on the antimicrobial action of plant secondary metabolites points to the fact that their primary target site is the cytoplasmic membrane of the microorganisms (Radulovic *et al*., 2013). Moreover, other mechanisms are also responsible for the antimicrobial action of plant extracts. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) assay using different methods determine the nature of the antimicrobial agent as bacteriostatic or bactericidal (Peterson and Shanholtzer, 1992; Wiegand *et al*., 2008). It has been reported that an appropriately performed routine bacteriostatic test including MIC and disk diffusion assay can detect resistance against antimicrobial agents resulting from many mechanisms (Peterson and Shanholtzer, 1992).

Discovery of the novel antimicrobial agent is an exclusively important objective of the present era. Bioactive molecules derived from natural products are still one of the major sources of new drug molecules and thus the plants are explored for this purpose

(Atanasov *et al*., 2015). Presence of secondary metabolites in plants is attributed to their biological activity. The antimicrobial and antioxidant properties of the plant extract are highly correlated with the total phenolic content and the flavonoid content of the plant extract (Silva *et al*., 2006). Flavonoid contents of plants largely influence the antioxidant activity than the antimicrobial activity (Silva *et al*., 2006).

Medicinal plants, fruits and vegetables possess a wide variety of phytochemicals that are rich in antioxidant activity (Reyes-Munguia *et al*., 2016). However, the use of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in the past decades has become a safety concern. Hence there has been a growing interest in natural antioxidants derived from the plant sources (Brewer, 2011). The dietary polyphenols have been reported to contribute to the prevention of oxidative stress thereby preventing many disease conditions (Brewer, 2011).

The cells are equipped with various mechanisms to fight against Reactive Oxygen Species (ROS) and to maintain the redox homeostasis of the cell (Phaniendra *et al*., 2015). Free radicals are the chemical species that tends to trap the electrons from surrounding molecules thereby damaging crucial biological molecules like DNA, proteins. These free radicals generated in our body are removed by the endogenous antioxidant defense system such as glutathione or catalase. If these molecules are not scavenged properly it may result in many disease conditions like tumor, inflammation, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, carcinogenesis, neurodegenerative diseases (Phaniendra *et al*., 2015).

Our body is continuously exposed to damaging environmental factors or exogenous factors such as tobacco, smoke, alcohol, pesticides, certain pollutants and microbial infection. Our endogenous antioxidant defense systems are not always completely effective in preventing oxidative damages. Hence there is a need for a supply of exogenous antioxidants containing the bioactive principle in the form of nutritional supplements or pharmaceutical products. Hence the alternative source for the natural antioxidant has to be investigated (Gilgun-Sherki *et al*., 2001; Pisoschi and Negulescu, 2011). The medicinal and the dietary plants are an excellent source of natural antioxidant and thus have been explored for therapeutic purposes (Uttara *et al.*, 2009).

Many plants, citrus fruits and vegetables are the sources of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics which possess the ability to scavenge the free radicals in the human body (Lobo *et al*., 2010). It has been reported that the antioxidant effects of plant extracts are mainly due to the presence of phytochemicals such as phenolic compounds, flavonoids, phenolic acids and tannin (Adedapo *et al*., 2008; Zheng and Wang, 2001). Antioxidants can decrease the oxidative damage directly by reacting with free radicals or indirectly by inhibiting the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes (Lu *et al*., 2010).

All the plants have the ability to synthesize nonenzymatic antioxidant substances whose concentration increases during biotic and abiotic stress resulting in the oxidative stress. In response to this oxidative stress, plant produce and accumulate various antioxidants as shown in Figure 2.1 (Kasote *et al.*, 2015).

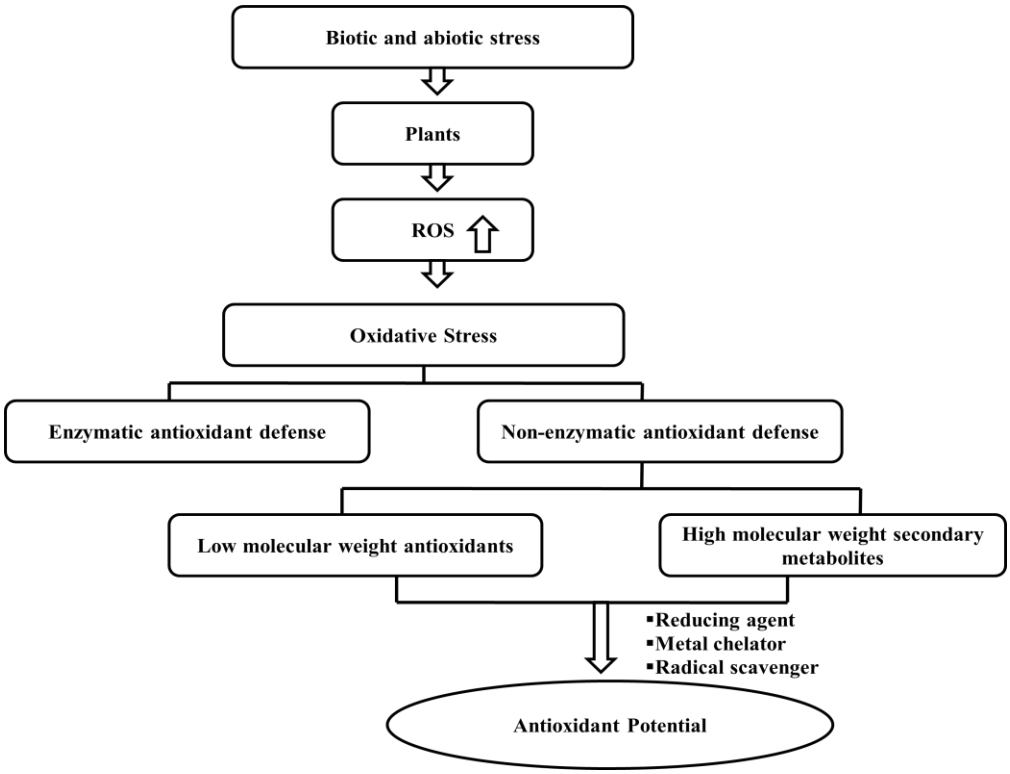


Figure 2.1: Synthesis of antioxidants by plants under biotic and abiotic stress (Kasote *et al.*, 2015).

The antioxidant activity can be studied using various methods. DPPH scavenging assay is one of the commonly used methods to investigate the antioxidant potential. DPPH is a stable free radical at room temperature. It accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It shows a strong absorption band at 517 nm due to its unpaired electron and hence gives deep violet color in the solution. With the addition of antioxidants, the electron gets paired which gives rise to the reduced form with the loss of violet color resulting in the decrease in the absorbance. This decrease in absorbance depends on the concentration of the antioxidant in the solution (Blois, 1958; Molyneux, 2004).

In ferric reducing antioxidant potential (FRAP) assay, the extract or the bioactive compound which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) that further reacts with ferric chloride to form a ferric ferrous complex. This complex has an absorption maximum at 700 nm that can be measured spectrophotometrically (Macwan and Patel, 2010).

Hydrogen peroxide scavenging assay is based on the principle that there is a decrease in absorbance of hydrogen peroxide upon oxidation of hydrogen peroxide which can be measured spectrophotometrically at 230 nm (Malik *et al*., 2011).

The hydroxyl radical scavenging assay is based on the measurement of metal chelating activity and hydroxyl radical scavenging activity of antioxidant compounds (Huang *et al*., 2005; Pisoschi and Negulescu, 2011).

Nitric oxide radical scavenging assay is based on the principle that, nitric oxide produced from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to give nitrite ions, which is quantified by Griess Illosvoy reaction. During the diazotization of nitrite ions with sulphanilamide and thus coupling with naphthyl ethylenediamine dihydrochloride (NED), the pink chromophore is generated. The colored product formed is measured spectrophotometrically at 540 nm (Hazra *et al*., 2008). These assays are widely used methods for the determination of antioxidant activity of plant extracts.

The presence of different phytochemicals in the plants contributes to the various biological activities (Compean and Ynalvez, 2014). Phytochemicals are non-nutritive bioactive compounds present in fruits, vegetables, grains, and other plants and are reported to reduce the risk of many chronic diseases (Doughari *et al*., 2009). These

bioactive compounds have antimicrobial and antioxidant properties. Phytochemicals are primarily referred to as plant chemicals (Hahn, 1998). Based on their role in plant metabolism phytochemicals are classified as primary and secondary constituents or metabolites. Primary metabolites are essential for the growth and survival of the plants. Primary constituents include chlorophyll, common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids. Secondary metabolites include phytochemicals such as alkaloids, terpenes, flavonoids, lignans, steroids, saponins, phenolics and glycosides (Saxena *et al*., 2013). Secondary metabolites are chemicals synthesized by the plant not as a requirement in the basic processes of growth and development of plants but as to protect themselves from environmental stress, pathogens, herbivores and symbiotic insects (Kennedy and Wightman, 2011).

Plants protect themselves by producing secondary metabolites including terpenoids, phenolics, and alkaloids (Mazid *et al*., 2011). Triterpenoids including cardiac glycosides are highly toxic to vertebrate herbivores, as well as humans if ingested in high amount. Glycosylated triterpenoids such as saponin which is present in many plant species disrupt the cell membranes of invading pathogenic microorganisms. Phenolics are a large class of secondary metabolites. Phenolic such as a flavonoid is produced by the plant as a defense against pathogens. Tannins are toxic to insects. It binds with salivary proteins and digestive enzymes causing inactivation of protein eventually causing death. Lignin provides a physical barrier against plant pathogen attack. Alkaloids are toxic to the insect as well as pathogens (Freeman and Beattie, 2008).

Alkaloids are the heterocyclic nitrogen compounds. It includes bitter-tasting nitrogenous compounds that are found in many plants. Caffeine, cocaine, morphine, and nicotine are

common examples of this class (Freeman and Beattie, 2008). The first alkaloid morphine was isolated from the opium poppy (*Papaver somniferum)* by Serturner in 1806 (Kutchan, 1995). Atropine an alkaloid from *Atropa belladonna* plant is a neurotoxin and cardiac stimulant in a high dose. However, in small quantity, it has medicinal use as pupil dilator and antidote for some nerve gas poisonings (Freeman and Beattie, 2008). Alkaloids from various plants have been reported to possess antimicrobial activity. Michellamine B, a novel plant alkaloid isolated from *Ancistrocladus korupensis*, has anti-HIV activity (McMahon *et al*., 1995). Alkaloid berberine is reported to possess antibacterial activity probably by damaging the bacterial cell membrane and inhibiting the synthesis of protein and DNA (Peng *et al*., 2015).

Flavonoids are hydroxylated phenolic substances and have the general structural backbone of C6–C3–C6 in which the two C6 units are phenolic in nature. Flavonoids can be further divided into different sub-groups: anthocyanins, flavan-3-ols, flavones, flavanones and flavonols (Tsao, 2010). Flavanols (mainly catechins and proanthocyanidins) and anthocyanins are the most abundant flavonoid present in the diet (Han *et al*., 2007). Flavonoids are found in vegetables, grains and in almost every part of plants including fruits, bark, roots, stems and flowers (Middleton, 1998). It has been reported that the flavonoids are synthesized by plants in response to microbial infection and hence are an effective antimicrobial agent (Dixion *et al*., 1983). It is known to probably complex with extracellular and soluble proteins and to complex with bacterial cell walls and may disrupt microbial membranes (Cowan, 1999) Flavonoids are also known to prevent damage caused by free radicals by various mechanisms which include direct scavenging of reactive oxygen species (ROS) by donating hydrogen atom,

activation of antioxidant enzymes, metal chelating activity, inhibition of oxidases, reduction of oxidative stress caused by nitric oxide, increase in uric acid levels, increase in antioxidant properties of low molecular antioxidants (Prochazkova *et al*., 2011).

Tannin is a phenolic substance having astringent property. They are found in the bark, wood, leaves, fruits, and roots of the plants (Scalbert, 1991). Tannins are categorized into two groups namely hydrolyzable tannins and condensed tannins. In hydrolyzable tannins, the hydroxyl group of carbohydrate is esterified with gallic acid while the condensed tannins (proanthocyanidins) are derived from flavonoid monomers (Cowan, 1999). Tannin exerts its antimicrobial effects by inhibition of extracellular microbial enzymes, by interfering with the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation (Scalbert, 1991). Dietary polyphenols including epigallocatechin, catechin, ellagic acid, gallic acid, caffeic acid, quercetin and epicatechin have been reported to possess antioxidant activity by various endogenous and exogenous mechanisms (Han *et al*., 2007). Polymeric tannin the minor component of green, black, and herbal teas of *Camellia sinensis* exhibited stronger antioxidant and antibacterial properties as compared to major nonpolymeric constituents (Chan *et al*., 2011). Tannin-rich extracts of fruits of *Phyllanthus emblica* constitute mucic acid gallate, mucic acid lactone gallate, monogalloylglucose, gallic acid, digalloylglucose, putranjivain A, elaeocarpusin, and chebulagic acid as major hydrolyzable tannins. The extract is reported to exhibit various *in vitro* and *in vivo* biological activities including antidiabetic, antimicrobial, anti-inflammatory and immunoregulatory activities (Yang and Liu, 2014).

Saponins are a structurally diverse class of compounds which are characterized by a skeleton derived of the 30-carbon precursor oxidosqualene to which glycosyl residues are attached (Vincken *et al*., 2007). Saponins are the secondary metabolites composed of a sugar moiety linked to a triterpene or steroid aglycone (Moghimipour *et al*., 2014). Saponins have foaming property in water. Saponin from soya beans, peas, *Solanum* species, tomato, *Allium* species, spinach has been well reported (Price *et al*., 1987). Saponin from plants has been reported to possess many biological activities. Anti-inflammatory activity of saponins from *Bupleurum fruticescens* has been reported (Just *et al*., 1998). Saponin extracted from *Sorghum bicolor* L. Moench exhibited antimicrobial activity against *Staphylococcus aureus* but did not inhibit the growth of Gram-negative bacteria and fungi (Soetan *et al*., 2006). Crude saponin extract from *Abutilon indicum* leaves has been reported to exhibit promising antimicrobial activity and DPPH free radical scavenging activity (Lokesh *et al*., 2016).

Steroids are members of a large class of compounds called terpenes and are characterized by the presence of perhydro-1, 2-cyclopentanophenanthrene ring (Morgan and Moynihan, 2000). Plant steroids are a unique class of compound. The structure of sterols is similar to cholesterol (Piironen *et al*., 2000). A wide variety of steroid molecules are produced by plants which are divided into three groups based on their biological role. Firstly those having a role on the plant itself, examples of which include brassinosteroids which are the growth-promoting phytohormones. Secondly, the substances related to animal hormones examples of which include androgens, estrogens and corticosteroids. Finally the allochemical substances specific to plants which work as a defense against phytophagous animals or parasitic fungi, examples of which include cucurbitacins,

sapogenins, steroidal alkaloids (Dinan *et al*., 2001). The commonly consumed plant sterols are sitosterol, stigmasterol and campesterol which are present in vegetable oils. The plant sterol has the capacity to lower plasma cholesterol and low-density lipoprotein (LDL) cholesterol thereby preventing cardiovascular diseases. Plant sterols possess many biological activities including anticancer, anti-inflammatory, anti-atherogenicity and antioxidant activities (Berger *et al*., 2004). Two steroid glycosides namely tomatidine and solasodine were isolated from berries of *Solanum aculeustrum*. The compound exhibited significant antioxidant activity individually and also exhibited a synergistic effect (Koduru *et al*., 2007). Antimicrobial activity of artificially synthesized steroid has been reported (Shamsuzzaman *et al*., 2014; Smith *et al*., 1963). Steroids isolated from *Chromolaena squalida* namely stigmasterol, β-sitosterol, campesterol, espinasterol and Δ^7- stigmasterol exhibited antimicrobial activity against Gram-positive bacteria (Taleb-Contini *et al*., 2003).

Plant secondary metabolites are unique sources for pharmaceuticals and nutraceutical products (Zhao *et al*., 2005). The role of phytochemicals in health promotion has been recognized by health professionals (Hasler, 1998; Howard and Kritchevsky, 1997). There has been a growing interest in the constituents of higher plants that are used in traditional medicine system or as dietary supplements. Characterization and structure elucidation of phytochemicals has been a matter of interest among the natural product scientists (Kinghorn *et al*., 2011). Plants contain a number of phytochemicals with different polarities. Therefore the isolation, purification and characterization of these bioactive compounds involve various chromatographic and spectroscopic techniques. The techniques such as Thin Layer Chromatography (TLC), Column Chromatography, High

Performance Liquid Chromatography (HPLC), Gas Chromatography Mass-Spectrometry (GC-MS), Liquid Chromatography Mass-Spectrometry (LC-MS), Fourier-Transform Infrared Spectroscopy (FTIR) facilitate the characterization and identification of the bioactive compounds present in the plant extracts (Sasidharan *et al*., 2011).

The medicinal properties of the plants are attributed to the presence of various phytochemicals (Vaghasiya *et al*., 2011). However, there are several factors which influence the concentration of the active phytoconstituents present in the plants and thus the biological activity. Some of the factors include period of plant collection, geographical origin and climatic conditions. It has been reported that all these factors sometimes attributes even to the absence of active constituents in the same plant collected from different regions and hence the absence of activity (Banerjee and Bonde, 2011; Houghton, 1998). The Himalayan region is rich in biodiversity and exhibits more diversity in its types of plants than any other parts of the Indian subcontinent (Rao, 1997). *Artemesia vulgaris* Linn. is a medicinal plant found in Sikkim and Darjeeling Himalayas. An isolated compound AV-2 from the plant exhibited antibacterial activity (Mitra, 2014). Antimicrobial and antioxidant activities of *Bergenia ciliata* Sternb. a medicinal herb of Sikkim has been reported. Three major bioactive components namely bergenin, catechin and gallic acid were detected by HPLC analysis (Singh *et al*., 2017). Plants collected from Darjeeling namely *Chenopodium album*, *Desmodium triflorum*, *Duchesnea indica*, *Plantago major*, *Pratia mumularia* with reported traditional antimicrobial use has been evaluated for antimicrobial activity. The methanol extract of the plants exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria (Saha *et al*., 2011).

In the present study, four test plants were selected namely *Cyphomandra betacea*, *Capsicum annuum* var. *cerasiforme*, *Dicentra scandens*, *Heracleum nepalense* D. Don from Sikkim, the North Eastern State of India.

Cyphomandra betacea is a shrub growing 2 to 3 m in height (Orwa *et al*., 2009). It is native to the Andes of Peru, Ecuador and Colombia (Vasco *et al*., 2009). It is cultivated in subtropical or warm temperate regions (Nascimento *et al*., 2013). Based on the fruit skin colors various types of tamarillo include solid deep-purple, blood-red, orange or yellow, or red and yellow and may have faint dark, longitudinal stripes. The fruit is ovoid in shape, 4-10 cm in length and 3-5 cm in diameter and exhibits a sour taste with a characteristic aroma (Morton, 1987). An invertase inhibitory protein isolated from *Cyphomandra betacea* has a broad-spectrum antimicrobial activity against plant pathogens namely *Ganoderma applanatum*, *Schizophyllum commune*, *Lenzites elegans*, *Pycnoporus sanguineous*, *Penicillium notatum*, *Aspergillus niger*, *Phomopsis sojae*, *Fusarium mango*, *Xanthomonas campestris* pvar vesicatoria CECT 792, *Pseudomonas solanacearum* CECT 125, *Pseudomonas corrugata* CECT 124, *Pseudomonas syringae* pv. *syringae* and *Erwinia carotovora* var carotovora (Ordonez *et al*., 2006). Antioxidant activity of alcoholic and aqueous extracts and pomace derived from ripe fruits of *Cyphomandra betacea* Sendt. from Argentina has been studied by Ordonez *et al*., (2010). Phenolic compound such as hydroxycinnamic acids and flavonol derivatives such as quercetin and myricetin derivatives were identified in tamarillo fruit (Vasco *et al*., 2009). Radical scavenging efficacy of different parts including placenta, endocarp, epicarp, seed and mesocarp of tamarillo fruit have been investigated. Phytonutrients like total carotene, lycopene, anthocyanin, total phenolics and flavonol have been reported in

tamarillo from Darjeeling, India (Mandal and Ghosal, 2012). The hydromethanolic extract of fruits exhibited antioxidant activity. The phenolic composition analysis by HPLC and LC-MS/MS identified ellagic acid, caffeic acid, ferulic acid, gallic acid, protocatechuic acid and quercetin from the fruits of *Cyphomandra betacea* from Sikkim (Prakash *et al*., 2012). Antioxidant activity and phenolic content have been investigated in the hydroethanolic extract of *Cyphomandra betacea* (Singh *et al*., 2014). Antioxidant property of tamarillo (*Solanum betaceum*) and a value-added product tamarillo sauce has been reported (Nallakurumban *et al*., 2015).

Several studies have been done to characterize phenolic constituents and anthocyanin from the fruits of *Cyphomandra betacea*. Anthocyanin pigments pelargonidin-3-rutinoside, pelargonidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-glucoside, delphinidin-3-rutinoside and delphinidin-3-glucoside from fruits of *Cyphomandra betacea* has been isolated and identified (Wrolstad and Heatherbell, 1974). Pelargonidin-3-glucosyl-glucose, an anthocyanin pigment from Brazilian tree tomato has been identified by using Gas-Liquid Chromatography (Bobbio *et al*., 1983). HPLC–PDA–MS/MS analysis of carotenoids and anthocyanins from tamarillo fruits detected 3 anthocyanins and 17 carotenoids with delphinidin 3-rutinoside and β-cryptoxanthin as the major anthocyanin and carotenoid respectively (Rosso and Mercadante, 2007). The composition of carotenoids, analysis of phenolic compound and antioxidant capacity were studied in the extracts of the edible part of the yellow and red variety of tree tomato (*Solanum betaceum* Cav.) (Mertz *et al*., 2009). Two major carotenoids, *all-trans-β*-cryptoxanthin esters and *all-trans-β*-carotene were identified. Thermal liability of carotenoids and vitamin C of tamarillo fruit (*Solanum betaceum* Cav.) has been studied

by Mertz *et al*., (2010). The anthocyanin delphinidin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside-3'-*O*-β-D-glucopyranoside from tamarillo fruit has been identified (Osorio *et al*., 2012). The characterization of volatile compounds from golden yellow and reddish purple varieties of *Solanum betaceum* Cav. by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) detected terpenoids as major compounds followed by aromatics, esters and aldehydes. However, the golden-yellow variety revealed higher levels of esters and terpenes while the reddish-purple variety contained a significant amount of aromatic compounds (Durant *et al*., 2013). A study from Brazil reports the chemical structure, antinociceptive and anti-inflammatory effects of a galactoarabinoglucuronoxylan polysaccharide isolated from the edible pulp of tamarillo (*Solanum betaceum*) fruits (Nascimento *et al*., 2013). Functional characterization of hydrocolloid from tamarillo revealed 66.48 % dietary fibre, 21.18 % protein and 0.83 % starch and possessed the oil-holding capacity and emulsifying activity thus serving as a potential source of food hydrocolloid (Gannasin *et al*., 2012). The tamarillo extract exhibited a protective effect against high-fat diet induced obesity in rats. The extract revealed lipid lowering effect and an increase in the activities of antioxidant enzymes in rats (Kadir *et al*., 2015).

It has been reported that the different variety of tree tomato exhibited high diversity in the chemical composition (Acosta-Quezada *et al*., 2015). Total phenolic content was found to be significantly correlated with antioxidant activity (Acosta-Quezada *et al*., 2015). The fruit of *Cyphomandra betacea* exhibited potential antiproliferative property (Mutalib *et al*., 2016). Gallic acid, caffeic acid and vanillic acid were most abundant phenolic acid and ferulic acid, p-coumaric acid and trans-ferulic acid were found in minor quantity in

the extract of *Cyphomandra betacea*. Caffeic and gallic acids have been reported to have various biological functions including antioxidant activity, which are mainly associated with the modulation of carcinogenesis (Mutalib *et al*., 2016). The ethanol extract of *Cyphomandra betacea* exhibited potential antioxidant and anticancer properties (Mutalib *et al*., 2017). Inhibitory effect of α-glucosidase enzyme activity by Indonesian tamarillo has been reported (Puspawati *et al*., 2018).

The genus *Capsicum* belongs to the Family Solanaceae and has five species namely *Capsicum annuum*, *Capsicum baccatum, Capsicum chinense, Capsicum frutescens* and *Capsicum pubescens* and are commonly recognized as domesticated. *Capsicum* has been used as a part of the human diet since 7500 BC (Al-Snafi, 2015). *Capsicum* is a small herb native to tropical America and cultivated throughout the tropics including many parts of India. It is grown in the hills up to the height of 6500 feet (Palevitch and Craker, 1995). *Capsicum* is a commonly used spice in India. It has carminative action (The Wealth of India, 1950). Fruit of *Capsicum* species is used as an appetizer (Sharma and Sharma, 2010). *Capsicum* fruits have been used in folk medicine by different tribes and ethnic communities worldwide (Thapa *et al*., 2009). *Capsicum* commonly called as peppers are rich sources of the provitamin A, carotenoids, α-carotene, β-carotene, and β-cryptoxanthin (Minguez-Mosquera and Hornero-Mendez, 1994). Capsaicin (*trans*-8-methyl-N-vanillyl-6-nonanamide) and dihydrocapsaicin (8-methyl-N-vanillyl-6-nonanamide) are the pungent principles responsible for the hot and spicy flavor present in most of the varieties of *Capsicum* fruit (Barbero *et al*., 2006). *Capsicum annuum* var. *cerasiforme* is cultivated in Sikkim and its neighboring regions including Darjeeling. It has very high pungency level (Bhutia *et al*., 2016; Lepcha *et al*., 2014). Capsaicin is

reported to have antibacterial activity and is found to be effective against *Bacillus subtilis* (Stephen and Kumar, 2014) and MDR-ESBL producing *Escherichia coli* isolates (Kar *et al*., 2016).

Dicentra scandens (D. Don) Walp. is a climbing perennial herb. It belongs to the Family Fumariaceae. It is dispersed in temperate Northern Asia and North America (The Wealth of India, 1952). Various tribal communities of Nepal and North East India use *Dicentra scandens* (D. Don) Walp. in traditional medicine. It has been reported that the extract prepared from the tuberous root and leaf of *Dicentra scandens* (D. Don) Walp. is used against fever, high blood pressure, gastrointestinal disorders and as a diuretic (Pfoze *et al*., 2010). In Nagaland, this medicinal herb has a prominent space in traditional medicine. It is used for the treatment of malaria, typhoid, viral fever, diabetes, pneumonia, high blood pressure, diarrhea, dysentery, flatulence, cut or injury (Pfoze and Chiezou, 2006). In Manipur, crushed tuberous root of *Dicentra scandens* (D. Don) Walp. is consumed with water against fever, stomach ache and high blood pressure (Pfoze *et al*., 2013). The crude alkaloid extract from the root of *Dicentra scandens* (D. Don) Walp. exhibited antimicrobial activity against *Bacillus mycoides, Bacillus subtilis, Escherichia coli, Enterobacter cloacae* and some fungal strains (Nakhuru *et al*., 2013). The extract also exhibited antibacterial activity against wound pathogens including *Pseudomonas aeruginosa* and *Proteus mirabilis* (Nakhuru *et al*., 2014). Ethnobotanical survey of various medicinal plants used in North-East India for the treatment of malaria have been reviewed and its medicinal preparation and the dose has been documented by Bora *et al*., (2016). A decoction of fresh leaves and tender stem of *Dicentra scandens* (D. Don) Walp. is prepared and approximately three teaspoonful of the decoction are consumed

thrice a day for the treatment of malaria by 'Chakhesang' tribe of Nagaland (Bora *et al*., 2016). *Dicentra scandens* is reported to contain alkaloids namely allocryptopine, protopine and l-dl-stylopine (The Wealth of India, 1952). The chemical and biological properties of protopine and allocryptopine have been reported. These isoquinoline alkaloids possess various biological activities including antithrombotic, anti-inflammatory, antispasmodic, neuroprotective, antibacterial, antiviral, antifungal and antiparasitic activities (Vacek *et al*., 2010).

The genus *Heracleum* is one of the largest genera of the Family Umbelliferae (Apiaceae). There are around 120-125 species of *Heracleum* in the world and 109 species in Asia (Pimenov and Leonov, 2004). In India, 15 species of *Heracleum* have been reported to be distributed in the North-western, Western and the Eastern Himalayas (Kharkwal *et al*., 2014). Different species of *Heracleum* have traditional medicinal importance. There are many scientific studies on pharmacological properties of this genus. The ethanol extract of *Heracleum sibiricum* exhibited significant apoptotic activity against two leukemic cell lines C8166 and J45 (Bogucka-Kocka *et al*., 2008). *In vitro* free radical scavenging activity of fruit oils of *Heracleum aquilegifolium* Wight collected from the Western Ghats of the Indian Peninsula has been investigated (Karuppusamy and Muthuraja, 2010). The GC-MS analysis detected β-Pinene, 1,8-cineole and β-phellandrene as the major components of the essential oil from the fruits of *Heracleum aquilegifolium* Wight (Karuppusamy and Muthuraja, 2010). The antioxidant properties and phytochemical analyses of root, stem, leaves and fruits of *Heracleum gorganicum* Rech. F. in Golestan province of Iran have been investigated (Mazandarani *et al*., 2012). The essential oil extracted from *Heracleum lanatum* Michx. of Uttarakhand Himalaya exhibited

antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella enterica* and *Staphylococcus aureus*. The characterization of the essential oil by GC-MS analysis detected β-phellandrene, sabinene and (E)-β-ocimene as the major compounds (Kharkwal *et al*., 2014).

Heracleum nepalense D. Don is a small shrub, 2-5 ft in height with pinnately divided leaves and ovoid fruits (The Wealth of India, 1959). It is a medicinal plant distributed in the temperate and sub-temperate zone (Yonzone *et al.*, 2012). The root of *Heracleum nepalense* is reported to have antimicrobial and antioxidant activity (Dash *et al*., 2005). Antibacterial activity of methanol extract of roots of *Heracleum nepalense* D. Don against bacteria causing diarrhea has been investigated. The extract was found to inhibit the growth of *Escherichia coli*, *Salmonella typhimurium*, *Shigella boydii*, *Shigella dysenteriae* and *Vibrio cholerae* (Bose *et al*., 2007). Quercetin-3-O-β-D-glucopyranoside, a known flavonoid glycoside was isolated from roots of *Heracleum nepalense* D. Don. The structure was elucidated on the basis of spectroscopic techniques (Dash and Bhise, 2006). The immuno-stimulatory activity of alcoholic extract of roots of *Heracleum nepalense* D. Don has been assessed *in vitro* and *in vivo* and exhibited dose-dependent immuno-stimulatory activity (Dash *et al*., 2006).

Dietary and medicinal plants would be a great source of novel drug discovery. With the use of many analytical methods including chromatographic and spectroscopic techniques, plant-derived compounds will be an essential aspect of future therapeutic medicines (Salim *et al*., 2008). Hence the plants with traditional medicinal value and the plants which are consumed as vegetables, fruits and spices should be investigated to better

understand their biological properties, efficacy and safety so as to develop a potent therapeutic agent.

3. MATERIALS AND METHODS

3.1. Chemicals, reagents and culture media

All the chemicals, reagents and the culture media used in the study were obtained from HiMedia, India, Merck, Germany and Sigma-Aldrich, USA (Appendix 1).

3.2. Collection and identification of plant samples

The plants under study were collected from different places of South Sikkim. The fruits of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don were collected during the fruiting season in the month of October. The roots of *Dicentra scandens* (D. Don) Walp.was collected in the month of November. The herbariums of the plant specimens were prepared. The plant specimens were identified and authenticated by the Taxonomist at the Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal, Siliguri, India. Copies of voucher specimens were deposited in the herbarium of the University of North Bengal Siliguri, India as well as in the Department of Microbiology, Sikkim University, Sikkim, India.

3.3. Preparation of plant sample

3.3.1. *Cyphomandra betacea* (Cav.) Sendth.

The fruits of *Cyphomandra betacea* (Cav.) Sendth. were cut into small pieces. The drying process takes longer time as the fruit has juicy pulp. Hence in order to accelerate the drying process as well as to avoid fungal growth, we developed a technique. The cut pieces were made into ‘garland-like’ with the help of needle and thread. The pieces were arranged in such a way so that one piece does not touch the other and hanged in shade for

drying (Figure 3.1). After drying the dried sample was ground into powder using Waring blender (Cole Parmer, RZ-04245-21). The powdered sample was kept in an airtight container till further use.

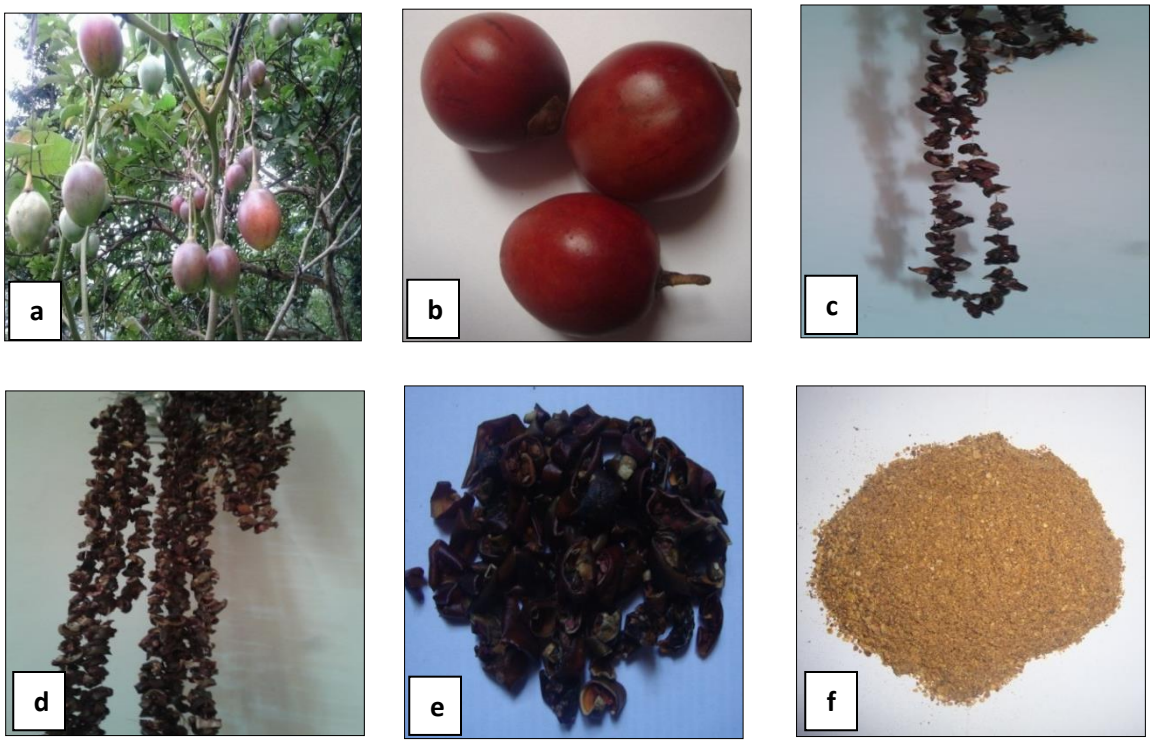


Figure 3.1. Preparation of plant sample (*Cyphomandra betacea* (Cav.) Sendth.) a. Plant of *Cyphomandra betacea* (Cav.) Sendth.; b. Fruits of *Cyphomandra betacea* (Cav.) Sendth. ; c-d. Drying process; e. Dried sample from fruits; f. Powdered sample from dried fruits.

3.3.2. *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

The fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish were cut into small pieces and dried in shade. After drying the dried sample was ground into powder using Waring blender (Cole Parmer, RZ-04245-21) (Figure 3.2). The powdered sample was stored in an airtight container till further use.

a.  b. 

c.  d. 

Figure 3.2. Preparation of plant sample (*Capsicum annuum* var. *cerasiforme* (Mill.) Irish) a. Plant of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish; b. Fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish; c. Dried sample from fruits; d. Powdered sample from dried fruits.

3.3.3. *Dicentra scandens* (D. Don) Walp.

The tuberous roots of *Dicentra scandens* (D. Don) Walp. were washed thoroughly with tap water. The roots were cut in small pieces and dried in shade. The dried plant material was ground into powder using Waring blender (Cole Parmer, RZ-04245-21) (Figure 3.3). The powdered sample was stored in an airtight container till further use.

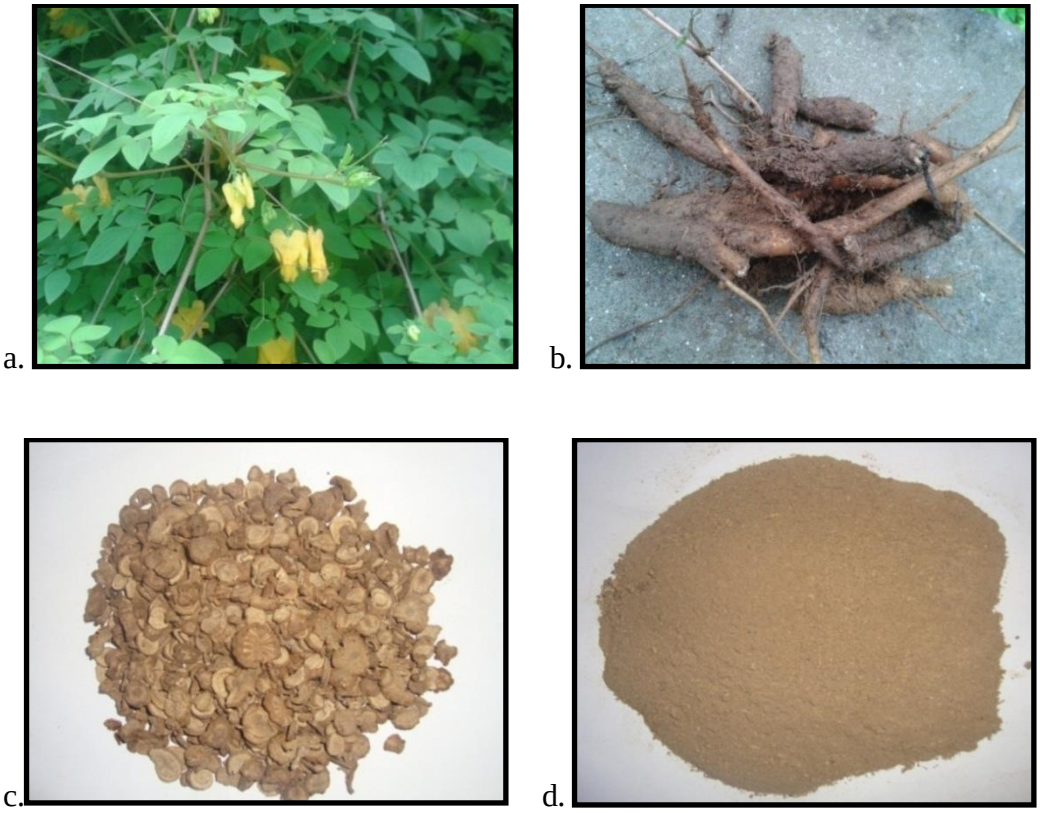


Figure 3.3. Preparation of plant sample (*Dicentra scandens* (D. Don) Walp.) a. Plant of *Dicentra scandens* (D. Don) Walp.; b. Tuberous root; c. Dried tuberous root; d. Powdered sample from tuberous root.

3.3.4. *Heracleum nepalense* D. Don

The fruits of *Heracleum nepalense* D. Don were dried in shade. After drying the dried sample was ground into powder using Waring blender (Cole Parmer, RZ-04245-21) (Figure 3.4). The powdered sample was stored in an airtight container till further use.

Figure 3.4. Preparation of plant sample (*Heracleum nepalense* D. Don) a. Plant of *Heracleum nepalense* D. Don; b. Fruits of *Heracleum nepalense* D. Don; c. Dried sample from fruits; d. Powdered sample from dried fruits.

3.4. Extraction of various phytochemical from test plants

Respective solvent and specific extraction procedures were used in order to extract various phytochemicals namely alkaloid, flavonoid, saponin, steroid and tannin from the test plants. Specific extraction procedures were followed to extract the phytochemicals in crude form and the extracted phytochemicals were termed accordingly as alkaloid, flavonoid, saponin, steroid and tannin extracts. The extracts were hence considered as the phytochemical extract.

3.4.1. Extraction of alkaloid

The extraction of alkaloid was done by the method adapted from Harborne (1998). In 30 g of powdered sample, 10 % acetic acid in ethanol (300 mL) was added. The mixture was incubated for 4 h in the dark. After incubation, the extract was filtered and the solution concentrated to one-fourth of its volume in a boiling Water bath (Remi/RSB-12). To the extract, 25 % ammonia was added until a precipitate was formed and was centrifuged using Centrifuge (Remi/C30-BL) at 2500 rpm for 5 min. The residue obtained was washed with 1 % ammonium hydroxide and filtered (Harborne, 1998). The residue was weighed using Balance (Mettler Toledo/ PL202-S/03) and stored at 4 ^{o}C until further analysis.

3.4.2. Extraction of flavonoid

The extraction of flavonoid was done by the method adapted from Waksmundzka-Hajnos *et al*., (2011). Powdered sample (30 g) was extracted with methanol (300 mL) with a continuous shaking using Rotary shaker (Remi/RS-24 BL) at 120 rpm for 24 h. After filtration, the methanolic extract was evaporated to dryness under reduced pressure in a

Rotary evaporator (Buchi, Switzerland, R-3). The dry residue was soaked in boiling water and cooled in the refrigerator, filtered and then extracted with ethyl acetate in shaking condition at 120 rpm for 24 h. The ethyl acetate fraction was collected using separating funnel and the solvent was evaporated using Rotary evaporator (Waksmundzka-Hajnos *et al*., 2011). The residue (extract) was weighed using Balance (Mettler Toledo/ PL202-S/03) and stored at 4 ^{o}C until further analysis.

3.4.3. Extraction of saponin

The extraction of saponin was done by the method adapted from Khanna and Kannabiran, (2008). The powdered sample (30 g) was defatted by 300 mL petroleum ether for 1 h at 40 ^{o}C in a Water bath (Remi/RSB-12). After filtering the petroleum ether, the sample was extracted with 300 mL of methanol for 1 h with gentle heating (40 ^{o}C) in a Water bath. After filtering, the methanol extract was concentrated. To the concentrated extract, methanol and acetone in the ratio of 1:5 v/v was added in order to precipitate saponin. The precipitate was dried by evaporating the solvent using Rotary evaporator (Buchi, Switzerland, R-3) (Khanna and Kannabiran, 2008). The residue (extract) obtained was weighed using Balance (Mettler Toledo/ PL202-S/03) and stored at 4 ^{o}C until further analysis.

3.4.4. Extraction of steroid

The extraction of steroid was done by the method adapted from Abdulmalik *et al*., (2011). Powdered sample (30 g) was extracted with petroleum ether (300 mL) in a Rotary shaker (Remi/RS-24 BL) at 120 rpm for 24 h. After filtering the solvent, the solvent was evaporated using Rotary evaporator (Buchi, Switzerland, R-3). The residue was extracted

with 96 % ethanol by continuous shaking in a Rotary shaker at 120 rpm for 24 h. The solvent was filtered and was concentrated in Rotary evaporator. To the ethanol extract, acetone (1:10 w/v) was added and vigorously shaken at 120 rpm for 24 h. The solvent was evaporated using Rotary evaporator (Abdulmalik *et al*., 2011). The residue (extract) obtained was weighed using Balance (Mettler Toledo/ PL202-S/03) and was stored at 4 ^{o}C until further analysis.

3.4.5. Extraction of tannin

The extraction of tannin was done by the method adapted from Cobzac *et al*., (2005). Powdered sample (30 g) was extracted with 300 mL distilled water in the Microwave (Samsung/ CE1031LFB) at 100 °C for three cycles of 1 min with 2 min pause. The solvent was filtered using double-layered gauze and centrifuged using Centrifuge (Remi/C30-BL) at 3000 rpm for 5 min to remove the debris. The solvent was evaporated using Rotary evaporator (Buchi, Switzerland, R-3) (Cobzac *et al*., 2005). The residue (extract) obtained was weighed using Balance (Mettler Toledo/ PL202-S/03) and stored at 4 °C until further analysis.

3.5. Preparation of general extracts (aqueous and methanol)

In the present study solvents (water and methanol respectively) were used for the preparation of general extracts namely aqueous and methanol extracts. The extracts were prepared by the method adapted from Bissa and Bohra, (2011). Powdered sample 30 g each was extracted with 300 mL of the respective solvents with continuous shaking at 120 rpm for 24 h using Rotary shaker (Remi/RS-24 BL). The solvents were filtered using double-layered gauze and centrifuged using Centrifuge (Remi/C30-BL) at 3000 rpm for 5

min to remove the debris. The solvent was then evaporated under reduced pressure in a Rotary evaporator (Buchi, Switzerland, R-3). The concentrated extracts were weighed using Balance (Mettler Toledo/ PL202-S/03) and were stored at 4 ^{o}C until further analysis (Bissa and Bohra, 2011).

3.6. Calculation of the extraction yield

The dried powdered sample of plants of known weight was used for extraction. After extraction, the final weight of the extract was measured using Balance (Mettler Toledo/ PL202-S/03). The percentage yield of extracts was calculated as follows (Terblanche *et al*., 2017).

Percentage (%) yield = $w_2 / w_1 \times 100$

Where w_2 is the final weight of the dried extract, w_1 is the known weight of plant sample used for extraction. The SD value was calculated from three different weight measurements.

3.7. Qualitative phytochemical analysis

Various phytochemical extracts, as well as the aqueous and the methanol extracts from the test plants, were subjected to qualitative phytochemical analyses to examine the presence of phytochemicals namely flavonoid, anthocyanin, tannin, phenol, saponin, steroid, alkaloid, glycoside, carbohydrate, protein and fat. The tests for detection of the phytochemicals were performed by adapting various standard procedures (De *et al*., 2010; Lalitha and Jayanthi, 2012; Sofowora, 1993; Tiwari *et al*., 2011; Trease and Evans, 2009).

3.7.1. Detection of flavonoid

3.7.1.1. Sodium hydroxide test: To 1 mL of extract 10 % aqueous sodium hydroxide was added. A yellow coloration developed. A change in color from yellow to colorless on the addition of dilute hydrochloric acid indicated the presence of flavonoid (De *et al*., 2010).

3.7.1.2. Zinc HCl test: To 1 mL of the plant extract, the solution mixture of zinc dust and concentrated hydrochloric acid were added. The formation of red color indicated the presence of flavonoid (De *et al*., 2010).

3.7.1.3. Sulphuric acid test: To 1 mL of the plant extract concentrated sulphuric acid was added. Formation of orange color indicated the presence of flavonoid (Lalitha and Jayanthi, 2012).

3.7.2. Detection of anthocyanin

To 1 mL of the plant extract, 2 M sodium hydroxide was added. Formation of blue-green color indicated the presence of anthocyanin (Lalitha and Jayanthi, 2012).

3.7.3. Detection of tannin

3.7.3.1. Gelatin test: To 1mL of the plant extract 1 % gelatin solution containing 10 % sodium chloride was added. White precipitate indicated the presence of tannin (De *et al*., 2010).

3.7.4. Detection of phenol

3.7.4.1. Ferric chloride test: To 1 mL of the plant extract a few drops of 1 % ferric chloride solution were added. The occurrence of blue-black color indicated the presence of phenol (Tiwari *et al*., 2011).

3.7.5. Detection of saponin

3.7.5.1. Froth test: 0.5 g of plant extract was boiled in 5 mL distilled water, and the content was filtered. To the filtrate, 3 mL of distilled water was added. After shaking it vigorously for 5 min frothing was developed. Frothing persisted on warming and was an indication for the presence of saponin (Trease and Evans, 2009).

3.7.5.2. Foam test: 0.5 g of extract was shaken with 2 mL of water. Formation of foam which persisted for 10 min was an indication for the presence of saponin (Trease and Evans, 2009).

3.7.6. Detection of steroid

3.7.6.1. Liebermann Burchard's test: 1 mL of the plant extract was treated with chloroform and then filtered. To the filtrate, few drops of acetic anhydride were added and boiled. The solution was cooled and a few drops of concentrated sulphuric acid were added. Formation of a brown ring at the junction of the solution indicated the presence of steroid (Tiwari *et al*., 2011).

3.7.6.2. Salkowski's test: 1 mL of the plant extract was treated with chloroform. Few drops of concentrated sulphuric acid were added and mixed well. The solution was

allowed to stand for a few minutes. The appearance of red color at the lower layer indicated the presence of steroid (De *et al*., 2010).

3.7.7. Detection of alkaloid: To 0.5 g of plant extract, 5 mL of 1 % aqueous hydrochloric acid was added and stirred on a Water bath (Remi/RSB-12) and then filtered. The filtrate was subjected to the following tests.

3.7.7.1. Mayer's test: To 1 mL of the filtrate Mayer's reagent was added. The appearance of buff-colored precipitate indicated the presence of alkaloid (Sofowora, 1993).

3.7.7.2. Wagner's test: To 1 mL of the filtrate Wagner's reagent was added. Formation of brown or reddish precipitate indicated the presence of alkaloid (Sofowora, 1993).

3.7.7.3. Hager's test: To 1 mL of the filtrate Hager's reagent was added. Yellow colored precipitate indicated the presence of alkaloid (Sofowora, 1993).

3.7.8. Detection of glycoside

3.7.8.1. Bromine water test: To 1 mL of the plant extract bromine water was added. Formation of yellow precipitate indicated the presence of glycoside (De *et al*., 2010).

3.7.9. Detection of carbohydrate

Plant extracts were dissolved in 5 mL distilled water and the content was filtered. The filtrates were used to test the presence of carbohydrates by the following tests.

3.7.9.1. Molisch's test: To 1 mL of the filtrate two drops of alcoholic α-naphthol solution was added. Formation of a violet ring at the junction of the filtrate and the solution indicated the presence of carbohydrate (Tiwari *et al*., 2011).

3.7.9.2. Benedict's test: To 1 mL of the filtrate Benedict's reagent was added and heated gently in a Water bath (Remi/RSB-12). Formation of orange-red precipitate indicated the presence of reducing sugar (Tiwari *et al*., 2011).

3.7.9.3. Fehling's test: To 1 mL of the filtrate dilute hydrochloric acid was added and was neutralized with the addition of sodium hydroxide. The solution was then heated in a Water bath (Remi/RSB-12) with Fehling's solutions A and B. Red precipitate indicated the presence of reducing sugar (Tiwari *et al*., 2011).

3.7.10. Detection of protein and amino acid

3.7.10.1. Xanthoproteic test: To 1 mL of the plant extract few drops of concentrated nitric acid was added. Formation of yellow color indicated the presence of protein (Tiwari *et al*., 2011).

3.7.10.2. Ninhydrin test: To 1 mL of the plant extract, 0.25 % ninhydrin reagent was added. The solution was boiled for a few minutes. Formation of blue color indicated the presence of amino acid (Tiwari *et al*., 2011).

3.7.11. Detection of fat and fixed oil

3.7.11.1. Stain test: A small quantity of plant extract was pressed between two filter papers. Presence of stain on the first filter paper indicated the presence of fixed oils and fat (De *et al*., 2010).

3.8. Quantitative phytochemical analysis

3.8.1. Determination of total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu method adapted from Slinkard and Singleton, (1977). To estimate the total phenolic content in the extracts, 0.1 mL of extract was mixed with 1 mL Folin-Ciocalteu reagent (10 %). After 3 min, 3 mL of aqueous sodium carbonate (2 %) was added. The reaction mixture was incubated at room temperature for 2 h and the absorbance was recorded at 760 nm using Lambda 25 UV/Vis Spectrophotometer (Perkin Elmer, L600-00BB). Gallic acid was used as a standard to prepare the calibration curve. Total phenolic content was represented as milligram of gallic acid equivalent (GAE) per gram of extract (Slinkard and Singleton, 1977). The entire test was performed in triplicate and their mean value was represented.

3.8.2. Determination of total flavonoid content

The total flavonoid content in the extracts was estimated by an aluminium chloride colorimetric method adapted from Chang *et al*., (2002). To estimate the total flavonoid content in the test extracts, 0.5 mL of sample was mixed with 1.5 mL of methanol (70 %), and 0.1 mL of aluminium chloride (10 %). After 3 min of incubation, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water was added and incubated for 30 min at room temperature. After incubation, the absorbance was recorded at 415 nm using Lambda 25 UV/Vis Spectrophotometer (Perkin Elmer, L600-00BB). Rutin was used as a standard to obtain a calibration curve. Total flavonoid content was expressed as milligram of rutin equivalent (RE) per gram of extract (Chang *et al*., 2002). The entire test was performed in triplicate and their mean value was represented.

3.8.3. Determination of total tannin content

The total tannin content was determined by the Folin-Ciocalteu method adapted from Tambe and Bhambar, (2014). In a 100 mL conical flask containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, 1 mL of 35 % sodium carbonate solution, 0.1 mL of the extract was added. The volume was made up to 10 mL with distilled water. The solution was mixed well and was kept at room temperature for 30 min. Absorbance was measured against the blank at 725 nm using Lambda 25 UV/Vis Spectrophotometer (Perkin Elmer, L600-00BB). Tannic acid was used as a standard for the preparation of a calibration curve. Total tannin content was expressed as milligram of tannic acid equivalent (TAE) per gram of extract (Tambe and Bhambar, 2014). The entire test was performed in triplicate and their mean value was represented.

3.9. Antimicrobial activity assay

3.9.1. Test microorganisms

The test microorganisms were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Gram-positive bacteria namely *Bacillus cereus* (MTCC-6840), *Staphylococcus aureus* (MTCC-7443) and Gram-negative bacteria namely *Escherichia coli* (MTCC-1089), *Klebsiella pneumoniae* (MTCC-3384), *Pseudomonas aeruginosa* (MTCC-1034), *Proteus vulgaris* (MTCC-742), *Salmonella enterica* ser. *typhi* (MTCC-733), *Shigella flexneri* (MTCC-1457) and *Vibrio cholerae* O139 (MTCC-3906) were used in the study. The cultures were revived in Nutrient Agar medium and Luria-Bertani medium. After revival, the Gram staining of bacterial cultures was performed.

3.9.2. Determination of Antimicrobial activity

The antimicrobial assay was performed by following standard agar well diffusion method (Perez *et al.*, 1990). The test plant extracts were dissolved in 0.25 % Dimethyl Sulphoxide (DMSO) to obtain a final stock concentration of 400 mg/mL and sterilized by filtration through 0.45 µm cellulose acetate membrane filters (Sartorius). Various concentrations (25 mg/mL, 50 mg/mL, 100 mg/mL, 200 mg/mL, 400 mg/mL) of phytochemical extracts namely alkaloid, flavonoid, saponin, steroid, tannin along with the aqueous and the methanol extracts were prepared using the stock concentration. The bacterial suspension (100 µL) maintained in the optical density of 0.08-0.13 at 625 nm corresponding to 1×10^8 CFU/mL (0.5 McFarland Standard) was spread onto the surface of Muller Hinton Agar (MHA) medium plate. 100 µL of plant extract (different concentrations) was introduced into the wells. DMSO (0.25 %) was used as the negative control. Gentamicin (0.1 mg/mL) (Shihabudeen *et al.*, 2010) was used as positive control. The plates were incubated in an Incubator (Remi/RIS-24 BL) at 37 ^{o}C for 24 h. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition (Perez *et al.*, 1990) inclusive of the well diameter of 8 mm. The diameter of the zone of inhibition values was expressed in millimeters (mm). All the experiments were performed in triplicate and the mean value was represented.

3.9.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC assay was performed by broth macrodilution method. The extract was two-fold serial diluted (0.048-400 mg/mL) into test tubes containing 1.0 mL of Muller Hinton

broth from the stock concentration of 800 mg/mL. To the different test tubes, 20 µL of bacterial inoculum was added and incubated in an Incubator (Remi/RIS-24 BL) at 37 °C for 24 h. The lowest concentration or highest dilution of the plant extract that inhibits the visible growth of test microorganism is known as minimum inhibitory concentration (Wiegand *et al*., 2008). For MBC determination, an aliquot (100 µL) of each sample that did not show visible growth was inoculated onto MHA plates. Plates were incubated in an Incubator for 24 h at 37 ^{o}C and then examined for microbial growth (Heredia *et al*., 2005). The MBC was defined as the lowest concentration of the extract at which no microbial growth was detected.

The MIC index was calculated by using the following formula to determine whether the plant extract is bactericidal or bacteriostatic in nature.

MIC index=MBC/MIC

Interpretation: Bactericidal, MIC index ≤ 4; Bacteriostatic, MIC index > 4 (Kone *et al*., 2007).

3.10. Time kill assay

To determine the bacterial killing property of the extracts the bacterial cells were inoculated into the nutrient broth and incubated in an Incubator (Remi/RIS-24 BL) at 37 ^{o}C for 24 h. After incubation, bacterial culture in the optical density range of 0.08-0.13 at 625 nm was maintained. The extracts at the concentrations of 1/2×MIC, MIC and 2×MIC were added to the bacterial suspension and incubated in an Incubator at 37 °C for 0–120 min. After incubation, the assay mixtures were serially diluted with 10 mM sodium

phosphate buffer (pH 7.4) and 100 µL of each from 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions were spread on to the nutrient agar plates. The plates were incubated at 37 ^{o}C for 24 h. After incubation, the colonies were hand counted and plotted as CFU/mL (Saritha *et al*., 2015).

3.11. Cellular leakage assay

The test microorganisms were grown overnight (24 h) in nutrient broth with continuous shaking at 120 rpm at 37 ^{o}C in an Orbital shaking incubator (Remi/RIS-24 BL). After incubation, the cultures were transferred to sterile tubes and were subjected to centrifugation at 10,000 rpm for 10 min using Centrifuge (Sorvall Legend XT Centrifuge-Thermo Scientific). The supernatant was discarded and the pellet was resuspended in sterile 0.85 % sodium chloride and the optical density of 1.5 was maintained at 600 nm. The extracts were added to 1 mL of bacterial suspension to achieve the final concentrations of MIC and 400 mg/mL. The suspension was incubated at different time intervals (0 min, 30 min, 60 min, 90 min and 120 min). After incubation, the aliquots of the samples were drawn at different time intervals, centrifuged using Centrifuge (Thermo Scientific/ Micro CL-21R) at 10,000 rpm for 10 min and cellular materials in the suspensions absorbing at 260 nm and 280 nm were measured using Lambda 25 UV/Vis Spectrophotometer (Perkin Elmer, L600-00BB). The net leakage of cellular materials absorbing at 260 nm and 280 nm was calculated by subtracting the absorbance of control untreated cells and UV absorbance of plant extract from the absorbance of bacterial cells treated with the test plant extrats (El-Nakeeb *et al*., 2011; Imelda *et al*., 2014).

3.11.1. Estimation of total protein content

The total protein content of the supernatant of the suspensions of the treated bacteria and untreated bacterial control was determined by using the Bradford assay. One mL of Bradford reagent was added to 1 mL of the supernatant and incubated at room temperature for 5 min. The absorbance of the solution was measured at 595 nm using Lambda 25 UV/Vis Spectrophotometer (Perkin Elmer, L600-00BB). Bovine serum albumin (BSA) was used as the protein standard (Bradford, 1976).

3.12. Scanning Electron Microscope Study

Scanning electron microscope (SEM) study was performed by the method adapted from Borthakur and Joshi, (2016). The analysis was performed to visualize the effect of the extracts on the bacterial cell membrane. Test bacteria were treated with plant extracts at the concentrations of MIC and 400 mg/mL which was the highest concentration of the extracts used in the study and was kept overnight for incubation at 37 ^{o}C. After the incubation, the bacterial cultures were harvested by centrifugation at 8000 rpm for 15 min at 4 ^{o}C using Centrifuge (Hermle Germany/ Z326K). The bacterial cells were washed three times with 0.1 M sodium cacodylate buffer (pH 7.3) followed by distilled water each time by centrifugation at 3000 rpm for 3 min. The cells were then fixed with 3 % glutaraldehyde and kept overnight at 4 ^{o}C. Glutaraldehyde was drained off and the bacterial pellets were subjected to three consecutive washes with 0.1 M sodium cacodylate buffer (pH 7.3), followed by one wash with distilled water each time by centrifugation at 1000 rpm for 15 min at 4 ^{o}C. The samples were then mounted in the coverslip. The samples were kept at room temperature for drying. Finally, the samples

were sputter coated (Ion Sputter JFC-1100/Jeol) with a thin layer of gold-palladium and scanned under a Scanning Electron Microscope (JSM-6360/Jeol) (Borthakur and Joshi, 2016).

3.13. Synergistic antimicrobial activity assay of various phytochemical extracts of the test plants

Possible synergistic antimicrobial activity of various phytochemical extracts from *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme*, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don were evaluated by the checkerboard assay.

3.13.1. Checkerboard assay

The possible synergistic antimicrobial activity of selected phytochemicals against different test microorganisms was evaluated by using the checkerboard broth microdilution method (Moody, 2010).

3.13.1.1. Preparation of checkerboard microdilution panels

Two-fold serial dilutions of the phytochemicals were prepared using Muller Hinton Broth (MHB) for each phytochemical extract tested. Concentrations of the phytochemicals ranging from four to eight times the MIC to 1/8 or 1/16 times the MIC in the final panel was prepared. 50 µL aliquot of each phytochemical extract in combination was placed into the wells of the sterile 96-well microtiter plate (100 µL final volume per well). The individual phytochemical extracts (100 µL) were dispensed into the first row and first

column. Growth control, sterility control of uninoculated broth, sterility control of test phytochemical extracts were included in the panel (Moody, 2010).

3.13.1.2. Preparation of inoculum

Three to five colonies of test microorganism was added to 5 mL of Muller Hinton Broth and incubated at 37 ^{o}C for 24 h in an Incubator (Remi/RIS-24 BL). The turbidity was adjusted to 0.5 McFarland turbidity standards (approximately 1.5×10^{8} CFU/mL). For the preparation of the intermediate inoculum dilution, 0.8 mL of the inoculum was added to 25 mL of water diluent to obtain a final concentration of 4×10^{6} to 5×10^{6} CFU/mL (Moody, 2010).

3.13.1.3. Inoculation and incubation

Into the wells of microdilution panels, 0.01 mL of the intermediate inoculum was added excluding the sterility control wells and plant extracts control wells. Inoculum count verification plate and purity control plates were prepared by streaking the inoculum into MHA plates. The plates were incubated in an Incubator (Remi/RIS-24 BL) at 37 ^{o}C for 20 h. After incubation, the microtiter plates were examined and the MIC was determined (Moody, 2010). 20 µL of 2,3,5-Triphenyl tetrazolium chloride (TTC) at a concentration of 2 mg/mL was added to each well and the plates were incubated at 37 ^{o}C for 20 min. After incubation, the wells that appear pink comparable to that of the growth control well were interpreted as positive for bacterial growth whereas wells with colorless solution were interpreted as negative for bacterial growth. The well containing the lowest concentration of the colorless solution was interpreted as the MIC. The MIC of individual phytochemical extracts and in combination were recorded (Basri and Sandra, 2016).

3.13.1.4. Determination of Fractional Inhibitory Concentration (FIC) and Fractional Inhibitory Concentration index (ΣFIC)

The FIC for the combination of phytochemicals was calculated using the following formula:

FIC of phytochemical extract A = MIC of phytochemical extract A in combination/ MIC of phytochemical extract A alone

FIC of phytochemical extract B = MIC of phytochemical extract B in combination/ MIC of phytochemical extract B alone

The FIC index (ΣFIC) for each combination of phytochemicals was calculated as follows:

ΣFIC = FIC of phytochemical extract A + FIC of phytochemical extract B

Interpretation:

Synergism; $\Sigma FIC \leq 0.5$, Additive; $0.5 < \Sigma FIC \leq 2$, Indifference; $2 < \Sigma FIC \leq 4$, Antagonism; $\Sigma FIC > 4$ (Basri and Sandra, 2016; Moody, 2010).

3.14. Evaluation of antioxidant activity of various phytochemical extracts and general extracts

Various phytochemical extracts namely alkaloid, flavonoid, steroid, saponin and tannin, as well as the aqueous and the methanol extracts, were evaluated for the antioxidant property. From the stock concentration of 1000 µg/mL of the extracts, different concentrations ranging from 10 µg/mL to 100 µg/mL were prepared for antioxidant assay.

3.14.1. DPPH free radical scavenging assay

The antioxidant activity of the plant extracts was estimated by DPPH (2,2-diphenyl-l-picryl hydrazyl) radical scavenging method adapted from Blois, (1958).

For analysis of antioxidant activity, 0.1 mM DPPH solution was prepared in methanol. In different test tubes, 3 mL of extract at various concentrations (10-100 µg/mL) was added. To each test tube, 1 mL of 0.1 mM DPPH in methanol solution was added. Control was prepared without adding the extracts. The reaction mixtures were incubated in dark at room temperature for 30 min and the absorbance of the reaction mixtures was taken at 517 nm by using Lambda 25 UV/Vis Spectrophotometer (Perkin Elmer, L600-00BB). Ascorbic acid at various concentrations was used as a standard. The decrease in optical density of DPPH on the addition of test extracts and the ascorbic acid in relation to control was used to calculate the antioxidant activity as percentage inhibition (% IP) or scavenging % of DPPH radical (Macwan and Patel, 2010).

$$\% \text{ inhibition} = \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})}{(\text{Absorbance}_{\text{control}})} \times 100$$

The entire test was performed in triplicate and the mean value was represented.

IC_{50} value refers to the concentration of the sample which is required to scavenge 50 % of DPPH free radicals (Shyur *et al*., 2005).

3.14.2. Ferric reducing antioxidant power (FRAP) assay

The reducing power of various extracts of the test plants was determined by the method adapted from Oyaizu, (1986). Various concentrations of extracts (10-100 µg/mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL potassium

ferricyanide (1 %). The reaction mixture was kept in a Water bath (Remi/RSB-12) at 50 ^{o}C for 20 min. After cooling, aliquot of 10 % trichloroacetic acid (TCA) (2.5 mL) was added and centrifuged using Centrifuge (Remi/C30-BL) at 3000 rpm for 10 min. After centrifugation, the upper layer of the solution (2.5 mL) was taken and was mixed with 2.5 mL of distilled water and 0.5 mL of freshly prepared 0.1 % ferric chloride solution. The absorbance of the reaction mixture was measured at 700 nm using Lambda 25 UV/Vis spectrophotometer (Perkin Elmer, L600-00BB). Ascorbic acid at various concentrations was used as a standard. Increase in the absorbance of the reaction mixture indicated an increase in the reducing power of extract (Macwan and Patel, 2010).The entire test was performed in triplicate and the mean value was represented.

3.14.3. Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenging activity was determined by the method adapted from Ruch *et al*., (1989). A solution of hydrogen peroxide (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). 0.1 mL of various concentrations (10-100 µg/mL) of extracts were transferred into the test tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). To the test tubes 0.6 mL hydrogen peroxide solution was added. The tubes were then vortexed using Vortex mixer (Eltex, VM 301) and after 10 min the absorbance was measured at 230 nm against a blank. 50 mM phosphate buffer without hydrogen peroxide was used as the blank. Control was prepared without using the extracts. Ascorbic acid at various concentrations was used as the standard (Ruch *et al*., 1989). Hydrogen peroxide scavenging activity or percentage inhibition of hydrogen peroxide was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance}_{control} - \text{Absorbance}_{sample})}{(\text{Absorbance}_{control})} \times 100$$

The entire test was performed in triplicate and the mean value was represented.

3.14.4. Nitric oxide radical scavenging assay

The nitric oxide radical scavenging activity was determined by the method adapted from Hazra *et al*., (2008). A reaction mixture containing 10 mM sodium nitroprusside (SNP), phosphate buffered saline (PBS) (pH 7.4) and various concentrations of the plant extracts (10-100 µg/mL) in a final volume of 3 mL were prepared. The reaction mixture was incubated at 25 ^{o}C in a Water bath (Remi/RIS-12) for 150 min. After incubation, to the incubated sample (0.5 mL), 1 mL of sulfanilamide (0.33 % in 20 % glacial acetic acid) was added and allowed to stand for 5 min. To the reaction mixture, 1 mL of naphthyl ethylenediamine dihydrochloride (NED) (0.1 %) was added and incubated at 25 ^{o}C for 30 min. The absorbance was measured spectrophotometrically at 540 nm against a blank using Lambda 25 UV/Vis Spectrophotometer (Perkin Elmer, L600-00BB). Ascorbic acid at various concentrations was used as the standard. Control was prepared without adding the plant extract (Hazra *et al*., 2008). The nitric oxide radical scavenging activity in terms of percentage inhibition of nitric oxide radical was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance}_{control} - \text{Absorbance}_{sample})}{(\text{Absorbance}_{control})} \times 100$$

The entire test was performed in triplicate and the mean value was represented.

3.14.5. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was determined the method adapted from Klein *et al*., (1981). Different concentrations (10-100 µg/mL) of the extracts were taken in capped tubes to which 1 mL of iron–EDTA solution (0.13 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 mL of 0.018 % EDTA and 1 mL of 0.85 % DMSO (in 0.1 M phosphate buffer , pH 7.4) were added, followed by addition of 0.5 mL of 0.22 % ascorbic acid . The tubes were capped tightly and incubated at 85 ^{o}C in a Water bath (Remi/RIS-12) for 15 min. Post incubation, the tubes were uncapped and immediately 1 mL of ice-cold trichloroacetic acid (TCA) (17.5 %) was added. Then, 3 mL of Nash reagent was added to all tubes and kept for incubation at room temperature for 15 min. Butylated hydroxytoluene (BHT) at various concentrations was used as the standard. Control was prepared without adding the plant extract. The absorbance of the reaction mixture was taken against the blank at 412 nm using Lambda 25 UV/Vis spectrophotometer (Perkin Elmer, L600-00BB) (Klein *et al*., 1981). The hydroxyl radical scavenging activity or percentage inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance}_{control} - \text{Absorbance}_{sample})}{(\text{Absorbance}_{control})} \times 100$$

The entire test was performed in triplicate and the mean value was represented.

3.15. Characterization of extracts by Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was performed at the Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India. The extracts were analyzed by GCMS- QP2010 Plus (Shimadzu) with head space auto sampler (AOC-20s) and auto-injector (AOC-20i), equipped with mass selective detector, having ion source temperature of 230 ^{o}C, interface temperature of 270 ^{o}C, a solvent cut time of 3.50 min, threshold of 1,000 eV and mass range of 40 to 650 m/z. Helium gas was used as a carrier at a linear velocity of 40.9 cm/s, with a split ratio of 10:1. The oven temperature was programmed from 100 ^{o}C (2 min), rising at the rate up to 10 ^{o}C/min with 5 min hold, raising at the rate of 15 ^{o}C/min up to 280 ^{o}C with 26 min hold. The injector temperature and volume of injected samples were maintained at 260 ^{o}C and 1 µL respectively. The interpretation for the mass spectrum analysis was done using the database of the National Institute of Standard Technology (NIST11) library and WILEY8 library as provided by the Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India.

3.16. Statistical analysis

The mean and standard deviation (SD) values were calculated using Microsoft Office Excel 2007. All statistical analyses were performed using GraphPad Prism V5.01 (San Diego, USA). The data were analyzed using Two-Way ANOVA followed by Bonferroni post-tests. A value of $p<0.05$ was considered to be statistically significant.

4. RESULTS

4.1. Collection and identification of plants

The details of the plants identified, places of collection, accession number of voucher specimens are shown in Table 4.1 and the parts of the plants used in the study are shown in Table 4.2.

Table 4.1: Identification of the plants used for the study

Sl. no.	Plants identified	Common name	Local name	Family	Accession number	Place of collection
1	*Cyphomandra betacea* (Cav.) Sendth.	Tamarillo or Tree Tomato	*Rookh tamater*	Solanaceae	09739	Temi Tarku, South Sikkim
2	*Capsicum annuum* var. *cerasiforme* (Mill.) Irish	Red Cherry Pepper	*Dalle Khorsani*	Solanaceae	09731	Lingee Payong, South Sikkim
3	*Dicentra scandens* (D. Don) Walp.	Bleeding Heart Vine or Athens yellow	*Jogi laharra*/ *Kanchi laharra*/ *Kundaley*	Fumariaceae	09734	Damthang, South Sikkim
4	*Heracleum nepalense* D. Don	Nepal Hogweed	*Chimphing*	Umbelliferae	09732	Damthang, South Sikkim

Table 4.2: Parts of the plants used for the study

Sl. No.	Name of plants	Parts of the plants used
1	*Cyphomandra betacea* (Cav.) Sendth.	Fruit
2	*Capsicum annuum* var. *cerasiforme* (Mill.) Irish	Fruit
3	*Dicentra scandens* (D. Don) Walp.	Tuberous root
4	*Heracleum nepalense* D. Don	Fruit

4.2. Extraction yield

The percentage yield of various extracts of the test plants is given in Table 4.3. Among all the extracts the highest yield of 29.09±1.40 % was obtained from tannin extract of *Dicentra scandens* (D. Don) Walp. While the steroid extract has the least percentage yield among all the extracts of the test plants. The least yield of 3.66±0.27 % was obtained in the steroid extract of *Heracleum nepalense* D. Don.

Table 4.3: Percentage yield of the various extracts of the plants under study

Extracts		Yield (%)			
		***Cyphomandra betacea* (Cav.) Sendth.**	***Capsicum annuum* var. *cerasiforme* (Mill.) Irish**	***Dicentra scandens* (D. Don) Walp.**	***Heracleum nepalense* D. Don**
Phytochemical extracts	Alkaloid extract	13.73±0.35	14.69±0.29	17.35±0.98	11.66±0.75
	Flavonoid extract	16.66±0.47	15.98±0.72	7.59±0.15	9.7±0.55
	Saponin Extract	19.91±0.51	15.89±0.60	12.47±0.63	11.85±0.89
	Steroid Extract	7.36±0.45	4.37±0.51	6.45±0.63	3.66±0.27
	Tannin Extract	15.35±0.66	22.95±1.29	29.09±1.40	16.46±1.33
General extracts	Aqueous extract	22.62±1.00	25.32±1.27	26.26±1.52	19.72±1.72
	Methanol extract	24.41±0.81	22.81±0.57	19.55±0.93	21.33±0.38

Each value represents mean ± SD (n=3).

4.3. Qualitative phytochemical analysis of the various extracts of the plants under study

The phytochemical extracts, as well as the aqueous and the methanol extracts, revealed the presence of various phytoconstituents. In the phytochemical extracts, respective phytochemicals along with some other components were detected. However in the aqueous and the methanol extracts most of the phytoconstituents such as alkaloid, flavonoid, tannin, phenol, saponin, steroid, glycoside, anthocyanin, protein, carbohydrate and fat were detected. The phytochemical profiles of the various extracts of plants under study are shown in Table 4.4, Table 4.5, Table 4.6 and Table 4.7.

Table 4.4: Phytochemical analysis of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

Test for Phytochemicals	Phytochemical extracts					General extracts	
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol
Flavonoid	-	+	-	-	-	+	+
Anthocyanin	+	+	+	+	+	+	+
Tannin	-	-	-	-	+	-	-
Phenol	-	+	-	-	+	+	+
Saponin	-	-	+	-	-	+	+
Steroid	-	-	-	+	-	+	+
Alkaloid	+	-	-	-	-	+	+
Glycoside	+	+	+	+	+	-	+
Carbohydrate	-	+	+	-	+	+	-
Protein	+	-	+	+	+	+	+
Fat	+	+	+	+	+	-	+

"+" Indicates present; "-" Indicates absent

Table 4.5: Phytochemical analysis of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Test for Phytochemicals	Phytochemical extracts					General extracts	
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol
Flavonoid	-	+	-	-	-	+	+
Anthocyanin	+	+	+	+	+	+	+
Tannin	-	-	-	-	+	+	+
Phenol	-	+	-	-	+	+	+
Saponin	-	-	+	+	-	+	+
Steroid	+	-	+	+	-	+	+
Alkaloid	+	-	-	-	-	+	+
Glycoside	+	-	-	-	-	+	+
Carbohydrate	+	+	+	+	+	+	-
Protein	-	+	-	-	+	+	+
Fat	+	+	+	+	+	+	+

"+" Indicates present; "-" Indicates absent

Table 4.6: Phytochemical analysis of various extracts of *Dicentra scandens* (D. Don) Walp.

Test for Phytochemicals	Phytochemical extracts					General extracts	
	Alkaloid	**Flavonoid**	**Saponin**	**Steroid**	**Tannin**	**Aqueous**	**Methanol**
Flavonoid	-	+	-	-	-	+	+
Anthocyanin	+	+	+	+	+	+	+
Tannin	-	-	-	-	+	+	+
Phenol	-	+	-	-	+	+	+
Saponin	-	-	+	-	+	+	-
Steroid	-	-	-	+	-	+	+
Alkaloid	+	-	-	-	-	+	+
Glycoside	+	+	+	+	+	+	+
Carbohydrate	+	-	+	+	+	+	+
Protein	-	+	+	-	+	-	+
Fat	+	-	-	+	-	-	-

"+" Indicates present; "-" Indicates absent

Table 4.7: Phytochemical analysis of various extracts of *Heracleum nepalense* D. Don

Test for Phytochemicals	Phytochemical extracts					General extracts	
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol
Flavonoid	-	+	-	-	-	-	-
Anthocyanin	+	+	+	+	+	+	+
Tannin	-	-	-	-	+	+	-
Phenol	-	+	-	-	+	+	+
Saponin	-	-	+	-	+	+	-
Steroid	+	-	+	+	-	-	-
Alkaloid	+	-	-	-	-	+	+
Glycoside	+	-	+	-	-	+	+
Carbohydrate	+	-	+	+	+	+	+
Protein	-	+	+	+	+	+	+
Fat	+	+	+	+	-	-	+

"+" Indicates present; "-" Indicates absent

4.4. Quantitative phytochemical analysis of the various extracts of the plants under study

The total phenolic, flavonoid and tannin contents of various extracts of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don are shown in Table 4.8,

Table 4.9, Table 4.10 and Table 4.11 respectively. The highest content of flavonoid (10.90±0.47 mg RE/g of extract) was observed in the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish. Similarly, the highest tannin content (36.24±0.03 mg TAE/g of extract) was observed in the tannin extract of *Dicentra scandens* (D. Don) Walp. However, among all the test plants the highest phenolic content (17.43±0.01 mg GAE/g of extract) was observed in the methanol extract of *Cyphomandra betacea* (Cav.) Sendth.

Table 4.8: Total phenolic, flavonoid and tannin contents of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

	***Cyphomandra betacea* (Cav.) Sendth. extracts**	**Total phenolic content (mg GAE/g) ± SD**	**Total flavonoid content (mg RE/g) ± SD**	**Total tannin content (mg TAE/g) ± SD**
Phytochemical extracts	Flavonoid extract	8.70±0.03	10.39±0.60	-
	Tannin extract	2.11±0.23	-	5.72±0.03
General extracts	Methanol extract	17.43±0.01	5.28±0.24	-
	Aqueous extract	3.02±0.18	2.18±0.15	-

Each value is expressed as mean ± SD (n=3). GAE; gallic acid equivalent, RE; rutin equivalent, TAE; tannic acid equivalent. '-' not detected in the qualitative phytochemical test. All the experiments were performed in triplicate.

Table 4.9: Total phenolic, flavonoid and tannin contents of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

	***Capsicum annuum* var. *cerasiforme* (Mill.) Irish extracts**	**Total phenolic content (mg GAE/g) ± SD**	**Total flavonoid content (mg RE/g) ± SD**	**Total tannin content (mg TAE/g) ± SD**
Phytochemical extracts	Flavonoid extract	2.75±0.25	10.90±0.47	-
	Tannin extract	2.98±0.04	-	17.97±0.87
General extracts	Methanol extract	7.41±0.03	6.81±0.24	-
	Aqueous extract	4.03±0.07	9.63±0.24	-

Each value is expressed as mean ± SD (n=3). GAE; gallic acid equivalent, RE; rutin equivalent, TAE; tannic acid equivalent. '-' not detected in the qualitative phytochemical test. All the experiments were performed in triplicate.

Table 4.10: Total phenolic, flavonoid and tannin contents of various extracts of *Dicentra scandens* (D. Don) Walp.

	***Dicentra scandens* (D. Don) Walp. extracts**	**Total phenolic content (mg GAE/g) ± SD**	**Total flavonoid content (mg RE/g) ± SD**	**Total tannin content (mg TAE/g) ± SD**
Phytochemical extracts	Flavonoid extract	4.86±0.02	2.51±0.05	-
	Tannin extract	6.75±0.01	-	36.24±0.03
General extracts	Methanol extract	12.40±0.03	2.36±0.05	23.92±0.03
	Aqueous extract	5.05±0.20	3.16±0.24	19.24±0.01

Each value is expressed as mean ± SD (n=3). GAE; gallic acid equivalent, RE; rutin equivalent, TAE; tannic acid equivalent. '-' not detected in the qualitative phytochemical test. All the experiments were performed in triplicate.

Table 4.11: Total phenolic, flavonoid and tannin contents of various extracts of *Heracleum nepalense* D. Don

	***Heracleum nepalense* D. Don extracts**	**Total phenolic content (mg GAE/g) ± SD**	**Total flavonoid content (mg RE/g) ± SD**	**Total tannin content (mg TAE/g) ± SD**
Phytochemical extracts	Flavonoid extract	3.15±0.01	4.12±0.34	-
	Tannin extract	3.76±0.05	3.06±0.27	8.07±0.02
General extracts	Methanol extract	7.01±0.17	1.18±0.31	4.27±0.01
	Aqueous extract	3.23±0.02	3.45±0.24	7.91±0.03

Each value is expressed as mean ± SD (n=3). GAE; gallic acid equivalent, RE; rutin equivalent, TAE; tannic acid equivalent. '-' not detected in the qualitative phytochemical test. All the experiments were performed in triplicate.

4.5. Antimicrobial activity assay

Antimicrobial activity of the various phytochemical extracts namely alkaloid, flavonoid, saponin, steroid and tannin of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme*, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don was evaluated against nine test microorganisms namely *Bacillus cereus* (MTCC-6840), *Staphylococcus aureus* (MTCC-7443), *Escherichia coli* (MTCC-1089), *Klebsiella pneumoniae* (MTCC-3384), *Pseudomonas aeruginosa* (MTCC-1034), *Proteus vulgaris* (MTCC-742), *Salmonella enterica* ser. *typhi* (MTCC-733), *Shigella flexneri* (MTCC-1457) and *Vibrio cholerae* O139 (MTCC-3906). Apart from the phytochemical extracts, the antimicrobial activity of the general extracts namely aqueous and methanol extracts was also evaluated. The results of the antimicrobial activity in terms of the diameter of the zone of inhibition in millimeters are depicted in Table 4.12 to Table 4.25 and Figure 4.1 to Figure 4.20.

4.5.1. Antimicrobial activity of various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth.

The zone of inhibition exhibited by the various phytochemical extracts (alkaloid, flavonoid and saponin) of *Cyphomandra betacea* (Cav.) Sendth. against different test microorganisms are shown in Table 4.12 to Table 4.14. At the concentration of 400 mg/mL the alkaloid extract showed the largest zone of inhibition (25.66±0.57 mm) against *Pseudomonas aeruginosa* (Table 4.12). Flavonoid extract exhibited the largest zone of inhibition (24.66±0.57 mm) against *Staphylococcus aureus* (Table 4.13). The saponin extract exhibited the largest zone of inhibition (20.66±0.57 mm) against *Staphylococcus aureus* (Table 4.14). At the concentration of 400 mg/mL, the tannin extract exhibited antimicrobial activity against *Shigella flexneri* and *Vibrio cholerae*

O139 with the zone of inhibition of 12 mm and 12.66±0.57 mm respectively. At the concentration of 200 mg/mL, the extract inhibited the growth of *Vibrio cholerae* O139 only with the zone of inhibition of 11.33±0.57 mm. However, no zone of inhibition was observed with the steroid extract.

4.5.2. Antimicrobial activity of the aqueous and the methanol extracts of *Cyphomandra betacea* (Cav.) Sendth.

At the concentration of 400 mg/mL the aqueous extract exhibited antimicrobial activity against *Bacillus cereus, Escherichia coli, Proteus vulgaris, Salmonella enterica* ser. *typhi, Shigella flexneri* and *Vibrio cholerae* O139 with the zone of inhibition of 12 mm, 14.33±0.57 mm, 15.33±0.57 mm, 14 mm, 12.66±0.57 mm and 13 mm respectively. At the concentration of 200 mg/mL, the zone of inhibition (13.33±0.57 mm) was observed against *Proteus vulgaris*. The aqueous extract at all the concentrations did not exhibit the zone of inhibition against *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

At the concentration of 400 mg/mL the methanol extract exhibited antimicrobial activity against all the test microorganisms with the largest zone of inhibition (26.33±0.57 mm) against *Proteus vulgaris* followed by *Staphylococcus aureus* (26 mm). At lower concentration of 25 mg/mL the extract exhibited antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* (Table 4.15).

Table 4.12: Zone of inhibition exhibited by the alkaloid extract of *Cyphomandra betacea* (Cav.) Sendth. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	12.66±0.57	11.33±0.57	12.66±0.57	-	14.33±0.57	-	14.33±0.57	-	-
100	14.66±0.57	13.33±0.57	15.66±0.57	-	19.66±0.57	-	18	12	13.66±0.57
200	16.33±0.57	18.66±0.57	17	12.66±0.57	23.33±0.57	-	20.66±0.57	14±1	15.33±0.57
400	18.66±0.57	23.66±0.57	19	18.66±0.57	25.66±0.57	12	22.33±0.57	17	17.66±0.57
Positive control	24.66±0.57	25	25	22.66±0.57	21.66±0.57	23.33±0.57	23.66±0.57	22.66±0.57	23
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.13: Zone of inhibition exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	-	11.66±0.57	13.33±0.57	-	-	-	-	-	-
100	15 ±1	15.33±0.57	17	-	11.33±0.57	-	12	12.33±0.57	14±1
200	17	19	18.33±0.57	11±1	14	13.33±0.57	12.33±0.57	15.33±0.57	16.33±0.57
400	19	24.66±0.57	20.66±0.57	13.66±0.57	16.66±0.57	16.66±0.57	18.33±0.57	18.66±0.57	18
Positive control	25.66±0.57	24	25 ±1	20.33±0.57	21	22.66±0.57	24	23.33±0.57	23.33±0.57
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.14: Zone of inhibition exhibited by the saponin extract of *Cyphomandra betacea* (Cav.) Sendth. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	-	11.33±0.57	-	-	-	-	-	-	-
100	12.66±0.57	14.33±0.57	-	-	11.33±0.57	-	-	-	-
200	15.33±0.57	17.66±0.57	13.66±0.57	-	12.33±0.57	11.33±0.57	13	-	13.33±0.57
400	17.66±0.57	20.66±0.57	16	15.33±0.57	14	15 ±1	14.33±0.57	13	16
Positive control	25	24.33±0.57	25.33±0.57	20.33±0.57	21.33±0.57	23	23.33±0.57	22.66±0.57	23.33±0.57
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.15: Zone of inhibition exhibited by the methanol extract of *Cyphomandra betacea* (Cav.) Sendth. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	12.66±0.57	12.66±0.57	12.33±0.57	-	-	13.66±0.57	-	-	-
50	18	14	14.66±0.57	-	13.66±0.57	16.66±0.57	-	-	-
100	21.66±0.57	19.66±0.57	18.66±0.57	12.66±0.57	17.66±0.57	19.66±0.57	12	11	13.66±0.57
200	22.66±0.57	23.33±0.57	21.66±0.57	13.33±0.57	19	25	15	12.66±0.57	15.33±0.57
400	23.66±0.57	26	25	15	20.33±0.57	26.33±0.57	21.66	15.66±0.57	17.33±0.57
Positive control	24.66±0.57	25	24.66±0.57	22	21.66±0.57	22.66±0.57	23	23.33±0.57	23
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

4.5.3. Comparative analysis of the antimicrobial activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

In the present study, there was a significant ($p<0.05$) difference in the antimicrobial activity in terms of the diameter of the zone of inhibition values exhibited by the various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth (Figure 4.1-4.4). At the concentration of 400 mg/mL the alkaloid, flavonoid and saponin extracts inhibited the growth of all the test microorganisms. The alkaloid extract exhibited largest zone of inhibition (25.66±0.57 mm) against *Pseudomonas aeruginosa*. However, the flavonoid extract exhibited significantly ($p<0.05$) higher antimicrobial activity than the alkaloid extract against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Shigella flexneri* and *Vibrio cholerae* O139 (Figure 4.1). The tannin extract inhibited the growth of *Vibrio cholerae* O139 at the concentrations of 200 mg/mL (Figure 4.2) and 400 mg/mL (Figure 4.1). At the concentration of 25 mg/mL of the phytochemical extracts, the zone of inhibition was not observed against any of the test microorganisms.

At the concentration of 400 mg/mL, the methanol extract exhibited antimicrobial activity against all the test microorganisms. The aqueous extract inhibited the growth of *Bacillus cereus, Escherichia coli, Proteus vulgaris, Salmonella enterica* ser. *typhi, Shigella flexneri* and *Vibrio cholerae* O139. The methanol extract exhibited significantly ($p<0.05$) higher antimicrobial activity as compared to the aqueous extract with the largest zone of inhibition (26.33±0.57 mm) against *Proteus vulgaris* (Figure 4.5). Furthermore, the methanol extract showed the zone of inhibition against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* at the

concentration of 25 mg/mL which was the lowest concentration of the extract used in the present study (Table 4.15).

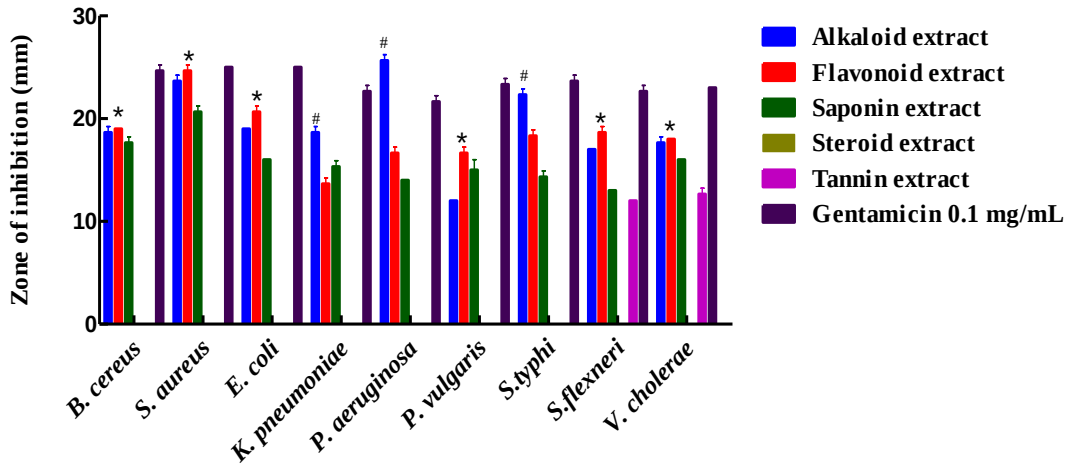


Figure 4.1: Zone of inhibition exhibited by the various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth. at the concentration of 400 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; # Alkaloid vs. Flavonoid, * Flavonoid vs Alkaloid. The data represent mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S.typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

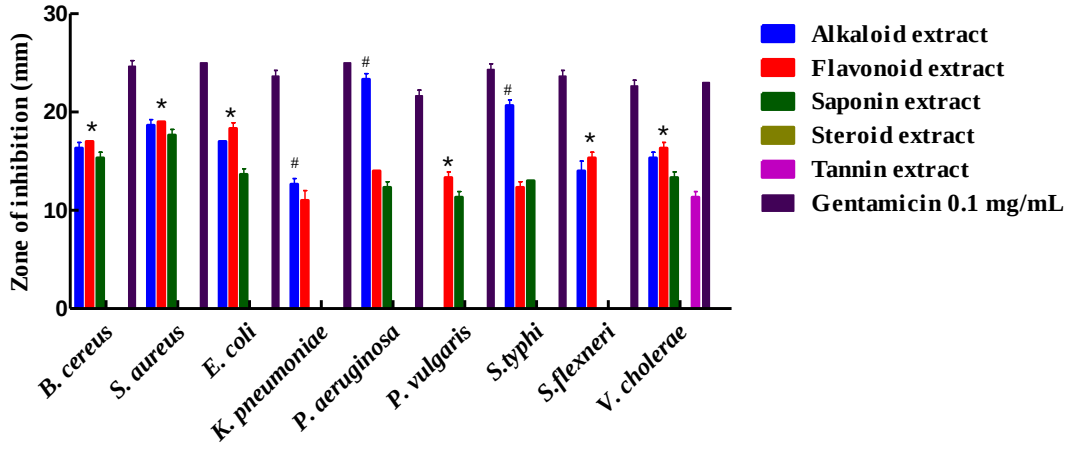


Figure 4.2: Zone of inhibition exhibited by the various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth. at the concentration of 200 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; # Alkaloid vs. Flavonoid, * Flavonoid vs Alkaloid. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S.typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

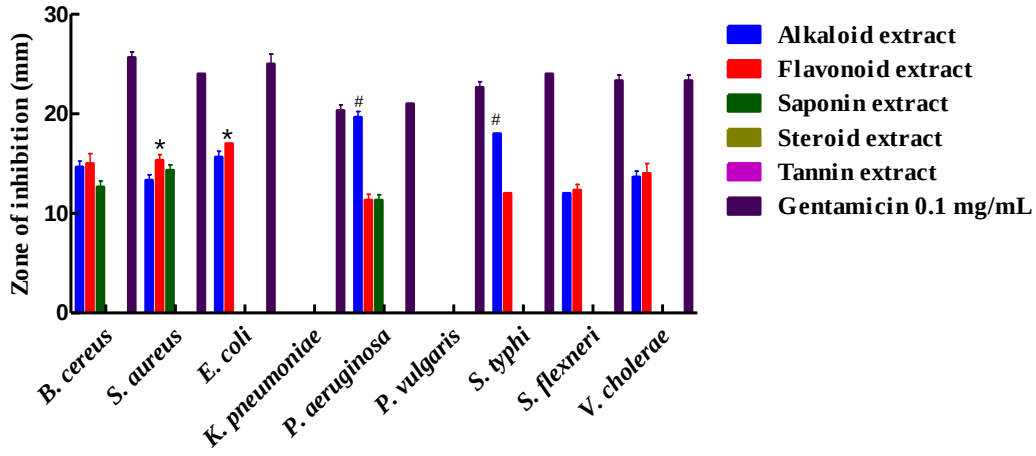


Figure 4.3: Zone of inhibition exhibited by the various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth. at the concentration of 100 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; $^{\#}$ Alkaloid vs. Flavonoid, *Flavonoid vs Alkaloid. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

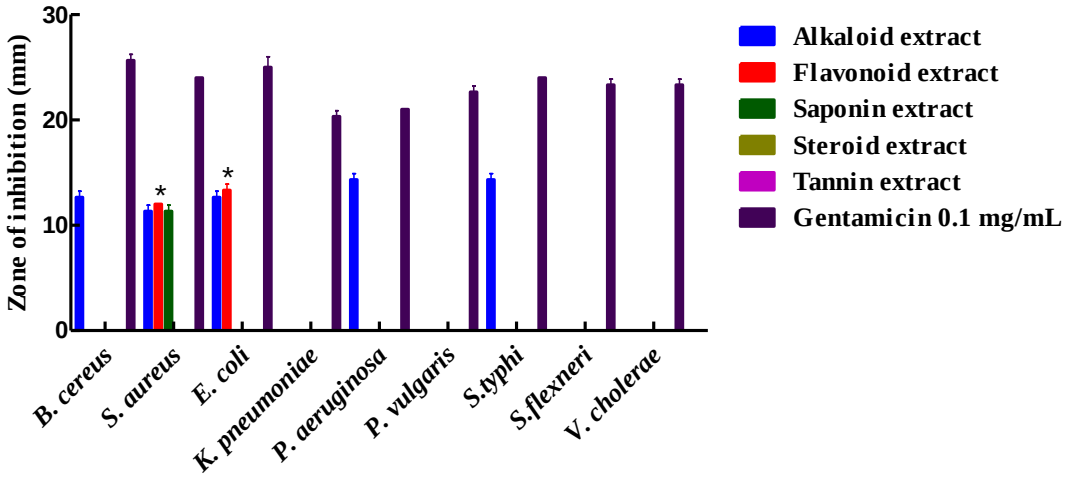


Figure 4.4: Zone of inhibition exhibited by the various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth. at the concentration of 50 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; * Flavonoid vs Alkaloid. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

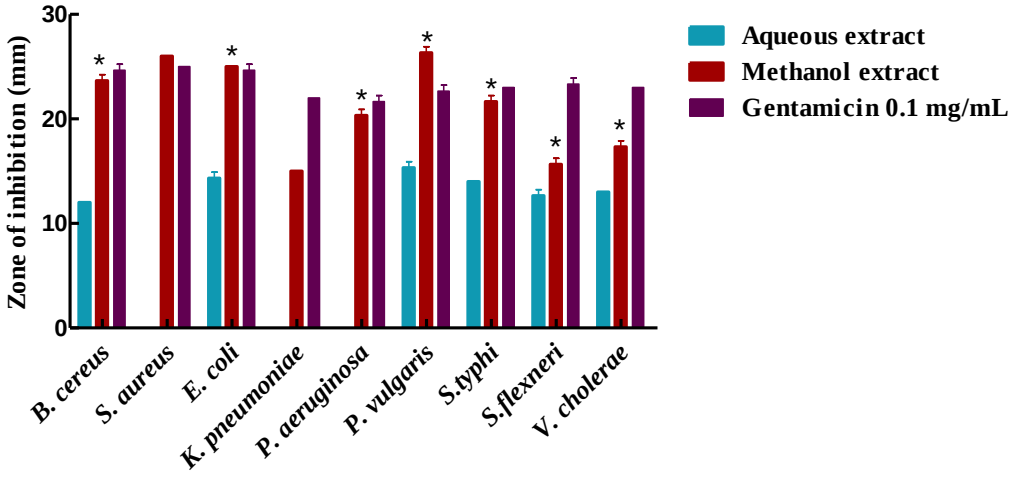
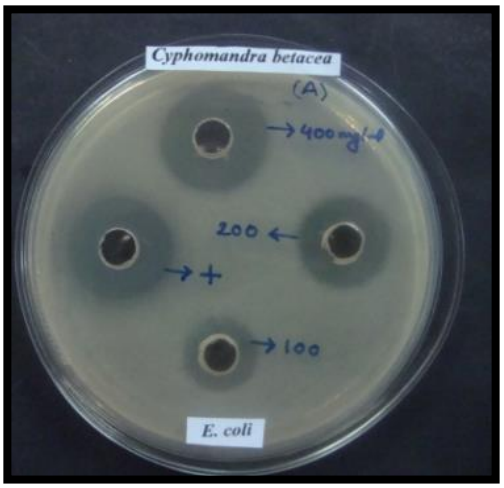
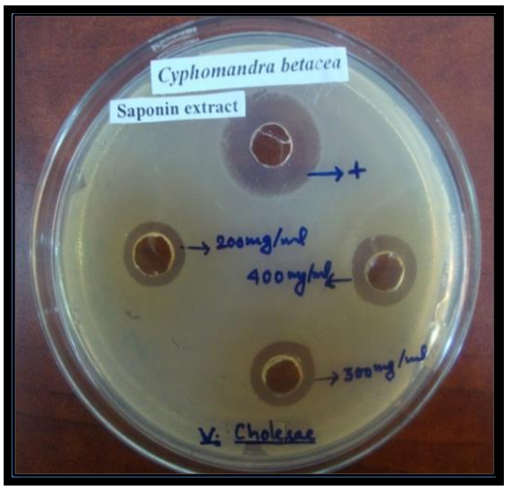


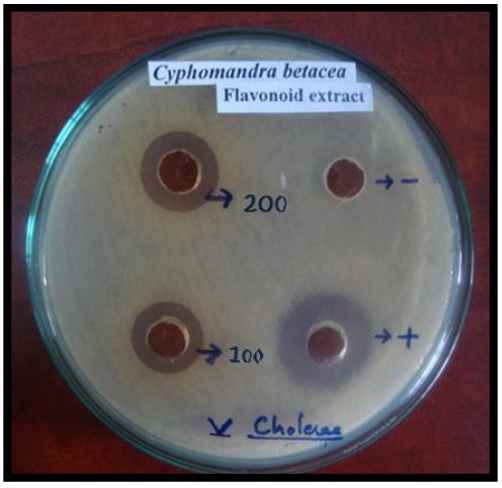
Figure 4.5: Zone of inhibition exhibited by the aqueous and the methanol extracts of *Cyphomandra betacea* (Cav.) Sendth. at the concentration of 400 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; * Methanol vs Aqueous. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

Antimicrobial activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

a. 

b. 

c. 

d. 

Photographic Plates 4.1: Zone of inhibition exhibited by the various extracts of *Cyphomandra betacea* (Cav.) Sendth. against different test microorganisms at different concentrations in mg/mL (a. alkaloid extract against *Escherichia coli*; b. saponin extract against *Vibrio cholerae* O139; c. flavonoid extract against *Vibrio cholerae* O139; d. methanol extract against *Vibrio cholerae* O139). '+', positive control gentamicin (0.1 mg/mL); '-', negative control (0.25 % DMSO).

4.5.4. Antimicrobial activity of various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Among the phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, the alkaloid extract exhibited antimicrobial activity against all the test microorganisms. The zone of inhibition exhibited by the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against different test microorganisms at different concentrations is depicted in Table 4.16. At the concentration of 400 mg/mL, the alkaloid extract exhibited antimicrobial activity against all the test microorganisms with the largest zone of inhibition (29.33±0.57 mm) observed against *Staphylococcus aureus* (Table 4.16).

The saponin extract inhibited the growth of *Bacillus cereus* at the concentrations of 400 mg/mL and 200 mg/mL with the zone of inhibition of 13.66±0.57 mm and 11.33±0.57 mm respectively. The extract exhibited antimicrobial activity against *Vibrio cholerae* O139 only at the concentration of 400 mg/mL with the zone of inhibition of 12 mm. However the flavonoid, steroid and tannin extracts did not show antimicrobial activity against all the test microorganisms.

4.5.5. Antimicrobial activity of the aqueous and the methanol extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Table 4.17 shows the zone of inhibition exhibited by the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against different test microorganisms. At the concentration of 400 mg/mL, the largest zone of inhibition (21.66±0.57 mm) was observed against *Escherichia coli*. Zone of inhibition was not observed with the methanol extract against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and

Proteus vulgaris. The aqueous extract at all the concentrations did not exhibit antimicrobial activity against any of the test microorganisms.

Table 4.16: Zone of inhibition exhibited by the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	-	-	13.33±0.57	-	-	-	-	-	-
100	14	12.66±0.57	18.66±0.57	12	13.66±0.57	-	13.33±0.57	14	13
200	17	21.33±0.57	22.66±0.57	16	19.66±0.57	-	14.66±0.57	16.66±0.57	16
400	20	29.33±0.57	25.66±0.57	24	28	16.66±0.57	16.66±0.57	22.66±0.57	22
Positive control	24.66±0.57	24.33±0.57	25.66±0.57	21.66±0.57	22	23.33±0.57	23	23.66±0.57	23.33±0.57
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.17: Zone of inhibition exhibited by the methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	13.66±0.57	-	-	-	-	-	-	-
50	-	15.66±0.57	13.33±0.57	-	-	-	-	-	-
100	11.66±0.57	16.66±0.57	19.33±0.57	-	-	-	11	11.66±0.57	12
200	12.66±0.57	17.66±0.57	20.33±0.57	-	-	-	12	13.66±0.57	12.66±0.57
400	14.66±0.57	20.33±0.57	21.66±0.57	-	-	-	13	15	15.66±0.57
Positive control	25.33±0.57	25	25.33±0.57	22	21.66±0.57	23.33±0.57	23.66±0.57	23	23.33±0.57
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

4.5.6. Comparative analysis of the antimicrobial activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Antimicrobial activity of the various phytochemicals extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish was compared at different concentrations (Figure 4.6 to Figure 4.9). At the concentration of 400 mg/mL the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited antimicrobial activity against all the nine test microorganisms (Figure 4.6). The saponin extract showed antimicrobial activity against *Bacillus cereus* and *Vibrio cholerae* O139. The zone of inhibition formed by alkaloid extract was significantly higher ($p<0.05$) than the saponin extract. At the concentration of 200 mg/mL, the alkaloid extract inhibited the growth of all the test microorganisms except *Proteus vulgaris* (Figure 4.7). The alkaloid extract inhibited the growth of *Escherichia coli* at the concentration of 50 mg/mL (Figure 4.9). At the concentration of 200 mg/mL, the saponin extract inhibited the growth of *Bacillus cereus* only (Figure 4.7). The saponin extract at the concentrations of 100 mg/mL and 50 mg/mL did not exhibit any zone of inhibition against all the test microorganisms (Figure 4.8 and Figure 4.9). At the concentration of 25 mg/mL of phytochemical extracts, the zone of inhibition was not observed against any of the test microorganisms.

At the concentration of 400 mg/mL the methanol extract inhibited the growth of *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella enterica* ser. *typhi, Shigella flexneri* and *Vibrio cholerae* O139 (Figure 4.10). At the concentration of 25 mg/mL, the methanol extract could inhibit the growth of *Staphylococcus aureus* (Table 4.17). The aqueous extract at all the test concentrations used in the study did not exhibit the zone of inhibition against all the test microorganisms (Figure 4.17).

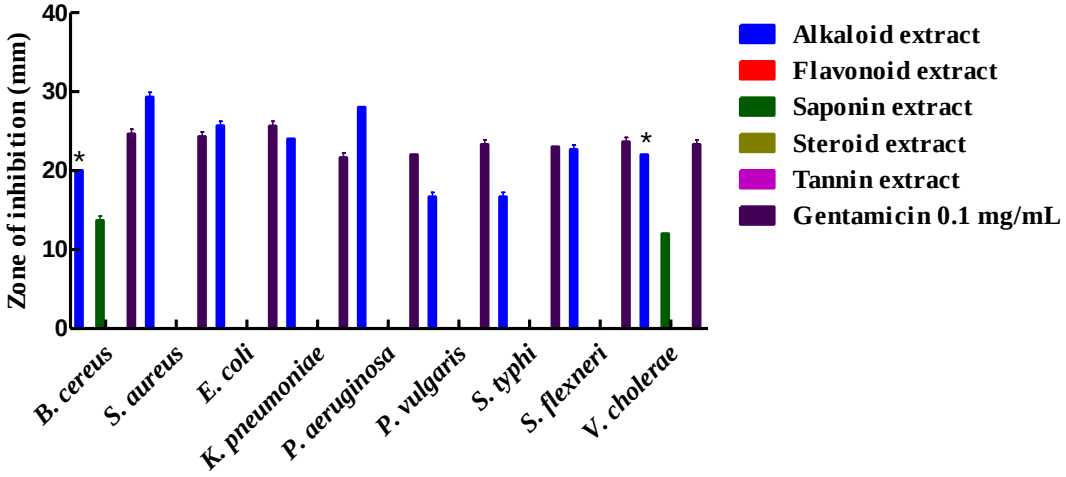


Figure 4.6: Zone of inhibition exhibited by the various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish at the concentration of 400 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Saponin. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus, S. aureus*: *Staphylococcus aureus, E. coli*: *Escherichia coli, K. pneumoniae*: *Klebsiella pneumoniae, P. aeruginosa*: *Pseudomonas aeruginosa, P. vulgaris*: *Proteus vulgaris, S. typhi*: *Salmonella enterica* ser. *typhi, S. flexneri*: *Shigella flexneri, V.cholerae*: *Vibrio cholerae* O139.

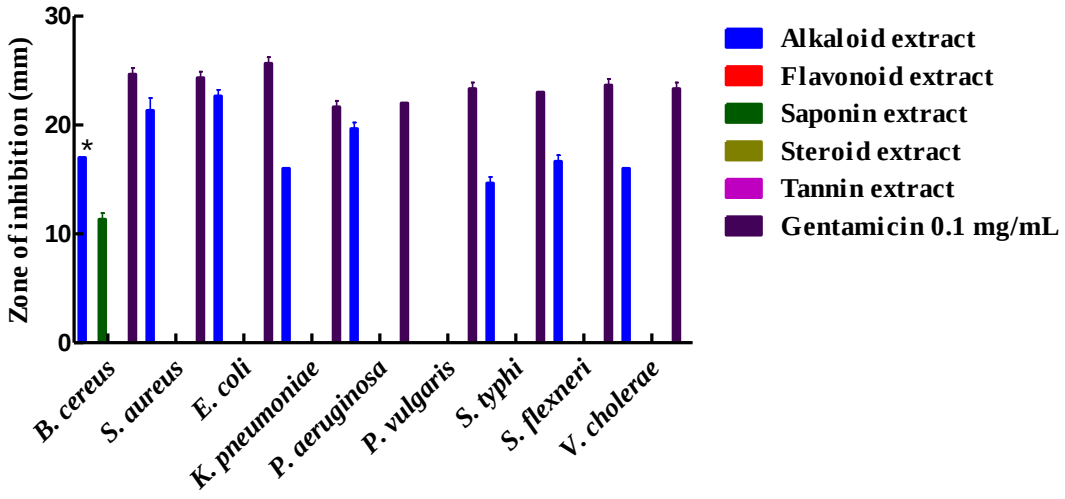


Figure 4.7: Zone of inhibition exhibited by the various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish at the concentration of 200 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Saponin. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

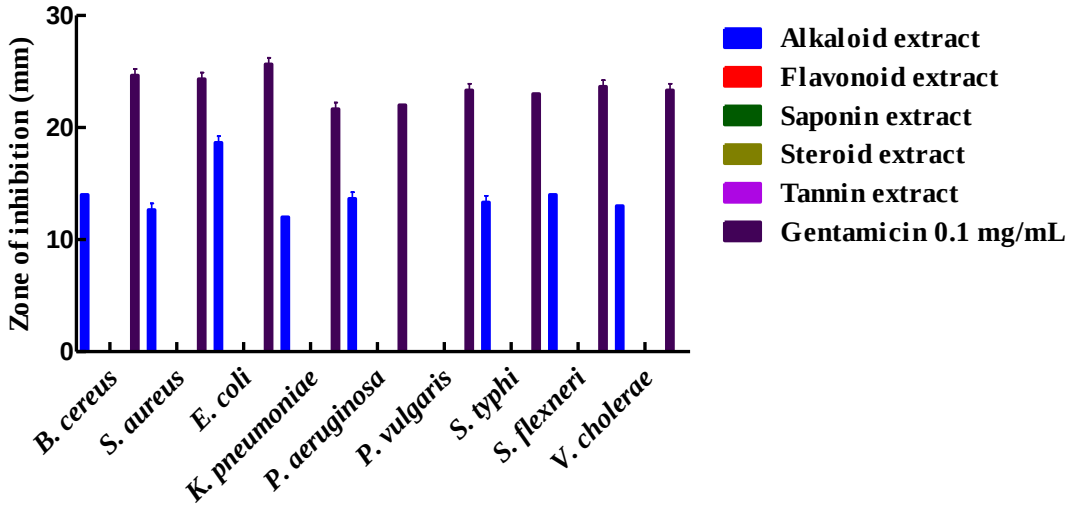


Figure 4.8: Zone of inhibition exhibited by the various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish at the concentration of 100 mg/mL against different test microorganisms.

The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V. cholerae*: *Vibrio cholerae* O139.

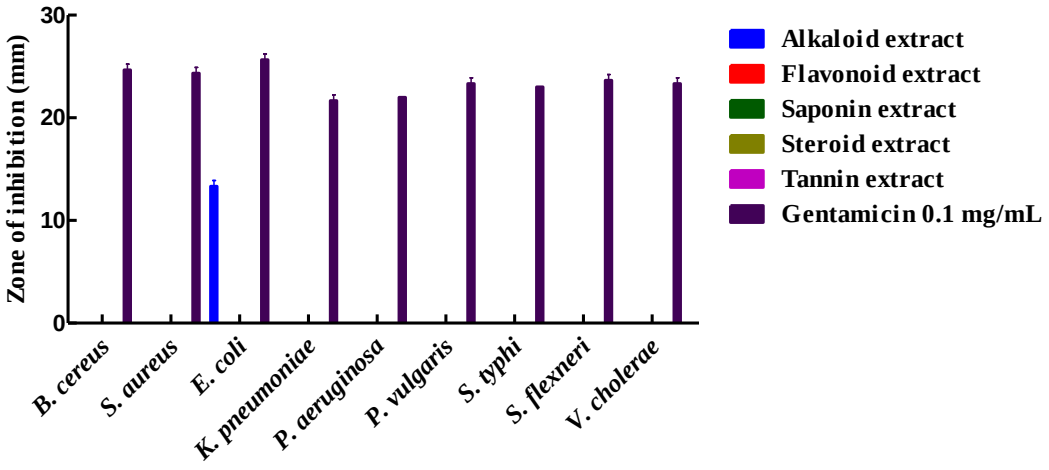


Figure 4.9: Zone of inhibition exhibited by the various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish at the concentration of 50 mg/mL against different test microorganisms.

The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V. cholerae*: *Vibrio cholerae* O139.

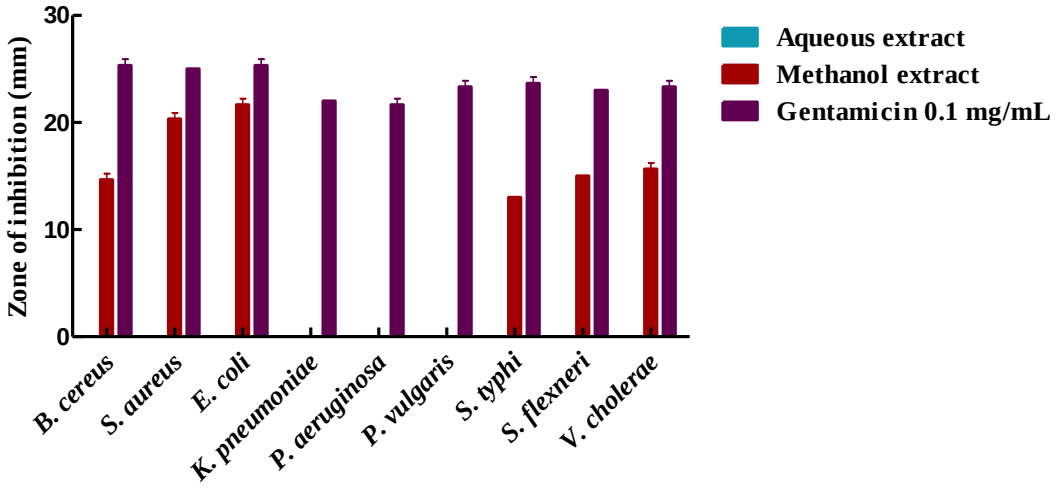
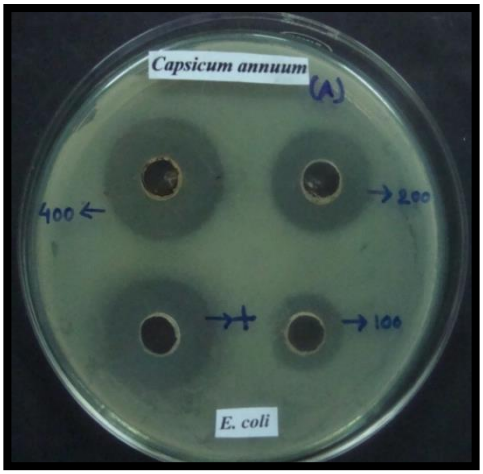


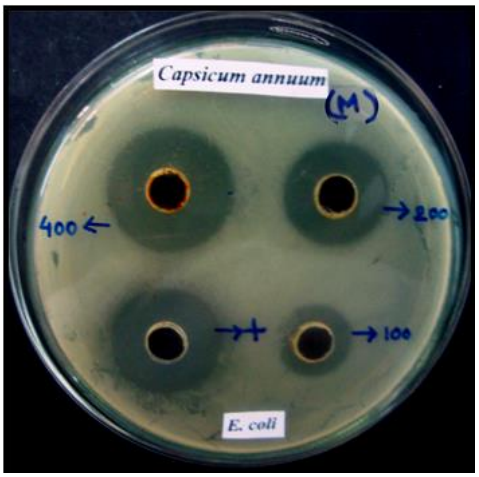
Figure 4.10: Zone of inhibition exhibited by the aqueous and the methanol extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish at the concentration of 400 mg/mL against different test microorganisms.

The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus, S. aureus*: *Staphylococcus aureus, E. coli*: *Escherichia coli, K. pneumoniae*: *Klebsiella pneumoniae, P. aeruginosa*: *Pseudomonas aeruginosa, P. vulgaris*: *Proteus vulgaris, S. typhi*: *Salmonella enterica* ser. *typhi, S. flexneri*: *Shigella flexneri, V. cholerae*: *Vibrio cholerae* O139.

Antimicrobial activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

a. 

b. 

c. 

d. 

Photographic Plate 4.2: Zone of inhibition exhibited by the various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against different test microorganisms at different concentrations in mg/mL (a. alkaloid extract against *Escherichia coli*; b. alkaloid extract against *Staphylococcus aureus*; c. methanol extract against *Escherichia coli*; d. methanol extract against *Shigella flexneri*). '+', positive control gentamicin (0.1 mg/mL); '-', negative control (0.25 % DMSO).

4.5.7. Antimicrobial activity of various phytochemical extracts of *Dicentra scandens* (D. Don) Walp.

The zone of inhibition exhibited by the alkaloid, flavonoid and saponin extracts of *Dicentra scandens* (D. Don) Walp. against different test microorganisms are shown in Table 4.18 to Table 4.20. The tannin extract inhibited the growth of *Shigella flexneri* with the zone of inhibition of 13 mm. The steroid extract did not exhibit the zone of inhibition against any of the test microorganisms. At the concentration of 400 mg/mL, the alkaloid extract exhibited the largest zone of inhibition of 28.66±0.57 mm against *Staphylococcus aureus* (Table 4.18) The flavonoid extract also showed the largest zone of inhibition (22.33±0.57 mm) against *Staphylococcus aureus* (Table 4.19). The saponin extract exhibited the largest zone of inhibition (17.66±0.57 mm) against *Bacillus cereus* (Table 4.20).

4.5.8. Antimicrobial activity of the aqueous and the methanol extracts of *Dicentra scandens* (D. Don) Walp.

At the concentration of 400 mg/mL, the aqueous extract inhibited the growth of only *Staphylococcus aureus* and *Proteus vulgaris* with the zone of inhibition of 13.66±0.57 mm and 17.33±0.57 mm respectively. At the concentration of 200 mg/mL the extract exhibited zone of inhibition of 14 mm against *Proteus vulgaris*.

The methanol extract of *Dicentra scandens* (D. Don) Walp., inhibited the growth of all the test microorganisms. At the concentration of 400 mg/mL, the extract exhibited the largest zone of inhibition (26 mm) against *Staphylococcus aureus* (Table 4.21).

Table 4.18: Zone of inhibition exhibited by the alkaloid extract of *Dicentra scandens* (D. Don) Walp. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	12	12.33±0.57	12.33±0.57	-	-	-	13.66±0.57	-	-
100	15.33±0.57	14.66±0.57	15.66±0.57	12.66±0.57	16.33±0.57	-	18.66±0.57	13.33±0.57	13
200	18	21.66±0.57	20.66±0.57	14.66±0.57	20.33±0.57	-	21	19.66±0.57	17
400	21.66±0.57	28.66±0.57	26	17.66±0.57	25.33±0.57	13	23.33±0.57	23	25.33±0.57
Positive control	24.66±0.57	25	25	23.66±0.57	21.66±0.57	24.33±0.57	23.66±0.57	22.66±0.57	23
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.19: Zone of inhibition exhibited by the flavonoid extract of *Dicentra scandens* (D. Don) Walp. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	11.66±0.57	12±1	-	-	-	-	-	-	-
100	15	14±1	-	-	-	-	11.33±0.57	-	-
200	18	18.66±0.57	12.33±0.57	-	-	-	12.33±0.57	13.66±0.57	15.33±0.57
400	19.33±0.57	22.33±0.57	13.66±0.57	11	-	10.33±0.57	16	15.66±0.57	17
Positive control	24.66±0.57	25	23.66±0.57	21	21.66±0.57	24.33±0.57	23.66±0.57	22.66±0.57	23
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.20: Zone of inhibition exhibited by the saponin extract of *Dicentra scandens* (D. Don) Walp. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-
100	11.66±0.57	11	-	-	-	-	-	-	-
200	13.33±0.57	11.66±0.57	-	-	-	-	-	-	12.33±0.57
400	17.66±0.57	13.66±0.57	11.66±0.57	-	-	-	12	-	17.33±0.57
Positive control	25.66±0.57	24.66±0.57	24.33±0.57	21.66±0.57	22	23.33±0.57	24	24	23.66±0.57
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.21: Zone of inhibition exhibited by the methanol extract of *Dicentra scandens* (D. Don) Walp. against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	12	-	-	-	-	-	-	-
50	-	13.66±0.57	11	-	-	-	-	-	11
100	11	18.33±0.57	14.33±0.57	15	13	12.33±0.57	12.66±0.57	-	12.33±0.57
200	11.66±0.57	21.33±0.57	16	19.33±0.57	14	13.66±0.57	14	-	13
400	14.33±0.57	26	17.33±0.57	20	14.66±0.57	14.66±0.57	15.66±0.57	11.33	14.33±0.57
Positive control	24.66±0.57	24	25.66±0.57	23	21.66±0.57	24.33±0.57	23.66±0.57	23.66±0.57	23
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

4.5.9. Comparative analysis of the antimicrobial activity of various extracts of *Dicentra scandens* (D. Don) Walp.

The alkaloid extract of *Dicentra scandens* (D. Don) Walp. exhibited significantly ($p<0.05$) higher antimicrobial activity than the other phytochemical extracts. At the concentration of 400 mg/mL, the alkaloid extract inhibited the growth of all the test microorganisms. Flavonoid extract showed antimicrobial activity against most of the test microorganisms except *Pseudomonas aeruginosa*. The saponin extract inhibited the growth of *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella enterica* ser. *typhi* and *Vibrio cholerae* O139. The tannin extract exhibited zone of inhibition only against *Shigella flexneri.* However, the steroid extract did not exhibit antimicrobial activity against any of the test microorganisms (Figure 4.11). The alkaloid extract inhibited that growth of *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Salmonella enterica* ser. *typhi* at the lower concentration of 50 mg/mL (Figure 4.14). At the concentration of 50 mg/mL the flavonoid extract exhibited the zone of inhibition against *Bacillus cereus* and *Staphylococcus aureus* (Figure 4.14). At the concentration of 100 mg/mL, the saponin extract inhibited the growth of *Bacillus cereus* and *Staphylococcus aureus* (Figure 4.13). However, at the concentration of 50 mg/mL the zone of inhibition was not observed with the saponin extracts (Figure 4.14). At the concentration of 25 mg/mL of phytochemical extracts, the zone of inhibition was not observed against any of the test microorganisms.

At the concentration of 400 mg/mL, the methanol extract inhibited the growth of all the test microorganisms. The aqueous extract could inhibit the growth of *Proteus vulgaris* and *Staphylococcus aureus* only. It was interesting to note that the zone of inhibition (17.33±0.57 mm) formed by the aqueous extract against *Proteus vulgaris* was significantly ($p<0.05$) larger than the zone of inhibition formed by the methanol

extract (Figure 4.15). The methanol extract inhibited the growth of most of the test microorganisms at the concentration of 100 mg/mL. At the concentration of 50 mg/mL, the extract inhibited the growth of *Staphylococcus aureus, Escherichia coli* and *Vibrio cholerae* O139. At the concentration of 25 mg/mL, the methanol extract inhibited the growth of *Staphylococcus aureus* (Table 4.21).

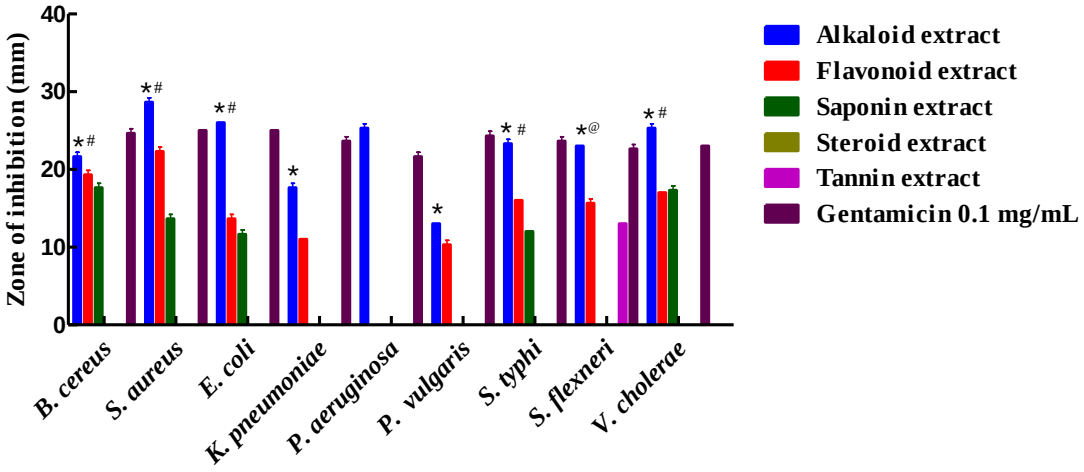


Figure 4.11: Zone of inhibition exhibited by the various phytochemical extracts of *Dicentra scandens* (D. Don) Walp. at the concentration of 400 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Flavonoid, #Alkaloid vs. Saponin,@Alkaloid vs, Tannin. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

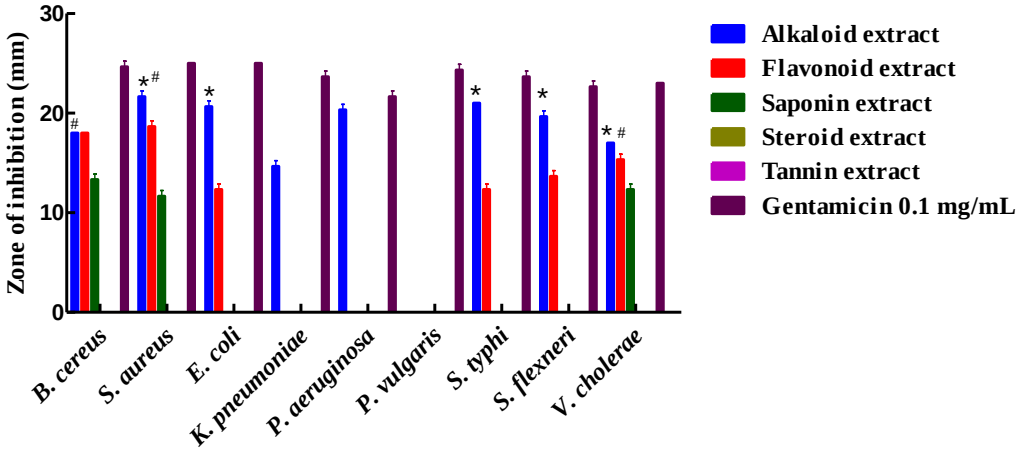


Figure 4.12: Zone of inhibition exhibited by the various phytochemical extracts of *Dicentra scandens* (D. Don) Walp.at the concentration of 200 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Flavonoid, #Alkaloid vs. Saponin.The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

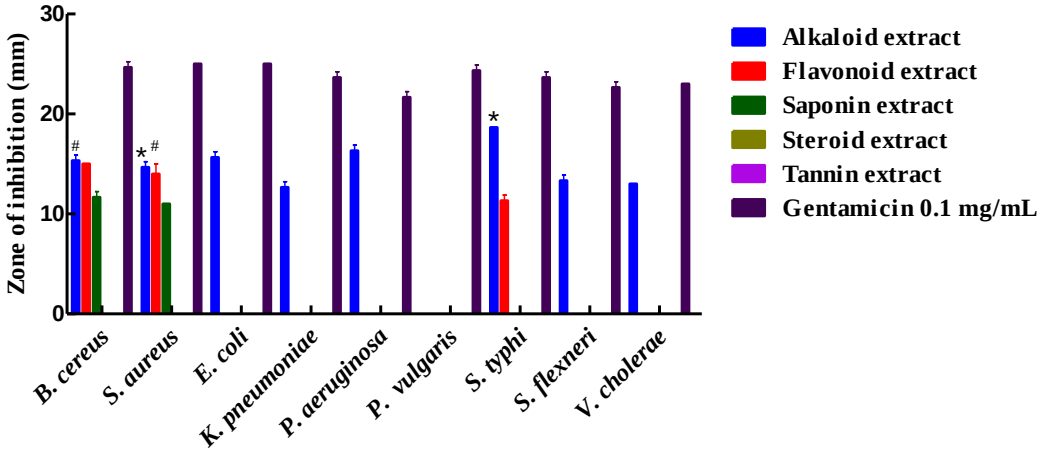


Figure 4.13: Zone of inhibition exhibited by the various phytochemical extracts of *Dicentra scandens* (D. Don) Walp. at the concentration of 100 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Flavonoid, #Alkaloid vs. Saponin. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S.typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V. cholerae*: *Vibrio cholerae* O139.

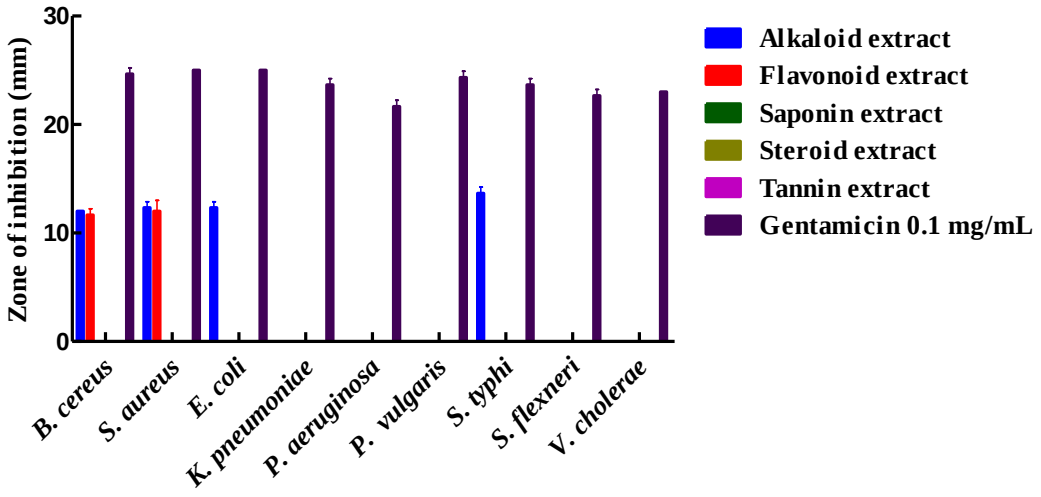


Figure 4.14: Zone of inhibition exhibited by the various phytochemical extracts of *Dicentra scandens* (D. Don) Walp. at the concentration of 50 mg/mL against different test microorganisms.

The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V. cholerae*: *Vibrio cholerae* O139.

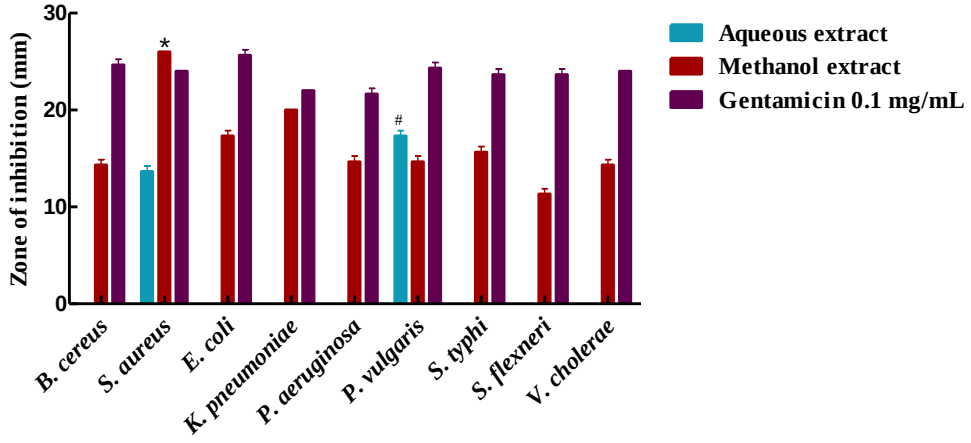
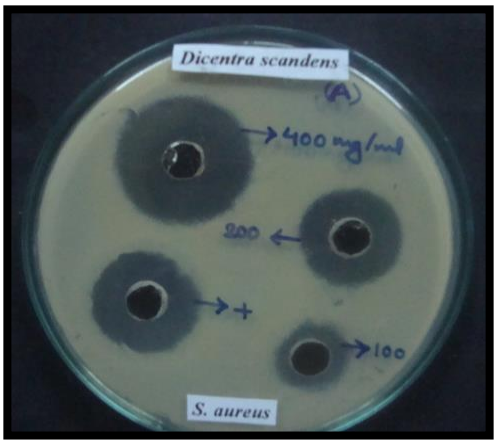
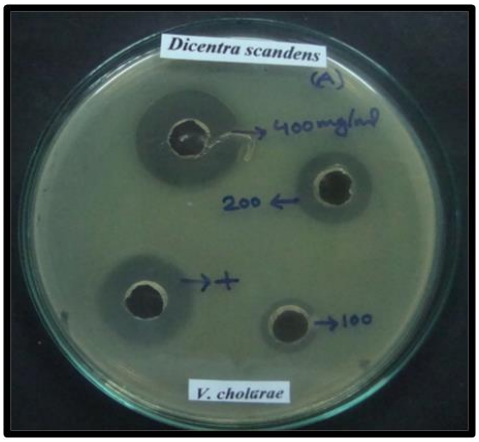


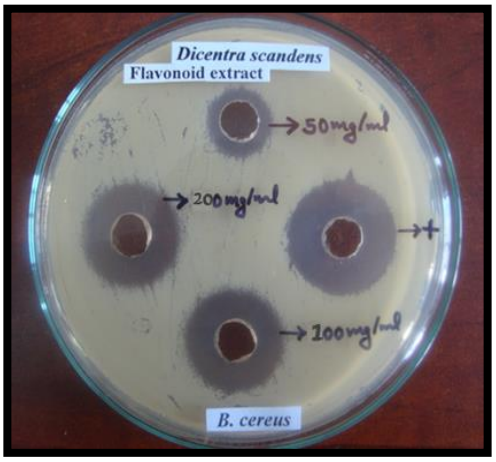
Figure 4.15: Zone of inhibition exhibited by the aqueous and the methanol extracts of *Dicentra scandens* (D. Don) Walp. at the concentration of 400 mg/mL against different test microorganisms.

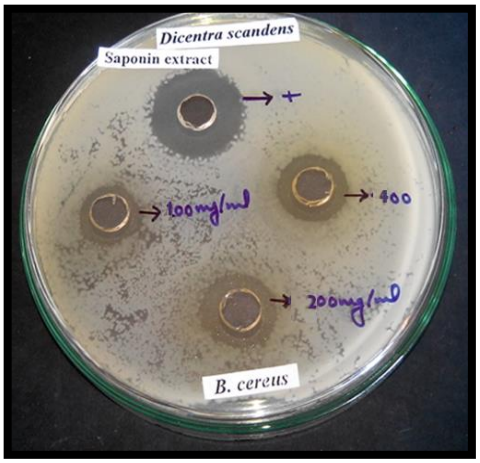
Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Methanol vs. Aqueous, #Aqueous vs. Methanol. The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

Antimicrobial activity of various extracts of *Dicentra scandens* (D. Don) Walp.

a. 

b. 

c. 

d. 

Photographic Plates 4.3: Zone of inhibition exhibited by the various extracts of *Dicentra scandens* (D. Don) Walp. against different test microorganisms at different concentrations in mg/mL (a. alkaloid extract against *Staphylococcus aureus*; b. alkaloid extract against *Vibrio cholerae* O139; c. flavonoid extract against *Bacillus cereus*; d. saponin extract against *Bacillus cereus*). '+', positive control gentamicin (0.1 mg/mL).

4.5.10. Antimicrobial activity of various phytochemical extracts of *Heracleum nepalense* D. Don

The zone of inhibition formed by the various phytochemical extracts namely alkaloid, flavonoid and saponin extracts of *Heracleum nepalense* D. Don against different test microorganisms are shown in Table 4.22 to Table 4.24. At the concentration of 400 mg/mL, the alkaloid extract exhibited the largest zone of inhibition (27.66±0.57 mm) against *Pseudomonas aeruginosa* (Table 4.22). The flavonoid extract showed the largest zone of inhibition (18.66±0.57 mm) against *Bacillus cereus* (Table 4.23). The saponin extract exhibited the largest zone of inhibition (17.66±0.57 mm) against *Bacillus cereus* (Table 4.24). The tannin extract inhibited the growth of only *Bacillus cereus* at the concentrations of 400 mg/mL, 200 mg/mL and 100 mg/mLwith the zone of inhibition of 12.66±0.57 mm, 10.66±0.57 mm and 10.33±0.57 mm respectively. The steroid extract at all the concentrations did not exhibit antimicrobial activity against any of the test microorganisms.

4.5.11. Antimicrobial activity of the aqueous and the methanol extracts of *Heracleum nepalense* D. Don

At the concentration of 400 mg/mL, the methanol extract of *Heracleum nepalense* D. Don exhibited the largest zone of inhibition (24 mm) against *Vibrio cholerae* O139 (Table 4.25). The aqueous extract at all the concentrations did not exhibit the zone of inhibition against the test microorganisms.

Table 4.22: Zone of inhibition exhibited by the alkaloid extract of *Heracleum nepalense* D. Don against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	13.33±0.57	-	13	-	-	-	-	-	-
100	16	-	18	-	13	-	12.66±0.57	-	-
200	17.66±0.57	16	22.33±0.57	-	19.66±0.57	-	19	-	13
400	18.66±0.57	24.33±0.57	24.33±0.57	15.66±0.57	27.66±0.57	12.33±0.57	20.66±0.57	-	17.66±0.57
Positive control	24.66±0.57	25	23.66±0.57	21.66±0.57	20	24.33±0.57	23.66±0.57	22.66±0.57	23.33±0.57
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.23: Zone of inhibition exhibited by the flavonoid extract of *Heracleum nepalense* D. Don against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-
100	14.33±0.57	-	-	-	-	-	-	-	-
200	16.33±0.57	12	11	-	-	-	-	-	-
400	18.66±0.57	13.66±0.57	13.66±0.57	-	-	-	-	-	-
Positive control	23.66±0.57	24.33±0.57	24	22.66±0.57	21.66±0.57	24.66±0.57	23.66±0.57	23.33±0.57	22±1
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.24: Zone of inhibition exhibited by the saponin extract of *Heracleum nepalense* D. Don against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-
100	13.33±0.57	11.33±0.57	-	-	-	-	-	-	-
200	15.66±0.57	11.66±0.57	-	-	-	-	-	-	-
400	17.66±0.57	14	-	-	-	-	-	-	11.66±0.57
Positive control	24.66±0.57	24.33±0.57	25.66±0.57	23	22.66±0.57	24	25.66±0.57	22.66±0.57	23.66±0.57
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

Table 4.25: Zone of inhibition exhibited by the methanol extract of *Heracleum nepalense* D. Don against different test microorganisms

Concentration (mg/mL)	Mean diameter of zone of inhibition (mm)								
	Gram positive bacteria		Gram negative bacteria						
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Salmonella enterica* ser. *typhi*	*Shigella flexneri*	*Vibrio cholerae* O139
25	11.33±0.57	-	-	-	-	-	-	-	-
50	12	-	-	-	-	-	-	-	-
100	13	11.33±0.57	12.33±0.57	-	-	-	-	-	-
200	13.66±0.57	13.66±0.57	14	-	-	-	-	-	20.33±0.57
400	15	15.33±0.57	15	-	-	-	-	-	24
Positive control	25	24.66±0.57	23.66±0.57	22.66±0.57	21	23.33±0.57	23.66±0.57	23±1	24
Negative control	-	-	-	-	-	-	-	-	-

Positive control (Gentamicin 0.1 mg/mL); Negative control (0.25 % DMSO).'-' Absence of zone of inhibition. All the experiments were performed in triplicate.

4.5.12. Comparative analysis of the antimicrobial activity of various extracts of *Heracleum nepalense* D. Don

The zone of inhibition exhibited by the various phytochemicals extracts of *Heracleum nepalense* D. Don against different test microorganisms was compared at different concentrations of the extracts used in the study (Figure 4.16 to Figure 4.19). At the concentration of 400 mg/mL, the alkaloid extract inhibited the growth of most of the test microorganisms and exhibited significantly ($p<0.05$) higher antimicrobial activity than the other phytochemical extracts (Figure 4.16). The flavonoid extract inhibited the growth of *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli*. The saponin extract exhibited the zone of inhibition against *Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae* O139. The tannin extract inhibited the growth of *Bacillus cereus* (Figure 4.16). At the concentration of 200 mg/mL, the alkaloid extract exhibited the zone of inhibition against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica* ser. *typhi* and *Vibrio cholerae* O139. The flavonoid extract inhibited the growth of *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli*. The saponin extract exhibited the zone of inhibition against *Bacillus cereus* and *Staphylococcus aureus.* The tannin extract inhibited the growth of only *Bacillus cereus* (Figure 4.17). At the concentration of 100 mg/mL, the alkaloid extract exhibited the zone of inhibition against *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica* ser. *typhi*. The saponin extract inhibited the growth of *Bacillus cereus* and *Staphylococcus aureus* while the flavonoid and tannin extracts inhibited the growth of *Bacillus cereus* only (Figure 4.18). However, at the concentration of 50 mg/mL, the alkaloid extract showed the zone of inhibition against *Bacillus cereus* and *Escherichia coli* (Figure 4.19). At the concentration of 25 mg/mL of the

phytochemical extracts, the zone of inhibition was not observed against any of the test microorganisms.

At the concentration of 400 mg/mL, the methanol extract of *Heracleum nepalense* D. Don exhibited antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio cholerae* O139 (Figure 4.20). The methanol extract inhibited the growth of *Bacillus cereus* at the concentration of 25 mg/mL which was the lowest concentration of the extract used in the study (Table 4.25). The aqueous extract at all the test concentrations used in the study did not exhibit the zone of inhibition against all the test microorganisms (Figure 4.20).

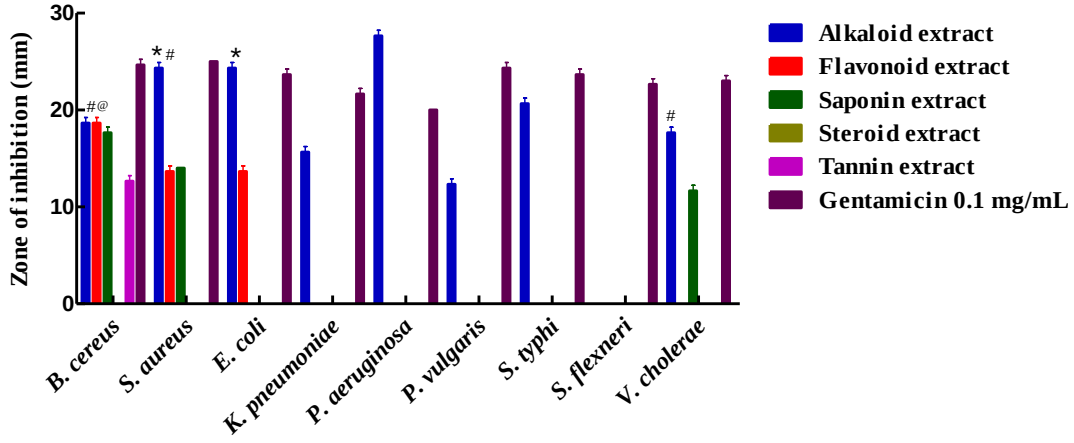


Figure 4.16: Zone of inhibition exhibited by the various phytochemical extracts of *Heracleum nepalense* D. Don at the concentration of 400 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Flavonoid, $^{\#}$Alkaloid vs Saponin, $^{@}$Alkaloid vs. Tannin. The data represents mean±SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V. cholerae*: *Vibrio cholerae* O139.

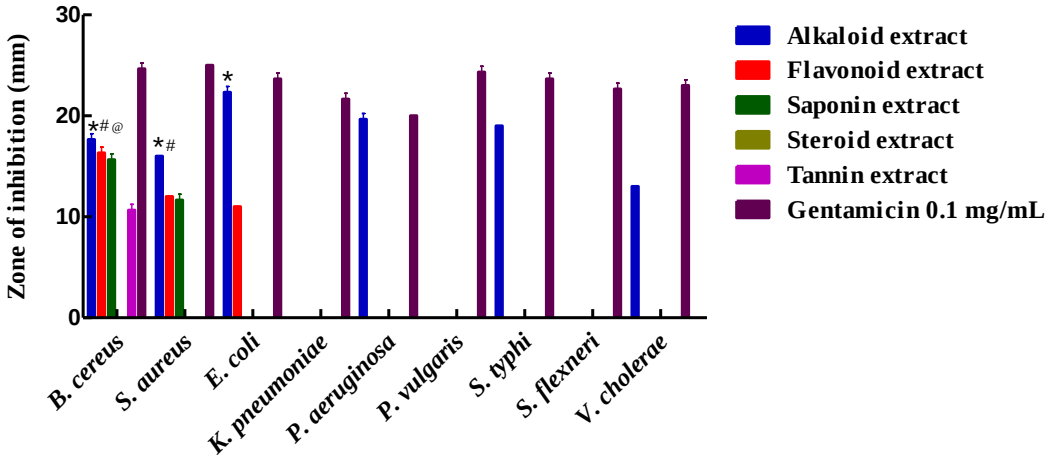


Figure 4.17: Zone of inhibition exhibited by the various extracts of *Heracleum nepalense* D. Don at the concentration of 200 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Flavonoid, $^{\#}$Alkaloid vs Saponin, $^{@}$Alkaloid vs. Tannin. The data represents mean±SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V. cholerae*: *Vibrio cholerae* O139.

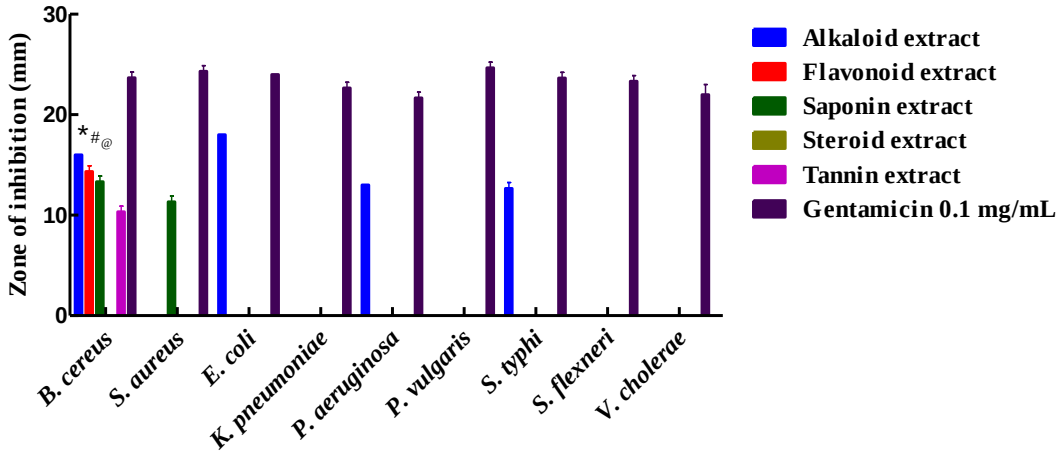


Figure 4.18: Zone of inhibition exhibited by the various phytochemical extracts of *Heracleum nepalense* D. Don at the concentration of 100 mg/mL against different test microorganisms.

Significant ($p<0.05$) difference in mean diameter of zone of inhibition; *Alkaloid vs. Flavonoid, #Alkaloid vs Saponin, @Alkaloid vs. Tannin. The data represents mean±SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V. cholerae*: *Vibrio cholerae* O139.

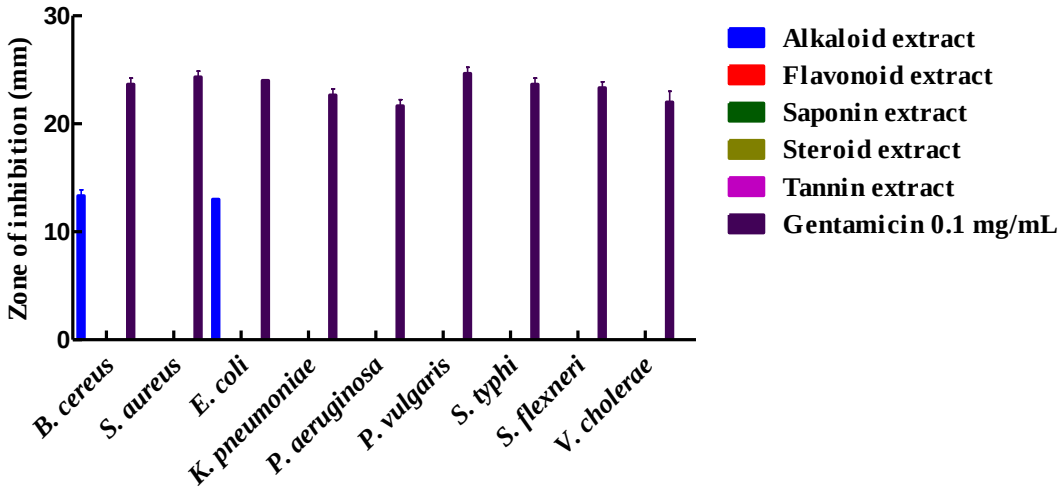


Figure 4.19: Zone of inhibition exhibited by the various phytochemical extracts of *Heracleum nepalense* D. Don at the concentration of 50 mg/mL against different test microorganisms.

The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus, S. aureus*: *Staphylococcus aureus, E. coli*: *Escherichia coli, K. pneumoniae*: *Klebsiella pneumoniae, P. aeruginosa*: *Pseudomonas aeruginosa, P. vulgaris*: *Proteus vulgaris, S. typhi*: *Salmonella enterica* ser. *typhi, S. flexneri*: *Shigella flexneri, V. cholerae*: *Vibrio cholerae* O139.

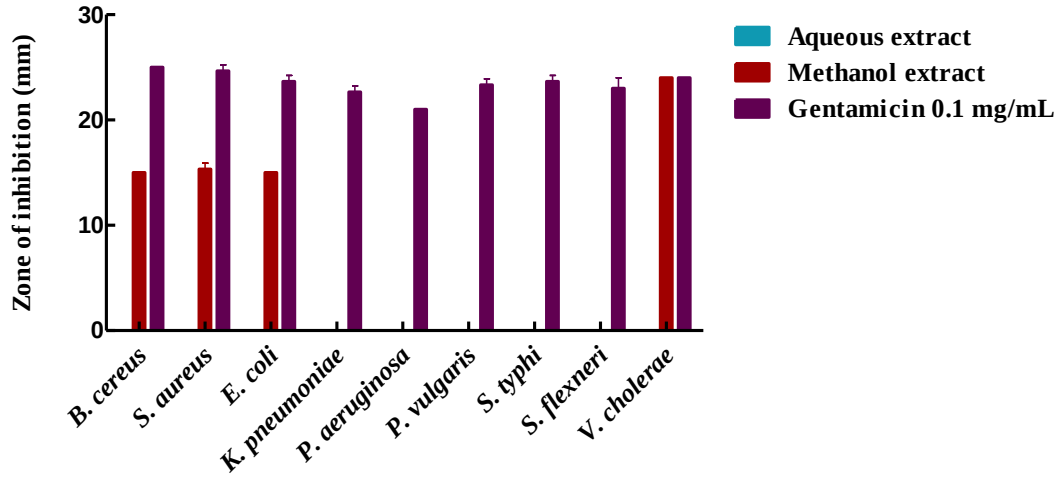
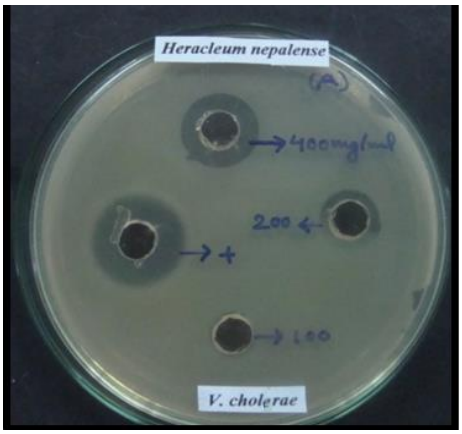
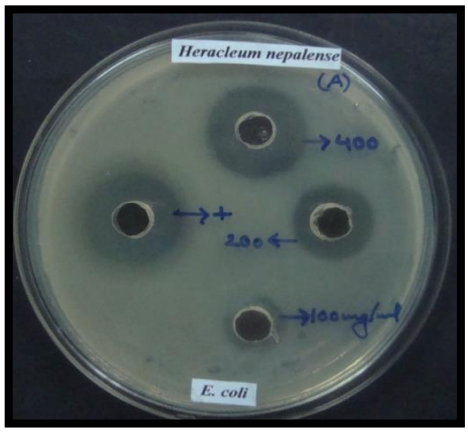


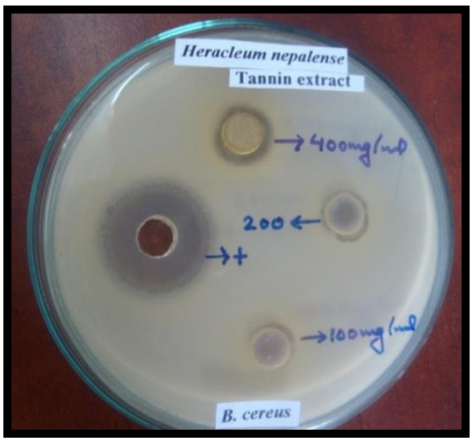
Figure 4.20: Zone of inhibition exhibited by the aqueous and the methanol extracts of *Heracleum nepalense* D. Don at the concentration of 400 mg/mL against different test microorganisms.

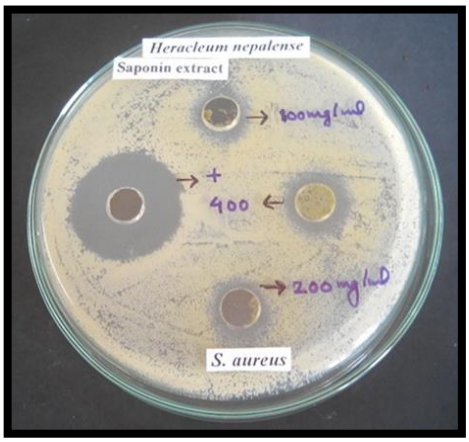
The data represents mean ± SD. All the experiments were performed in triplicate. *B. cereus*: *Bacillus cereus*, *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *S. typhi*: *Salmonella enterica* ser. *typhi*, *S. flexneri*: *Shigella flexneri*, *V.cholerae*: *Vibrio cholerae* O139.

Antimicrobial activity of various extracts of *Heracleum nepalense* D. Don

a. 

b. 

c. 

d. 

Photographic Plates 4.4: Zone of inhibition exhibited by the various extracts of *Heracleum nepalense* D. Don against different test microorganisms at different concentrations in mg/mL (a. alkaloid extract against *Vibrio cholerae* O139; b. alkaloid extract against *Escherichia coli*; c. tannin extract against *Bacillus cereus*; d. Saponin extract against *Staphylococcus aureus*). '+', positive control gentamicin (0.1 mg/mL).

4.6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC, MBC and MIC index values of the various extracts of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don against test microorganisms are depicted in Table 4.26 to Table 4.29.

The extracts of *Cyphomandra betacea* (Cav.) Sendth. exhibited MIC values ranging from 3.125 mg/mL to 100 mg/mL. The MBC values ranged from 6.25 mg/mL to 200 mg/mL. The lowest MIC value of 3.125 mg/mL was exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. against *Staphylococcus aureus.* Mostly the tannin extracts showed higher MIC and MBC values. The lowest MBC value of 6.25 mg/mL was exhibited by the methanol extract of *Cyphomandra betacea* (Cav.) Sendth. against *Vibrio cholerae* O139. Aquoeus extract exhibited higher MIC and MBC values. However, various extract of *Cyphomandra betacea* (Cav.) Sendth. were found to exhibit bactericidal activity against the test microorganisms as indicated by the MIC index value of ≤ 4 (Table 4.26).

In the case of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, the MIC values of the extracts ranged from 6.25 mg/mL to 200 mg/mL and the MBC values ranged from 12.5 mg/mL to 200 mg/mL. The least MIC value (6.25 mg/mL) and MBC value (12.5 mg/mL) was exhibited by the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against *Vibrio cholerae* O139 and *Proteus vulgaris* respectively. The extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish were found to exhibit bactericidal activity against the test microorganisms as indicated by the MIC index value of ≤ 4 (Table 4.27).

The extracts of *Dicentra scandens* (D. Don) Walp.exhibited MIC values ranging from 6.25 mg/mL to 100 mg/mL. The MBC values ranged from 6.25 mg/mL to 200 mg/mL. The least MIC value of 6.25 mg/mL was exhibited by the alkaloid extract against most of the test microorganisms. The least MBC value of 6.25 mg/mL was exhibited by the alkaloid extract against *Pseudomonas aeruginosa* and *Proteus vulgaris.* Similarly, the least MIC and MBC values of 6.25 mg/mL each were exhibited by the methanol extract against *Vibrio cholerae* O139. Various extracts of *Dicentra scandens* (D. Don) Walp. were found to exhibit bactericidal activity against the test microorganisms as indicated by the MIC index value of ≤ 4 (Table 4.28).

In the case of *Heracleum nepalense* D. Don, the MIC values ranged from 6.25 mg/mL to 200 mg/mL and the MBC values ranged from 12.5 mg/mL to 200 mg/mL. The least value of MIC (6.25 mg/mL) and MBC (12.5 mg/mL) was observed against *Proteus vulgaris* with the alkaloid extract of *Heracleum nepalense* D. Don. The extracts of *Heracleum nepalense* D. Don were found to exhibit bactericidal activity against the test microorganisms as indicated by the MIC index value of ≤ 4 (Table 4.29).

Table 4.26: Minimum Inhibitory Concentration, Minimum Bactericidal Concentration in mg/mL and MIC index of the various extracts of *Cyphomandra betacea* (Cav.) Sendth. against test microorganisms

Test microorganisms		Phytochemical extracts					General extracts	
		Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol
Bacillus cereus	MIC	12.5	25	25	-	-	100	6.25
	MBC	12.5	25	50	-	-	100	12.5
	MIC Index	1	1	2	-	-	1	2
Staphylococcus aureus	MIC	6.25	3.125	25	-	-	-	6.25
	MBC	12.5	12.5	50	-	-	-	12.5
	MIC Index	2	4	2	-	-	-	2
Escherichia coli	MIC	6.25	25	25	-	-	100	6.25
	MBC	12.5	25	50	-	-	200	12.5
	MIC Index	2	1	2	-	-	2	2
Klebsiella pneumoniae	MIC	6.25	25	-	-	-	-	12.5
	MBC	12.5	50	-	-	-	-	12.5
	MIC Index	2	2	-	-	-	-	1
Pseudomonas aeruginosa	MIC	25	25	25	-	-	-	25
	MBC	50	50	50	-	-	-	25
	MIC Index	2	2	2	-	-	-	1
Proteus vulgaris	MIC	6.25	6.25	12.5	-	-	25	6.25
	MBC	12.5	25	25	-	-	100	12.5
	MIC Index	2	4	2	-	-	4	2
Salmonella enterica* ser. *typhi	MIC	12.5	12.5	25	-	-	100	12.5
	MBC	12.5	25	50	-	-	200	12.5
	MIC Index	1	2	2	-	-	2	1
Shigella flexneri	MIC	6.25	25	12.5	-	50	50	12.5
	MBC	25	50	25	-	100	100	12.5
	MIC Index	4	2	2	-	2	2	1
***Vibrio cholerae* O139**	MIC	6.25	25	25	-	100	25	6.25
	MBC	12.5	25	50	-	200	100	6.25
	MIC Index	2	1	2	-	2	4	1

'-' absence of zone of inhibition in agar well diffusion assay at higher concentration (400 mg/mL).

Table 4.27: Minimum Inhibitory Concentration, Minimum Bactericidal Concentration in mg/mL and MIC index of the various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against test microorganisms

Test microorganisms		Phytochemical extracts					General extracts	
		Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol
Bacillus cereus	MIC	12.5	-	200	-	-	-	100
	MBC	50	-	200	-	-	-	100
	MIC Index	4	-	1	-	-	-	1
Staphylococcus aureus	MIC	25	-	-	-	-	-	100
	MBC	50	-	-	-	-	-	100
	MIC Index	2	-	-	-	-	-	1
Escherichia coli	MIC	12.5	-	-	-	-	-	50
	MBC	25	-	-	-	-	-	100
	MIC Index	2	-		-	-	-	2
Klebsiella pneumoniae	MIC	50	-	-	-	-	-	-
	MBC	50	-	-	-	-	-	-
	MIC Index	1	-	-	-	-	-	-
Pseudomonas aeruginosa	MIC	12.5	-	-	-	-	-	-
	MBC	50	-	-	-	-	-	-
	MIC Index	4	-		-	-	-	-
Proteus vulgaris	MIC	12.5	-	-	-	-	-	-
	MBC	12.5	-	-	-	-	-	-
	MIC Index	1	-	-	-	-	-	-
Salmonella enterica* ser. *typhi	MIC	100	-	-	-	-	-	100
	MBC	100	-	-	-	-	-	200
	MIC Index	1	-	-	-	-	-	2
Shigella flexneri	MIC	12.5	-	-	-	-	-	25
	MBC	50	-	-	-	-	-	50
	MIC Index	4	-	-	-	-	-	2
***Vibrio cholerae* O139**	MIC	6.25	-	200	-	-	-	50
	MBC	25	-	200	-	-	-	100
	MIC Index	4	-	1	-	-	-	2

‘-’absence of zone of inhibition in agar well diffusion assay at higher concentration (400 mg/mL).

Table 4.28: Minimum Inhibitory Concentration, Minimum Bactericidal Concentration in mg/mL and MIC index of the various extracts of *Dicentra scandens* (D. Don) Walp. against test microorganisms

Test microorganisms		Phytochemical extracts					General extracts	
		Alkaloid	**Flavonoid**	**Saponin**	**Steroid**	**Tannin**	**Aqueous**	**Methanol**
Bacillus cereus	MIC	6.25	100	100	-	-	-	100
	MBC	12.5	200	200	-	-	-	200
	MIC Index	2	2	2	-	-	-	2
Staphylococcus aureus	MIC	6.25	12.5	100	-	-	100	25
	MBC	25	50	100	-	-	200	100
	MIC Index	2	4	1	-	-	2	4
Escherichia coli	MIC	12.5	50	50	-	-	-	25
	MBC	25	100	50	-	-	-	50
	MIC Index	2	2	1	-	-	-	2
Klebsiella pneumoniae	MIC	6.25	100	-	-	-	-	100
	MBC	25	100	-	-	-	-	100
	MIC Index	4	1	-	-	-	-	1
Pseudomonas aeruginosa	MIC	6.25	-	-	-	-	-	100
	MBC	6.25	-	-	-	-	-	100
	MIC Index	2	-	-	-	-	-	1
Proteus vulgaris	MIC	6.25	50	-	-	-	50	50
	MBC	6.25	50	-	-	-	200	100
	MIC Index	1	1	-	-	-	4	2
Salmonella enterica* ser. *typhi	MIC	6.25	25	50	-	-	-	25
	MBC	12.5	50	100	-	-	-	100
	MIC Index	2	2	2	-	-	-	4
Shigella flexneri	MIC	12.5	25	-	-	100	-	12.5
	MBC	12.5	50	-	-	200	-	50
	MIC Index	1	2	-	-	2	-	4
***Vibrio cholerae* O139**	MIC	12.5	25	50	-	-	-	6.25
	MBC	12.5	100	100	-	-	-	6.25
	MIC Index	1	4	2	-	-	-	1

'-' absence of zone of inhibition in agar well diffusion assay at higher concentration (400 mg/mL).

Table 4.29: Minimum Inhibitory Concentration, Minimum Bactericidal Concentration in mg/mL and MIC index of the various extracts of *Heracleum nepalense* D. Don against test microorganisms

Test microorganisms		Phytochemical extracts					General extracts	
		Alkaloid	**Flavonoid**	**Saponin**	**Steroid**	**Tannin**	**Aqueous**	**Methanol**
Bacillus cereus	MIC	25	100	50	-	50	-	25
	MBC	100	100	100	-	100	-	100
	MIC Index	4	1	2	-	2	-	4
Staphylococcus aureus	MIC	25	100	100	-	-	-	100
	MBC	100	200	100	-	-	-	200
	MIC Index	4	2	1	-	-	-	2
Escherichia coli	MIC	25	12.5	-	-	-	-	50
	MBC	50	25	-	-	-	-	200
	MIC Index	2	2	-	-	-	-	4
Klebsiella pneumoniae	MIC	25	-	-	-	-	-	-
	MBC	25	-	-	-	-	-	-
	MIC Index	1	-	-	-	-	-	-
Pseudomonas aeruginosa	MIC	12.5	-	-	-	-	-	-
	MBC	50	-	-	-	-	-	-
	MIC Index	4	-	-	-	-	-	-
Proteus vulgaris	MIC	6.25	-	-	-	-	-	-
	MBC	12.5	-	-	-	-	-	-
	MIC Index	2	-	-	-	-	-	-
Salmonella enterica* ser. *typhi	MIC	25	-	-	-	-	-	-
	MBC	50	-	-	-	-	-	-
	MIC Index	2	-	-	-	-	-	-
Shigella flexneri	MIC	-	-	-	-	-	-	-
	MBC	-	-	-	-	-	-	-
	MIC Index	-	-	-	-	-	-	-
***Vibrio cholerae* O139**	MIC	25	-	200	-	-	-	50
	MBC	50	-	200	-	-	-	100
	MIC Index	2	-	1	-	-	-	1

‘-’absence of zone of inhibition in agar well diffusion assay at higher concentration (400 mg/mL).

4.7. Time kill assay

Among all the four test plants, various extracts of *Cyphomandra betacea* (Cav.) Sendth. and *Dicentra scandens* (D. Don) Walp. exhibited promising antimicrobial activity in terms of the mean diameter of the zone of inhibition against the different test microorganisms. In the case of *Cyphomandra betacea* (Cav.) Sendth. among the phytochemical extracts, the alkaloid, flavonoid and saponin extracts inhibited the growth of all the test microorganisms. Alkaloid extract exhibited larger zone of inhibition against some of the test microorganisms than the other extracts. However, the flavonoid extract exhibited higher antimicrobial activity against the majority of the test microorganisms. The extract exhibited higher antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* than the alkaloid and other phytochemical extracts. Furthermore, the flavonoid extract also exhibited the lowest MIC value of 3.125 mg/mL against *Staphylococcus aureus.*

Similarly the alkaloid extract of *Dicentra scandens* (D. Don) Walp. exhibited significantly ($p<0.05$) higher antimicrobial activity than the other phytochemical extracts and exhibited the largest zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*. Hence, these plants extract namely the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. were analyzed further for the time kill assay using three different concentrations (2×MIC, MIC and 1/2×MIC) against one Gram-negative (*Escherichia coli*) and one Gram-positive (*Staphylococcus aureus*) representative bacteria.

4.7.1. Time kill assay against *Escherichia coli* and *Staphylococcus aureus* treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth.

The complete killing of *Escherichia coli* was observed at 30 min at the concentration of 2×MIC. However, the time taken to accomplish complete killing by MIC and 1/2×MIC was 90 min and 120 min respectively (Figure 4.21). The untreated control showed a gradual increase in the bacterial count as time increased.

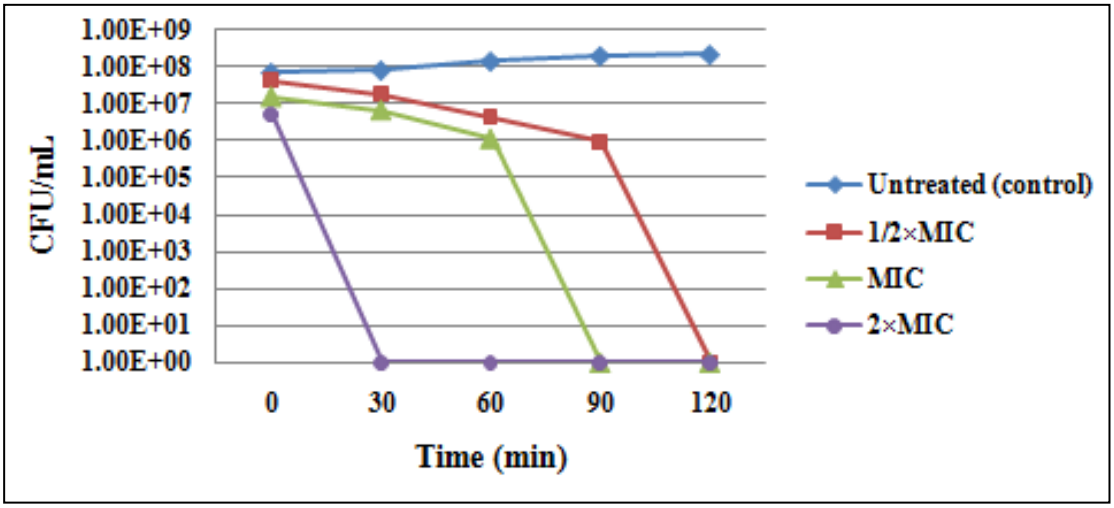


Figure 4.21: Time-kill curve of *Escherichia coli* untreated control and after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at the concentrations of 1/2×MIC, MIC and 2×MIC for 0-120 min.

MIC=25 mg/mL, 1/2×MIC=12.5 mg/mL and 2×MIC=50 mg/mL. All the experiments were performed in triplicate.

Based on the standard reference antibacterial activity is considered to be potent if there is more than two log reduction in the bacterial count. In the present study more than two log reduction refers to a value of log reduction ≥ 2.5. In the case of *Escherichia coli*, with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth., there was more than two log reduction in the bacterial count at the concentrations of

2×MIC, MIC and 1/2×MIC after 30 min, 90 min and 120 min respectively (Table 4.30).

Table 4.30: Log reduction and percentage reduction of *Escherichia coli* treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth.

Time (min)	1/2×MIC		*MIC		2×MIC	
	Log reduction	Percentage reduction	Log reduction	Percentage reduction	Log reduction	Percentage reduction
0	0.22	39.91	0.65	77.77	1.12	92.49
30	0.65	78.00	1.08	91.68	2.89	99.87
60	1.53	97.05	2.11	99.22	3.15	99.92
90	2.32	99.53	3.29	99.94	3.29	99.94
120	3.34	99.95	3.34	99.95	3.34	99.95

*MIC=25 mg/mL, 1/2×MIC=12.5 mg/mL and 2×MIC=50 mg/mL.

In the case of *Staphylococcus aureus* with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth., the complete bacterial killing was observed at 60 min at the concentration of 2×MIC, whereas MIC and 1/2×MIC did not exhibit complete bacterial killing (Figure 4.22). On the other hand, untreated growth control shows a gradual increase in the bacterial count as time increases.

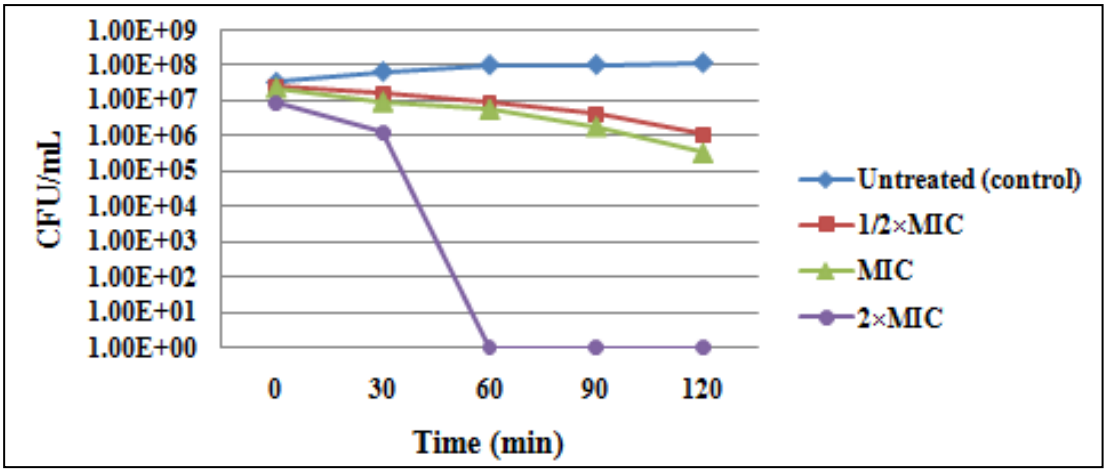


Figure 4.22: Time-kill curve of *Staphylococcus aureus* untreated control and after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at the concentrations of 1/2×MIC, MIC and 2×MIC for 0-120 min.

MIC=3.125 mg/mL, 1/2×MIC=1.562 mg/mL and 2×MIC=6.25 mg/mL. All the experiments were performed in triplicate.

In the case of *Staphylococcus aureus*, more than two log reductions in the bacterial count were observed after 60 min at the concentration of 2×MICand at 120 min with MIC (Table 4.31).

Table 4.31: Log reduction and percentage reduction of *Staphylococcus aureus* treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth.

Time (min)	1/2×MIC		*MIC		2×MIC	
	Log reduction	Percentage reduction	Log reduction	Percentage reduction	Log reduction	Percentage reduction
0	0.10	22.09	0.14	28.83	0.58	74.09
30	0.58	73.94	0.83	85.28	1.71	98.06
60	1.06	91.46	1.22	94.09	3.01	99.90
90	1.37	95.79	1.73	98.14	3.02	99.90
120	2.03	99.07	2.51	99.69	3.07	99.91

*MIC=3.125 mg/mL, 1/2×MIC=1.562 mg/mL and 2×MIC=6.25 mg/mL.

4.7.2. Time kill assay against *Escherichia coli* and *Staphylococcus aureus* treated with the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

With the alkaloid extract of *Dicentra scandens* (D. Don) Walp.the complete killing of *Escherichia coli* was observed at the concentration of 2×MIC at 90 min. There was a gradual decrease in the bacterial count at the concentrations of MIC and 1/2×MIC with an increase in the time interval. Untreated growth control shows a gradual increase in the bacterial count as the time increases (Figure 4.23).

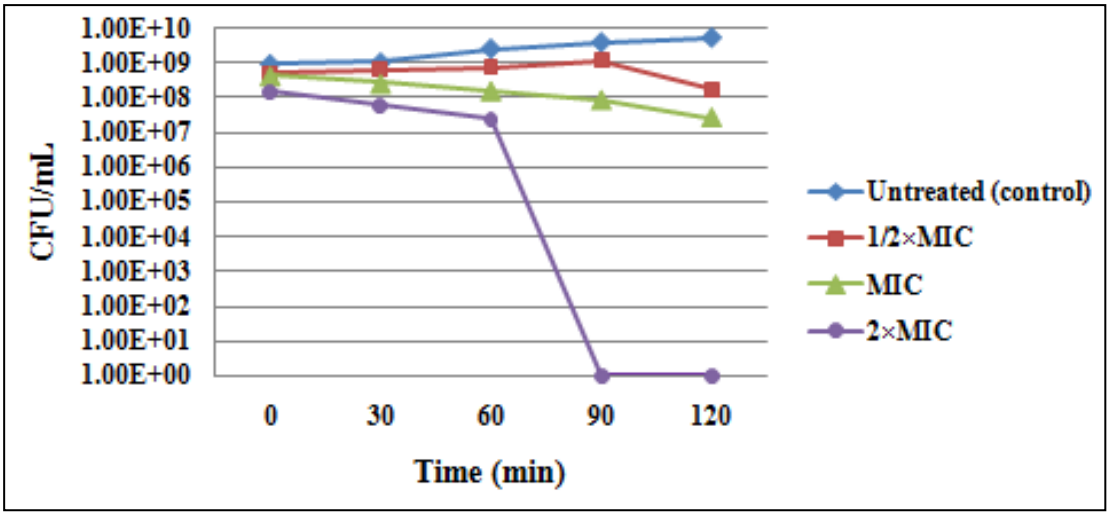


Figure 4.23: Time-kill curve of *Escherichia coli* untreated control and after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at the concentrations of 1/2×MIC, MIC and 2×MIC for 0-120 min.

MIC=12.5 mg/mL, 1/2×MIC=6.25 mg/mL and 2×MIC=25 mg/mL. All the experiments were performed in triplicate.

With the alkaloid extract of *Dicentra scandens* (D. Don) Walp. there was more than two log reduction in the bacterial count of *Escherichia coli* at the concentration of 2×MIC at 90 min (Table 4.32).

Table 4.32: Log reduction and percentage reduction of *Escherichia coli* treated with the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

Time (min)	1/2×MIC		*MIC		2×MIC	
	Log reduction	Percentage reduction	Log reduction	Percentage reduction	Log reduction	Percentage reduction
0	0.25	44.60	0.32	52.51	0.80	84.17
30	0.21	39.10	0.59	74.67	1.23	94.23
60	0.53	70.91	1.21	93.87	2.04	99.10
90	0.52	70.30	1.66	97.83	2.60	99.74
120	1.49	96.81	2.31	99.51	2.73	99.81

*MIC=12.5 mg/ml, 1/2×MIC=6.25 mg/ml and 2×MIC=25 mg/mL.

In the case of *Staphylococcus aureus*, the complete bacterial killing was observed at 120 min at the concentration of 2×MIC. At the MIC, gradual decrease in the bacterial count was observed, whereas with 1/2×MIC, an increase in the bacterial count was observed after 90 min (Figure 4.24). The untreated growth control showed a gradual increase in the bacterial count as the time interval increased.

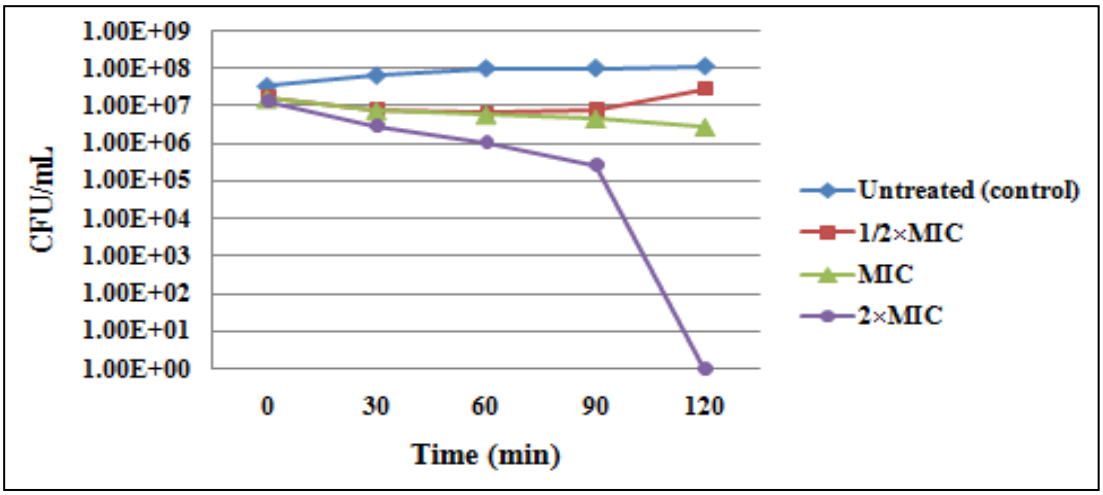


Figure 4.24: Time-kill curve of *Staphylococcus aureus* untreated control and after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at the concentrations of 1/2×MIC, MIC and 2×MIC for 0-120 min.

MIC=6.25 mg/mL, 1/2×MIC=3.125 mg/mL and 2×MIC=12.5 mg/mL. All the experiments were performed in triplicate.

In the case of *Staphylococcus aureus* more than two log reduction in the bacterial count was observed at the concentration of 2×MIC at 90 min (Table 4.33).

Table 4.33: Log reduction and percentage reduction of *Staphylococcus aureus* treated with the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

Time (min)	1/2×MIC		*MIC		2×MIC	
	Log reduction	Percentage reduction	Log reduction	Percentage reduction	Log reduction	Percentage reduction
0	0.27	47.21	0.33	53.37	0.39	59.92
30	0.90	87.66	0.93	88.45	1.32	95.29
60	1.17	93.35	1.23	94.22	1.98	98.97
90	1.10	92.21	1.34	95.44	2.59	99.74
120	0.60	75.32	1.61	97.58	3.07	99.91

*MIC=6.25 mg/mL, 1/2×MIC=3.125 mg/mL and 2×MIC=12.5 mg/mL.

4.8. Cellular leakage assay

The flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) exhibited time and dose-dependent killing of the test microorganisms. Hence to see the possible mechanism of their action, the cellular leakage assay was performed. The assay was performed by using two concentrations (MIC and 400 mg/mL) of the extracts. The leakage of cellular materials absorbing at 260 nm and 280 nm was calculated by subtracting the absorbance of control untreated cells and UV absorbance of plant extract from the absorbance of the bacterial cells treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

4.8.1. Cellular leakage assay with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. against *Escherichia coli* and *Staphylococcus aureus*

The net cellular leakage of the materials absorbing at 260 nm and 280 nm in the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspension after incubation with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at MIC and at the concentration of 400 mg/mL are shown in Table 4.34 and Table 4.35. The absorbance at 260 nm and 280 nm of the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspension were increased after 30 min of incubation with the flavonoid extract of the *Cyphomandra betacea* (Cav.) Sendth. at MIC and at the concentration of 400 mg/mL (Figure 4.25 and Figure 4.26). There was a significant time-dependent increase in the absorbance ($p<0.05$). Leakage of cellular materials absorbing at 280 nm was higher as compared to the leakage of cellular materials absorbing at 260 nm from both the test bacteria (Figure 4.25 and Figure 4.26).

Table 4.34: Net cellular leakage from *Escherichia coli* treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth.

Time (min)	Treated at MIC (25 mg/mL)		Treated at 400 mg/mL	
	Net leakage of UV absorbing material at 260 nm	Net leakage of UVabsorbing material at 280 nm	Net leakage of UV absorbing material at 260 nm	Net leakage of UV absorbing material at 280 nm
0	0.0119±0.0004	0.0152±0.0009	0.0257±0.0005	0.0364±0.0005
30	0.0447±0.0006	0.0667±0.0006	0.0809±0.0049	0.0886±0.0016
60	0.0583±0.0034	0.0758±0.0093	0.0967±0.0079	0.1223±0.0169
90	0.0744±0.0027	0.0970±0.0044	0.1534±0.0032	0.1918±0.0255
120	0.1810±0.0091	0.2363±0.0077	0.2662±0.0061	0.3843±0.0190

Net leakage=Absorbance (Treated-untreated-plant extract). Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

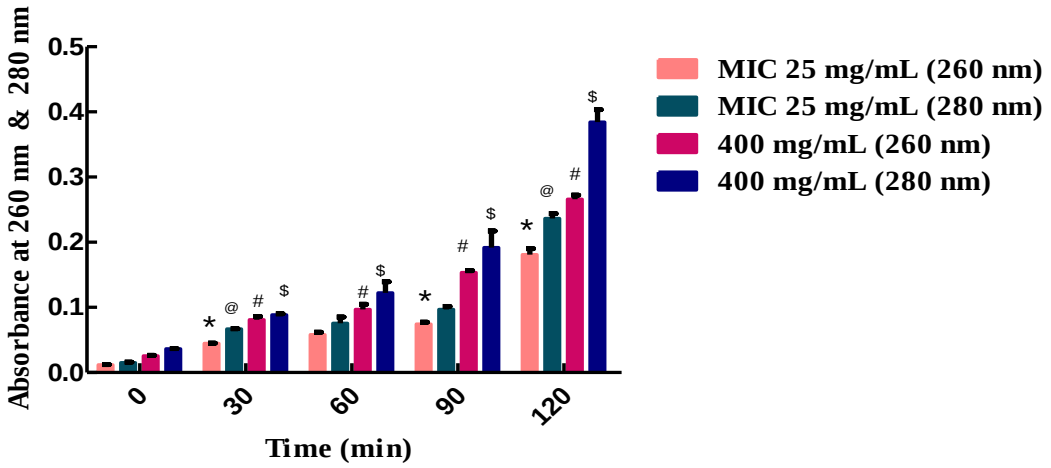


Figure 4.25: Absorbance at 260 nm and 280 nm of the supernatant of *Escherichia coli* suspension after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at the concentrations of MIC and 400 mg/mL measured at different time intervals.

Significant ($p<0.05$) time dependent (0 min vs. 30 min; 30 min vs. 60 min; 60 min vs. 90 min; 90 min vs. 120 min) increase in the absorbance, *, MIC (25 mg/mL) at 260 nm; #, 400 mg/mL at 260 nm; @, MIC (25 mg/mL) at 280 nm; $, 400 mg/mL at 280 nm.

Table 4.35: Net cellular leakage from *Staphylococcus aureus* treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth.

Time (min)	Treated at MIC (3.125 mg/mL)		Treated at 400 mg/mL	
	Net leakage of UV absorbing material at 260 nm	Net leakage of UV absorbing material at 280 nm	Net leakage of UV absorbing material at 260 nm	Net leakage of UV absorbing material at 280 nm
0	0.0114±0.0005	0.0132±0.0005	0.0254±0.0001	0.0357±0.0005
30	0.0360±0.0008	0.0429±0.0005	0.0770±0.0063	0.0865±0.0036
60	0.0569±0.0068	0.0676±0.0048	0.1105±0.0079	0.1260±0.0066
90	0.0709±0.0093	0.0960±0.0038	0.1625±0.0059	0.1856±0.0134
120	0.1007±0.0079	0.1226±0.0148	0.2012±0.0089	0.3235±0.0132

Net leakage=Absorbance (Treated-untreated-plant extract). Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

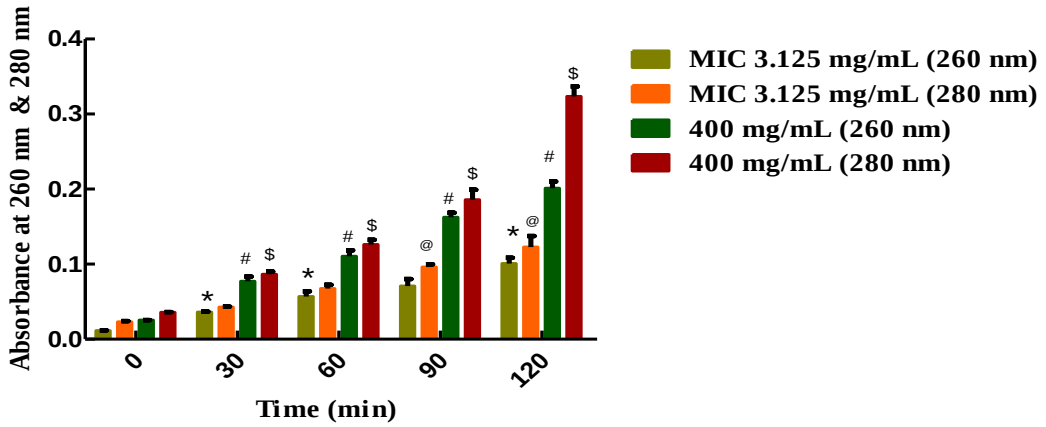


Figure 4.26: Absorbance at 260 nm and 280 nm of the supernatant of *Staphylococcus aureus* suspension after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at the concentrations of MIC and 400 mg/mL measured at different time intervals.

Significant ($p<0.05$) time-dependent (0 min vs. 30 min; 30 min vs. 60 min; 60 min vs. 90 min; 90 min vs. 120 min) increase in the absorbance, *, MIC (3.125 mg/mL) at 260 nm; $^{\#}$, 400 mg/mL at 260 nm; $^{@}$, MIC (3.125 mg/mL) at 280 nm; $^{\$}$, 400 mg/mL at 280 nm.

4.8.1.1. Estimation of total protein content

The protein content of supernatant of *Escherichia coli* and *Staphylococcus aureus* suspension after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. are shown in Table 4.36, Table 4.37 and Figure 4.27. There was a significant ($p<0.05$) increase in the protein content with an increase in the time interval. The protein content of treated bacterial cells was significantly ($p<0.05$) higher than that of untreated cells.

Table 4.36: Total protein content in µg/mL of the supernatant of *Escherichia coli* suspension after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. compared with untreated control

Time (min)	Total protein content (µg/mL)		
	Untreated *Escherichia coli*	Treated *Escherichia coli* at MIC (25 mg/mL)	Treated *Escherichia coli* at 400 mg/mL
0	5.95±0.31	11.04±0.28	22.17±0.17
30	7.86±0.32	25.18±0.32	34.77±0.45
60	13.03±0.38	27.15±0.45	39.34±0.35
90	15.06±0.48	32.03±0.18	47.20±0.29
120	16.04±0.27	40.91±0.32	63.93±0.90

Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

Table 4.37: Total protein content in µg/mL of the supernatant of *Staphylococcus aureus* suspension after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. compared with untreated control

Time (min)	Total protein content (µg/mL)		
	Untreated *Staphylococcus aureus*	Treated *Staphylococcus aureus* at MIC (3.125 mg/mL)	Treated *Staphylococcus aureus* at 400 mg/mL
0	4.85±0.22	7.94±0.01	19.20±0.35
30	6.52±0.23	20.64±0.85	29.93±0.31
60	8.45±0.30	22.41±0.26	34.28±0.38
90	9.60±0.25	25.46±0.38	40.00±0.49
120	11.03±0.60	29.98±0.13	49.26±0.14

Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

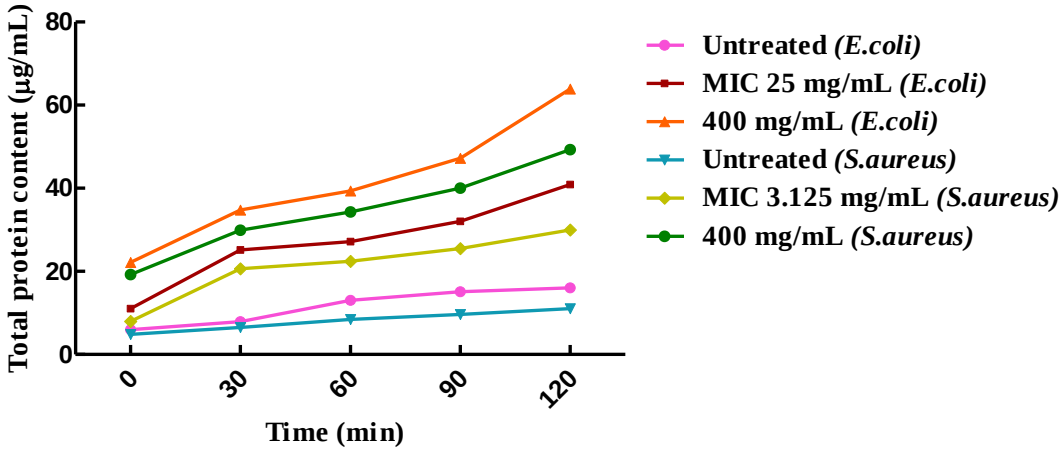


Figure 4.27: Total protein content of supernatant from *Escherichia coli* and *Staphylococcus aureus* suspensions of the control (untreated) and after treatment with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at the concentrations of MIC and 400 mg/mL measured at different time intervals. *E. coli*; *Escherichia coli*, *S. aureus*; *Staphylococcus aureus.*

4.8.2. Cellular leakage assay with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. against *Escherichia coli* and *Staphylococcus aureus*

The net cellular leakage of the materials absorbing at 260 nm and 280 nm in the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspension after incubation with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at MIC and at the concentration of 400 mg/mL are shown in Table 4.38 and Table 4.39. The absorbance at 260 nm and 280 nm of the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspension were increased after 30 min of incubation with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at MIC and at the concentration of 400 mg/mL (Figure 4.28 and Figure 4.29). There was a significant time-dependent increase in the absorbance ($p<0.05$). Leakage of cellular materials absorbing at 280nm

was higher as compared to the leakage of cellular materials absorbing at 260 nm in case of both the test bacteria (Figure 4.28 and Figure 4.29).

Table 4.38: Net cellular leakage from *Escherichia coli* treated with the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

Time (min)	Treated at MIC (12.5 mg/mL)		Treated at 400 mg/mL	
	Net leakage of UV absorbing material at 260 nm	Net leakage of UV absorbing material at 280 nm	Net leakage of UV absorbing material at 260 nm	Net leakage of UV absorbing material at 280 nm
0	0.0100±0.0008	0.0106±0.0009	0.0280±0.0003	0.0309±0.0004
30	0.0246±0.0007	0.0366±0.0007	0.1379±0.0093	0.1679±0.0114
60	0.0467±0.0052	0.0551±0.0019	0.2506±0.0093	0.2967±0.0222
90	0.0580±0.0071	0.0789±0.0061	0.3094±0.0106	0.3285±0.0118
120	0.1322±0.0104	0.1505±0.0102	0.3927±0.0251	0.4164±0.0116

Net leakage=Absorbance (Treated-untreated-plant extract). Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

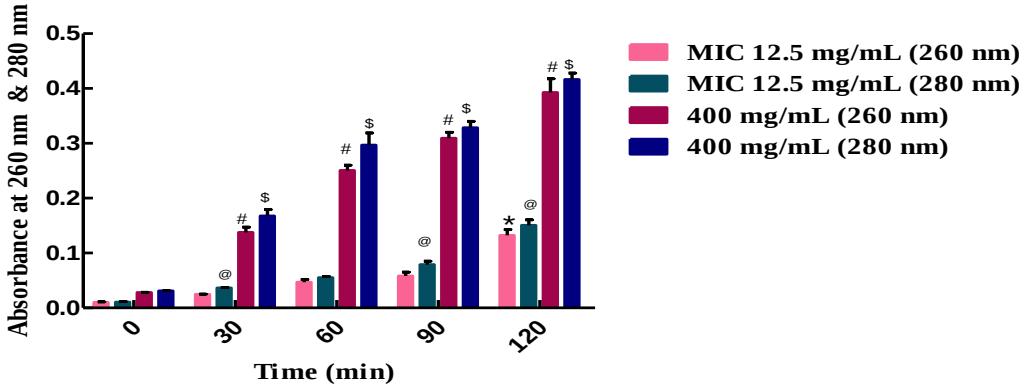


Figure 4.28: Absorbance at 260 nm and 280 nm of the supernatant of *Escherichia coli* suspension after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at the concentrations of MIC and 400 mg/mL measured at different time intervals. Significant ($p<0.05$) time dependent (0 min vs. 30 min; 30 min vs. 60 min; 60 min vs. 90 min; 90 min vs. 120 min) increase in the absorbance, *, MIC (12.5 mg/mL) at 260 nm; $^{\#}$, 400 mg/mL at 260 nm; $^{@}$, MIC (12.5 mg/mL) at 280 nm; $^{\$}$, 400 mg/mL at 280 nm.

Table 4.39: Net cellular leakage from *Staphylococcus aureus* treated with the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

Time (min)	Treated at MIC (6.25 mg/mL)		Treated at 400 mg/mL	
	Net leakage of UV absorbing material at 260 nm	Net leakage of UV absorbing material at 280 nm	Net leakage of UV absorbing material at 260 nm	Net leakage of UV absorbing material at 280 nm
0	0.0100±0.0008	0.0102±0.0009	0.0201±0.0008	0.0235±0.0002
30	0.0237±0.0009	0.0348±0.0007	0.1338±0.0109	0.1595±0.0091
60	0.0383±0.0014	0.0513±0.0017	0.2112±0.0081	0.2325±0.0080
90	0.0546±0.0010	0.0734±0.0053	0.2615±0.0157	0.2885±0.0098
120	0.1090±0.0042	0.1157±0.0068	0.3261±0.0078	0.3332±0.0107

Net leakage=Absorbance (Treated-untreated-plant extract). Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

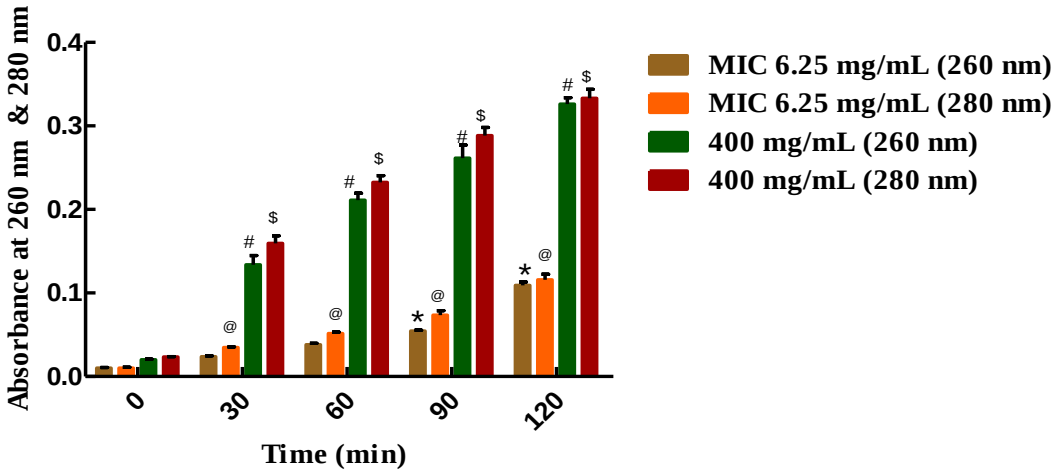


Figure 4.29: Absorbance at 260 nm and 280 nm of the supernatant of *Staphylococcus aureus* suspension after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at the concentrations of MIC and 400 mg/mL measured at different time intervals.

Significant ($p<0.05$) time-dependent (0 min vs. 30 min; 30 min vs. 60 min; 60 min vs. 90 min; 90 min vs. 120 min) increase in the absorbance, *, MIC (6.25 mg/mL) at 260 nm; $^{\#}$, 400 mg/mL at 260 nm; $^{@}$, MIC (6.25 mg/mL) at 280 nm; $^{\$}$, 400 mg/mL at 280 nm.

4.8.2.1. Estimation of total protein content

The protein content of supernatant of *Escherichia coli* and *Staphylococcus aureus* suspension after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. are shown in Table 4.40, Table 4.41 and Figure 4.30. There was significant ($p<0.05$) increase in protein content with an increase in the time interval. The protein content of treated bacterial cells was significantly ($p<0.05$) higher than that of untreated cells.

Table 4.40: Total protein content in µg/mL of the supernatant of *Escherichia coli* suspension after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. compared with untreated control

Time (min)	Total protein content (µg/mL)		
	Untreated *Escherichia coli*	**Treated *Escherichia coli* at MIC (12.5 mg/mL)**	**Treated *Escherichia coli* at 400 mg/mL**
0	5.53±0.33	9.07±0.35	16.70±0.23
30	7.15±0.22	22.02±0.61	37.97±0.46
60	12.86±0.79	25.82±0.29	46.30±0.48
90	14.68±0.79	30.97±0.42	60.87±0.23
120	16.15±0.38	33.27±0.38	72.12±0.51

Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

Table 4.41: Total protein content in µg/ml of the supernatant of *Staphylococcus aureus* suspension after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. compared with untreated control

Time (min)	Total protein content (µg/mL)		
	Untreated *Staphylococcus aureus*	Treated *Staphylococcus aureus* at MIC (6.25 mg/mL)	Treated *Staphylococcus aureus* at 400 mg/mL
0	4.34±0.15	6.92±0.13	12.54±0.62
30	7.92±0.20	13.92±0.30	31.38±0.53
60	9.58±0.30	18.94±0.67	36.83±0.11
90	10.72±0.36	21.00±0.44	46.75±0.10
120	11.93±0.12	25.35±0.24	57.42±0.82

Each value represents mean ± SD (n=3). All experiments were performed in triplicate.

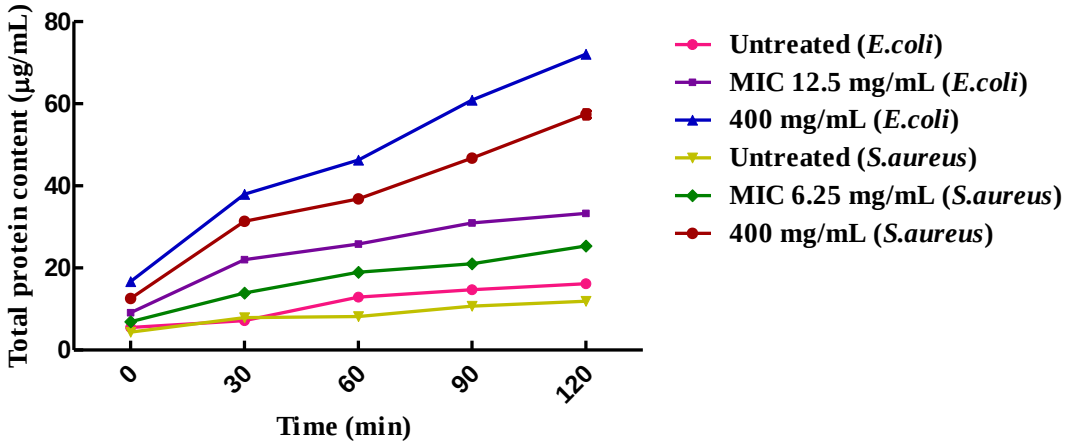


Figure 4.30: Total protein content of supernatant from *Escherichia coli* and *Staphylococcus aureus* suspensions of the control (untreated) and after treatment with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at the concentrations of MIC and 400 mg/mL measured at different time intervals. *E. coli*; *Escherichia coli*, *S. aureus*; *Staphylococcus aureus.*

4.9. Scanning electron microscope study

Scanning electron microscope (SEM) study was performed to visualize the effects of the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. on the bacterial cell membrane. The supernatant of the bacterial suspensions (*Escherichia coli* and *Staphylococcus aureus*) treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. revealed the leakage of cellular material absorbing at 260 nm and 280 nm. To further validate the possibility of the cellular material leakage due to the possible bacterial membrane damaging property of the extracts, scanning electron microscope study was performed.

Control bacteria *Escherichia coli* (Figure 4.31.a) and *Staphylococcus aureus* (Figure 4.32.a) which were not treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. exhibited normal cell morphology. The bacteria treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. showed deformed cell structure, cell wall breakage and leakage of cellular contents. *Escherichia coli* cells treated with the extract at MIC (25 mg/mL) showed breakage and deformed cell structure (Figure 4.31 b-c). An increased extent of membrane damage was observed at 400 mg/mL (Figure 4.31 d) resulting in membrane disruption and leakage. *Escherichia coli* cells treated with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at MIC (12.5 mg/mL) exhibited deformed cell structure and breakage (Figure 4.31 e). The extent of damage was increased causing more damage and leakage at 400 mg/mL (Figure 4.31 f).

Similarly, *Staphylococcus aureus* treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at MIC (3.125 mg/mL) revealed deformed cell structure and

leakage (Figure 4.32 b). The extent of damage was increased at 400 mg/mL concentration with increased cellular leakage and deformed cell structure (Figure 4.32 c-d). *Staphylococcus aureus* cell treated with the alkaloid extract of *Dicentra scandens* (D. Don) Walp. at MIC (6.25 mg/mL) exhibited deformed cell structure, swelling of cells and leakage (Figure 4.32 e). The cells treated at 400 mg/mL showed increased damage causing breakage and leakage of cellular contents (Figure 4.32 f).

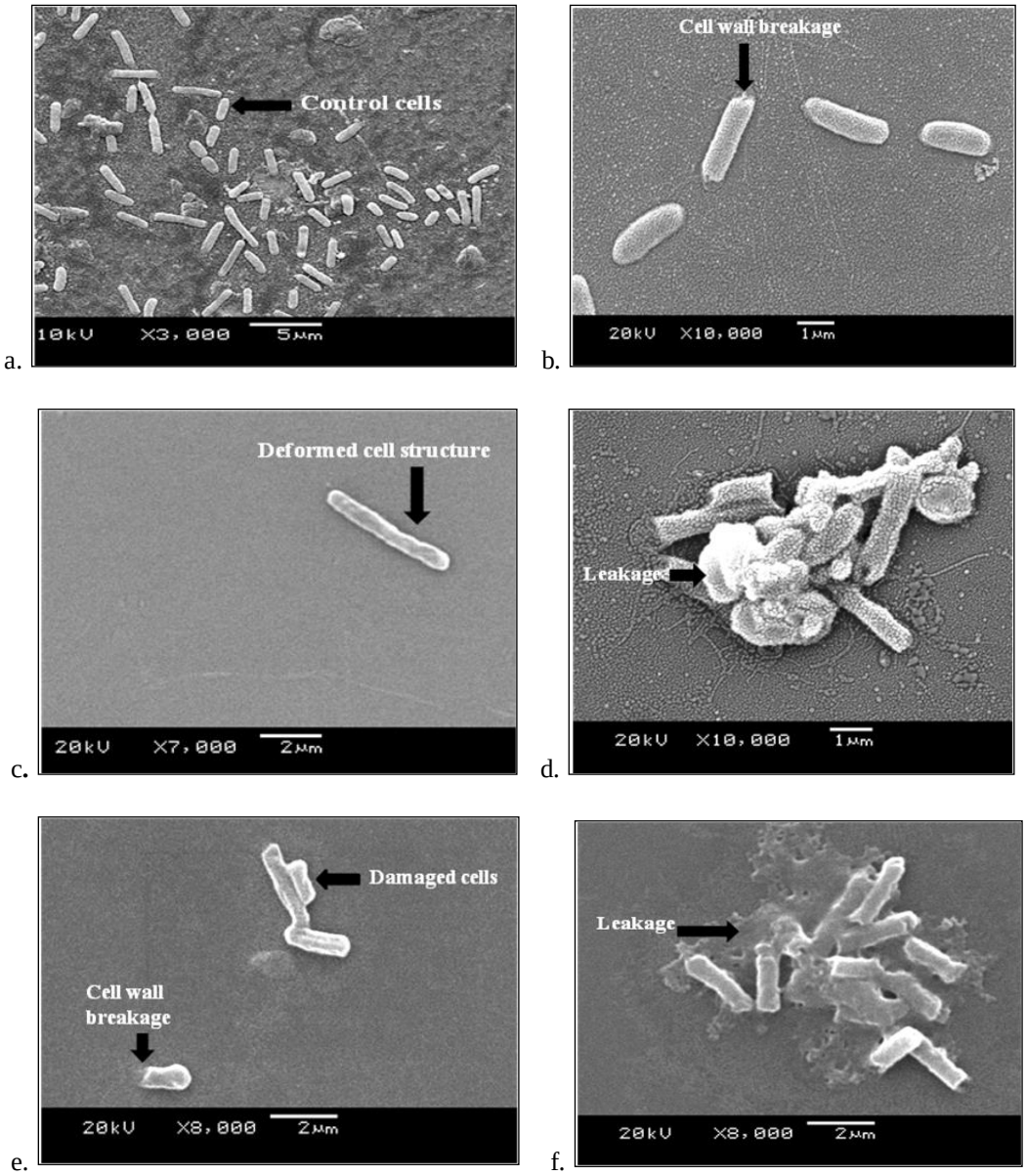


Figure 4.31. Scanning electron micrographs (a) Untreated *Escherichia coli* (b-c) *Escherichia coli* treated with flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at MIC (25 mg/mL) (d) *Escherichia coli* treated with flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. (400 mg/mL) (e) *Escherichia coli* treated with alkaloid extract of *Dicentra scandens* (D. Don) Walp. at MIC 12.5 mg/mL (f) *Escherichia coli* treated with alkaloid extract of *Dicentra scandens* (D. Don) Walp. at 400 mg/mL.

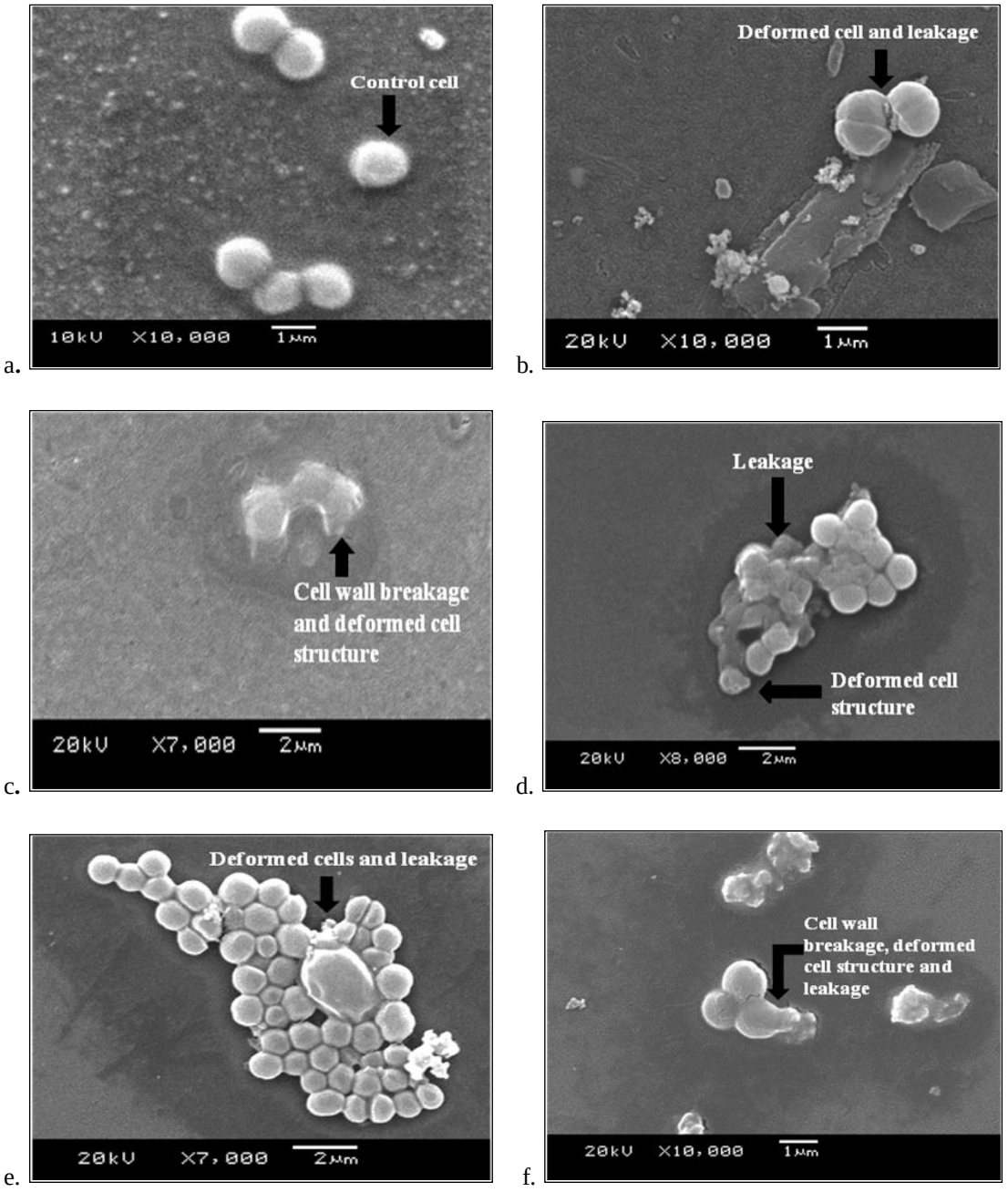


Figure 4.32. Scanning electron micrographs (a) Untreated *Staphylococcus aureus*. (b) *Staphylococcus aureus* treated with flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. at MIC (3.125 mg/mL) (c-d) *Staphylococcus aureus* treated with flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. (400 mg/mL). (e) *Staphylococcus aureus* treated with alkaloid extract of *Dicentra scandens* (D. Don) Walp. at MIC (6.25 mg/mL). (f) *Staphylococcus aureus* treated with alkaloid extract of *Dicentra scandens* (D. Don) Walp. (400 mg/mL).

4.10. Synergistic antimicrobial activity assay

The possible synergistic antimicrobial activity of the selected phytochemicals extracted from the test plants was evaluated by the checkerboard assay.

4.10.1. Checkerboard assay

Checkerboard assay was performed by taking the phytochemical extract exhibiting antimicrobial activity for almost all the test microorganisms in the agar well diffusion assay. The test microorganisms for which the largest zone of inhibition was observed at the concentration of 400 mg/mL were considered for the study. In the case of *Cyphomandra betacea* (Cav.) Sendth. among the Gram-positive test microorganisms, the largest zone of inhibition was observed against *Staphylococcus aureus* with the alkaloid, flavonoid and saponin extracts. Hence the combination of alkaloid and flavonoid extract as well as the combination of flavonoid and saponin extracts were evaluated against *Staphylococcus aureus*. Similarly, with the flavonoid and saponin extracts, the largest zone of inhibition was observed against *Escherichia coli* among the Gram-negative test microorganism. Therefore, the combination of these extracts was evaluated against *Escherichia coli*.

In the case of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, the alkaloid extract exhibited the largest zone of inhibition against *Staphylococcus aureus*. On the other hand, saponin extract did not exhibit antimicrobial activity against *Staphylococcus aureus.* Hence the checkerboard assay was performed against *Bacillus cereus* and *Vibrio cholerae* O139 for which the zone of inhibition was observed with both the extracts.

In the case of *Dicentra scandens* (D. Don) Walp., with the alkaloid and the flavonoid extracts the largest zone of inhibition was observed against *Staphylococcus aureus* among the Gram-positive test microorganism. Hence the checkerboard assay was performed against *Staphylococcus aureus* with these extracts. Among the Gram-negative test microorganism, the largest zone of inhibition was observed against *Vibrio cholerae* O139 with the flavonoid and the saponin extracts of *Dicentra scandens* (D. Don) Walp. Hence, the combination of alkaloid and flavonoid, as well as the combination of flavonoid and saponin extracts, were investigated by checkerboard assay against *Vibrio cholerae* O139.

The alkaloid extract of *Heracleum nepalense* D. Don exhibited antimicrobial activity against most of the test microorganisms. However, the flavonoid extract exhibited antimicrobial activity only against *Escherichia coli* among the Gram-negative test microorganisms, hence the checkerboard assay was performed against *Escherichia coli* with the combination of the flavonoid and alkaloid extracts. With the saponin extract of *Heracleum nepalense* D. Don, the largest zone of inhibition was observed against *Bacillus cereus.* The tannin extract of *Heracleum nepalense* D. Don exhibited antimicrobial activity only against *Bacillus cereus*, hence the checkerboard assay was performed against *Bacillus cereus* with tannin and saponin extracts. The interpretation of the results of the checkerboard assay was based on the fractional inhibitory concentration index (ΣFIC). The combination of the phytochemical extracts and the test microorganisms used for the checkerboard assay is shown in Table 4.42.

Table 4.42: Combination of the phytochemical extracts and the test microorganisms used for checkerboard assay

Sl. no.	Name of plants	Combination of phytochemical extracts	Test microorganisms
1	*Cyphomandra betacea* (Cav.) Sendth.	Flavonoid+Alkaloid	*Staphylococcus aureus*
2	*Cyphomandra betacea* (Cav.) Sendth.	Flavonoid+Saponin	*Escherichia coli*
3	*Cyphomandra betacea* (Cav.) Sendth.	Flavonoid+Saponin	*Staphylococcus aureus*
4	*Capsicum annuum* var. *cerasiforme* (Mill.) Irish	Alkaloid+Saponin	*Vibrio cholerae* O139
5	*Capsicum annuum* var. *cerasiforme* (Mill.) Irish	Alkaloid+Saponin	*Bacillus cereus*
6	*Dicentra scandens* (D. Don) Walp.	Flavonoid+Alkaloid	*Staphylococcus aureus*
7	*Dicentra scandens* (D. Don) Walp.	Flavonoid+Alkaloid	*Vibrio cholerae* O139
8	*Dicentra scandens* (D. Don) Walp.	Flavonoid+Saponin	*Vibrio cholerae* O139
9	*Heracleum nepalense* D. Don	Flavonoid+Alkaloid	*Escherichia coli*
10	*Heracleum nepalense* D. Don	Saponin+Tannin	*Bacillus cereus*

4.10.1.1. *Cyphomandra betacea* (Cav.) Sendth.

For *Cyphomandra betacea* (Cav.) Sendth., a combination of the flavonoid and the alkaloid extracts was evaluated against *Staphylococcus aureus*. The value of FIC indices ranged from 0.75 to 1.125. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.43 and Figure 4.33).

Similarly, a combination of flavonoid and saponin extracts was evaluated against *Escherichia coli*. The value of FIC indices ranged from 1.003 to 1.5. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.44 and Figure 4.34).

The combination of flavonoid and saponin extract of *Cyphomandra betacea* (Cav.) Sendth. was evaluated for possible synergistic activity against *Staphylococcus aureus.* The extracts in combination exhibited synergistic interaction ($\Sigma FIC \leq 0.5$) at two concentrations (flavonoid extract 3.125 mg/mL and saponin extract 6.25 mg/mL) with FIC index of 0.375 and (flavonoid extract 3.125 mg/mL and saponin extract 12.5 mg/mL) with FIC index of 0.5. All other concentrations in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.45 and Figure 4.35).

Table 4.43: FIC Indices for the flavonoid and the alkaloid extracts of *Cyphomandra betacea* (Cav.) Sendth. in combination against *Staphylococcus aureus*

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B8	12.5	12.5	1	0.781	25	0.031	1.031	ADD
C8	12.5	12.5	1	1.562	25	0.062	1.062	ADD
D8	12.5	12.5	1	3.125	25	0.125	1.125	ADD
E8	12.5	12.5	1	6.25	25	0.25	1.25	ADD
F6	3.125	12.5	0.25	12.5	25	0.5	0.75	ADD
F7	6.25	12.5	0.5	12.5	25	0.5	1	ADD
G2	0.195	12.5	0.015	25	25	1	1.015	ADD
G3	0.390	12.5	0.031	25	25	1	1.031	ADD
G4	0.781	12.5	0.062	25	25	1	1.062	ADD
G5	1.562	12.5	0.125	25	25	1	1.125	ADD

A; Flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth., B; Alkaloid extract of *Cyphomandra betacea* (Cav.) Sendth., ADD; Additive.

Flavonoid extract (mg/mL)

Alkaloid extract (mg/mL)

1 **A** Growth control	**2** 0.1953	**3** 0.3906	**4** 0.78125	**5** 1.5625	**6** 3.125	**7** 6.25	**8** 12.5 MIC	**9** 25	**10** 50	**11** 100	**12** Sterility control
B 0.78125							ADD				Sterility control
C 1.5625							ADD				Sterility control
D 3.125							ADD				Plant extract control A
E 6.25							ADD				Plant extract control B
F 12.5					ADD	ADD					Plant extract control A
G 25 MIC	ADD	ADD	ADD	ADD							Plant extract control B
H 50											Sterility control

Figure 4.33: The checkerboard panel showing the activity of the flavonoid and the alkaloid extracts of *Cyphomandra betacea* (Cav.) Sendth. in combination against *Staphylococcus aureus.* Sterility control; Muller Hinton Broth, Plant extract control A; Flavonoid extract, Plant extract control B; Alkaloid extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

Table 4.44: FIC Indices for the flavonoid and the saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. in combination against *Escherichia coli*

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B10	50	50	1	1.562	100	0.015	1.015	ADD
C10	50	50	1	3.125	100	0.031	1.031	ADD
D10	50	50	1	6.25	100	0.062	1.062	ADD
E10	50	50	1	12.5	100	0.125	1.125	ADD
F10	50	50	1	25	100	0.25	1.25	ADD
G10	50	50	1	50	100	0.5	1.5	ADD
H2	0.195	50	0.003	100	100	1	1.003	ADD
H3	0.390	50	0.007	100	100	1	1.007	ADD
H4	0.781	50	0.015	100	100	1	1.015	ADD
H5	1.562	50	0.031	100	100	1	1.031	ADD
H6	3.125	50	0.062	100	100	1	1.062	ADD
H7	6.25	50	0.125	100	100	1	1.125	ADD
H8	12.5	50	0.25	100	100	1	1.25	ADD
H9	25	50	0.5	100	100	1	1.5	ADD

A; Flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth., B; Saponin extract of *Cyphomandra betacea* (Cav.) Sendth., ADD; Additive.

Flavonoid extract (mg/mL)

Saponin extract (mg/mL)

1 **A** Growth control	**2** 0.1953	**3** 0.3906	**4** 0.78125	**5** 1.5625	**6** 3.125	**7** 6.25	**8** 12.5	**9** 25	**10** 50 MIC	**11** 100	**12** Sterility control
B 1.5625									ADD		Sterility control
C 3.125									ADD		Sterility control
D 6.25									ADD		Plant extract control A
E 12.5									ADD		Plant extract control A
F 25									ADD		Plant extract control B
G 50									ADD		Plant extract control B
H 100 MIC	ADD	ADD	ADD	ADD	ADD	ADD	ADD	ADD			Sterility control

Figure 4.34: The checkerboard panel showing the activity of the flavonoid and the saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. in combination against *Escherichia coli.* Sterility control; Muller Hinton Broth, Plant extract control A; Flavonoid extract, Plant extract control B; Saponin extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

Table 4.45: FIC Indices for the flavonoid and the saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. in combination against *Staphylococcus aureus*

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B8	12.5	12.5	1	1.562	50	0.031	1.031	ADD
C8	12.5	12.5	1	3.125	50	0.062	1.062	ADD
D6	3.125	12.5	0.25	6.25	50	0.125	0.375	**SYN**
D7	6.25	12.5	0.5	6.25	50	0.125	0.625	ADD
E6	3.125	12.5	0.25	12.5	50	0.25	0.5	**SYN**
F6	3.125	12.5	0.25	25	50	0.5	0.75	ADD
G2	0.195	12.5	0.015	50	50	1	1.015	ADD
G3	0.390	12.5	0.031	50	50	1	1.031	ADD
G4	0.781	12.5	0.062	50	50	1	1.062	ADD
G5	1.562	12.5	0.125	50	50	1	1.125	ADD

A; Flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth., B, Saponin extract of *Cyphomandra betacea* (Cav.) Sendth., ADD; Additive, SYN; Synergism.

Flavonoid extract (mg/mL)

Saponin extract (mg/mL)

1 **A** Growth control	**2** 0.1953	**3** 0.3906	**4** 0.78125	**5** 1.5625	**6** 3.125	**7** 6.25	**8** 12.5 MIC	**9** 25	**10** 50	**11** 100	**12** Sterility control
B 1.5625							ADD				Sterility control
C 3.125							ADD				Sterility control
D 6.25					SYN	ADD					Plant extract control A
E 12.5					SYN						Plant extract control A
F 25					ADD						Plant extract control B
G 50 MIC	ADD	ADD	ADD	ADD							Plant extract control B
H 100											Sterility control

Figure 4.35: The checkerboard panel showing the activity of the flavonoid and the saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. in combination against *Staphylococcus aureus*. Sterility control; Muller Hinton Broth, Plant extract control A; Flavonoid extract, Plant extract control B; Saponin extract, A-H & 1-12; Well number, ADD; Additive, SYN; Synergism, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

4.10.1.2. *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

The alkaloid extract and the saponin extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish in combination was tested against *Vibrio cholerae* O139. The value of FIC indices ranged from 1.007 to 1.5. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.46 and Figure 4.36).

The alkaloid extract and the saponin extract of *Capsicum annuum* var. *cerasiforme* in combination was tested against *Bacillus cereus*. The value of FIC indices ranged from 0.75 to 1.25. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.47 and Figure 4.37).

Table 4.46: FIC Indices for the alkaloid and the saponin extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish in combination against *Vibrio cholerae* O139

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B9	25	25	1	6.25	200	0.031	1.031	ADD
C9	25	25	1	12.5	200	0.062	1.062	ADD
D9	25	25	1	25	200	0.125	1.125	ADD
E9	25	25	1	50	200	0.25	1.25	ADD
F9	25	25	1	100	200	0.5	1.5	ADD
G2	0.195	25	0.007	200	200	1	1.007	ADD
G3	0.390	25	0.015	200	200	1	1.015	ADD
G4	0.781	25	0.031	200	200	1	1.031	ADD
G5	1.562	25	0.062	200	200	1	1.062	ADD
G6	3.125	25	0.125	200	200	1	1.125	ADD
G7	6.25	25	0.25	200	200	1	1.25	ADD
G8	12.5	25	0.5	200	200	1	1.5	ADD

A; Alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, B; Saponin extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, ADD; Additive.

Alkaloid extract (mg/mL)

Saponin extract (mg/mL)											
	1 **A** Growth control	**2** 0.1953	**3** 0.3906	**4** 0.78125	**5** 1.5625	**6** 3.125	**7** 6.25	**8** 12.5	**9** 25 MIC	**10** 50	**11** 100	**12** Sterility control
	B 6.25								ADD			Sterility control
	C 12.5								ADD			Sterility control
	D 25								ADD			Plant extract control A
	E 50								ADD			Plant extract control A
	F 100								ADD			Plant extract control B
	G 200 MIC	ADD	ADD	ADD	ADD	ADD	ADD	ADD				Plant extract control B
	B 400											Sterility control

Figure 4.36: The checkerboard panel showing the activity of the alkaloid and the saponin extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish in combination against *Vibrio cholerae* O139. Sterility control; Muller Hinton Broth, Plant extract control A; Alkaloid extract, Plant extract control B; Saponin extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

Table 4.47: FIC Indices for the alkaloid and the saponin extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish in combination against *Bacillus cereus*

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B9	50	50	1	6.25	400	0.015	1.015	ADD
C9	50	50	1	12.5	400	0.031	1.031	ADD
D9	50	50	1	25	400	0.062	1.062	ADD
E9	50	50	1	50	400	0.125	1.125	ADD
F8	25	50	0.5	100	400	0.25	0.75	ADD
G8	25	50	0.5	200	400	0.5	1	ADD
H2	0.390	50	0.007	400	400	1	1.007	ADD
H3	0.781	50	0.015	400	400	1	1.015	ADD
H4	1.562	50	0.031	400	400	1	1.031	ADD
H5	3.125	50	0.062	400	400	1	1.062	ADD
H6	6.25	50	0.125	400	400	1	1.125	ADD
H7	12.5	50	0.25	400	400	1	1.25	ADD

A; Alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, B; Saponin extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, ADD; Additive.

Alkaloid extract (mg/mL)

Saponin extract (mg/mL)

1 **A** Growth control	**2** 0.3906	**3** 0.78125	**4** 1.5625	**5** 3.125	**6** 6.25	**7** 12.5	**8** 25	**9** 50 MIC	**10** 100	**11** 200	**12** Sterility control
B 6.25								ADD			Sterility control
C 12.5								ADD			Sterility control
D 25								ADD			Plant extract control A
E 50								ADD			Plant extract control A
F 100							ADD				Plant extract control B
G 200							ADD				Plant extract control B
H 400 MIC	ADD	ADD	ADD	ADD	ADD	ADD					Sterility control

Figure 4.37: The checkerboard panel showing the activity of the alkaloid and the saponin extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish in combination against *Bacillus cereus.* Sterility control; Muller Hinton Broth, Plant extract control A; Alkaloid extract, Plant extract control B; Saponin extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

4.10.1.3. *Dicentra scandens* (D. Don) Walp.

For *Dicentra scandens* (D. Don) Walp. combination of the flavonoid and the alkaloid extract was evaluated against *Staphylococcus aureus*. The value of FIC indices ranged from 1.062 to 2.007 and thus exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$). The FIC index values for the combination of some concentrations of these phytochemicals were in the range of 2.015 to 2.5 hence interpreted as indifference ($2 < \Sigma FIC \leq 4$) (Table 4.48 and Figure 4.38).

Similarly, the combination of the flavonoid and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. were evaluated against *Vibrio cholerae* O139. The value of FIC indices ranged from 1.062 to 2. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.49 and Figure 4.39).

The combination of the flavonoid and the saponin extract of *Dicentra scandens* (D. Don) Walp. were evaluated against *Vibrio cholerae* O139. The value of FIC indices ranged from 1.062 to 1.5. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.50 and Figure 4.40).

Table 4.48: FIC Indices for the flavonoid and the alkaloid extracts of *Dicentra scandens* (D. Don) Walp. in combination against *Staphylococcus aureus*

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B9	25	25	1	0.78125	12.5	0.0625	1.062	ADD
C9	25	25	1	1.5625	12.5	0.125	1.125	ADD
D9	25	25	1	3.125	12.5	0.25	1.25	ADD
E9	25	25	1	6.25	12.5	0.5	1.5	ADD
F2	0.195	25	0.007	25	12.5	2	2.007	ADD
F3	0.390	25	0.015	25	12.5	2	2.015	IND
F4	0.781	25	0.031	25	12.5	2	2.031	IND
F5	1.562	25	0.062	25	12.5	2	2.062	IND
F6	3.125	25	0.125	25	12.5	2	2.125	IND
F7	6.25	25	0.25	25	12.5	2	2.25	IND
F8	12.5	25	0.5	25	12.5	2	2.5	IND

A; Flavonoid extract of *Dicentra scandens* (D. Don) Walp., B; Alkaloid extract of *Dicentra scandens* (D. Don) Walp., ADD; Additive, IND; Indifference.

Flavonoid extract (mg/mL)

Alkaloid extract (mg/mL)

1 **A** Growth control	**2** 0.1953	**3** 0.3906	**4** 0.78125	**5** 1.5625	**6** 3.125	**7** 6.25	**8** 12.5	**9** 25 MIC	**10** 50	**11** 100	**12** Sterility control
B 0.78125								ADD			Sterility control
C 1.5625								ADD			Sterility control
D 3.125								ADD			Plant extract control A
E 6.25								ADD			Plant extract control B
F 12.5 MIC	ADD	IND	IND	IND	IND	IND	IND				Plant extract control A
G 25											Plant extract control B
H 50											Sterility control

Figure 4.38: The checkerboard panel showing the activity of the flavonoid and the alkaloid extracts of *Dicentra scandens* (D. Don) Walp. in combination against *Staphylococcus aureus*. Sterility control; Muller Hinton Broth, Plant extract control A; Flavonoid extract, Plant extract control B; Alkaloid extract, A-H & 1-12; Well number, ADD; Additive, IND; Indifference, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

Table 4.49: FIC Indices for the flavonoid and the alkaloid extracts of *Dicentra scandens* (D. Don) Walp. in combination against *Vibrio cholerae* O139

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B6	6.25	6.25	1	1.562	25	0.062	1.062	ADD
C6	6.25	6.25	1	3.125	25	0.125	1.125	ADD
D6	6.25	6.25	1	6.25	25	0.25	1.25	ADD
E5	6.25	6.25	1	12.5	25	0.5	1.5	ADD
F2	0.390	6.25	0.062	25	25	1	1.062	ADD
F3	0.781	6.25	0.125	25	25	1	1.125	ADD
F4	1.562	6.25	0.25	25	25	1	1.25	ADD

A; Flavonoid extract of *Dicentra scandens* (D. Don) Walp., B; Alkaloid extract of *Dicentra scandens* (D. Don) Walp., ADD; Additive.

Flavonoid extract (mg/mL)

Alkaloid extract (mg/mL)

1 **A** Growth control	**2** 0.3906	**3** 0.78125	**4** 1.5625	**5** 3.125	**6** 6.25 MIC	**7** 12.5	**8** 25	**9** 50	**10** 100	**11** 200	**12** Sterility control
B 1.5625					ADD						Sterility control
C 3.125					ADD						Sterility control
D 6.25					ADD						Plant extract control A
E 12.5				ADD							Plant extract control A
F 25 MIC	ADD	ADD	ADD								Plant extract control B
G 50											Plant extract control B
H 100											Sterility control

Figure 4.39: The checkerboard panel showing the activity of the flavonoid and the alkaloid extracts of *Dicentra scandens* (D. Don) Walp. in combination against *Vibrio cholerae* O139. Sterility control; Muller Hinton Broth, Plant extract control A; Flavonoid extract, Plant extract control B; Alkaloid extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

Table 4.50: FIC Indices for the flavonoid and the saponin extracts of *Dicentra scandens* (D. Don) Walp. in combination against *Vibrio cholerae* O139

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B6	6.25	6.25	1	3.125	50	0.062	1.062	ADD
C6	6.25	6.25	1	6.25	50	0.125	1.125	ADD
D6	6.25	6.25	1	12.5	50	0.25	1.25	ADD
E6	6.25	6.25	1	25	50	0.5	1.5	ADD
F2	0.390	6.25	0.062	50	50	1	1.062	ADD
F3	0.781	6.25	0.125	50	50	1	1.125	ADD
F4	1.562	6.25	0.25	50	50	1	1.25	ADD
F5	3.125	6.25	0.5	50	50	1	1.5	ADD

A; Flavonoid extract of *Dicentra scandens* (D. Don) Walp., B; Saponin extract of *Dicentra scandens* (D. Don) Walp., ADD; Additive.

Flavonoid extract (mg/mL)

Saponin extract (mg/mL)

1 **A** Growth control	**2** 0.3906	**3** 0.78125	**4** 1.5625	**5** 3.125	**6** 6.25 MIC	**7** 12.5	**8** 25	**9** 50	**10** 100	**11** 200	**12** Sterility control
B 3.125					ADD						Sterility control
C 6.25					ADD						Sterility control
D 12.5					ADD						Plant extract control A
E 25					ADD						Plant extract control A
F 50 MIC	ADD	ADD	ADD	ADD							Plant extract control B
G 100											Plant extract control B
H 200											Sterility control

Figure 4.40: The checkerboard panel showing the activity of the flavonoid and the saponin extracts of *Dicentra scandens* (D. Don) Walp. in combination against *Vibrio cholerae* O139. Sterility control; Muller Hinton Broth, Plant extract control A; Flavonoid extract, Plant extract control B; Saponin extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

4.10.1.4. *Heracleum nepalense* D. Don

To see the possible synergistic activity, the flavonoid and the alkaloid extracts of *Heracleum nepalense* D. Don in combination were tested against *Escherichia coli.* The value of FIC indices ranged from 0.625 to 1.25. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 4$) (Table 4.51 and Figure 4.41).

Similarly, the combination of saponin and the tannin extracts of *Heracleum nepalense* D. Don were tested against *Bacillus cereus.* The value of FIC indices ranged from 1.001 to 1.5. The extracts in combination exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) (Table 4.52 and Figure 4.42).

Table 4.51: FIC Indices for the flavonoid and the alkaloid extracts of *Heracleum nepalense* D. Don in combination against *Escherichia coli*

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B8	12.5	12.5	1	1.562	25	0.062	1.062	ADD
C7	6.25	12.5	0.5	3.125	25	0.125	0.625	ADD
D7	6.25	12.5	0.5	6.25	25	0.25	0.75	ADD
E7	6.25	12.5	0.5	12.5	25	0.5	1	ADD
F2	0.195	12.5	0.015	25	25	1	1.015	ADD
F3	0.390	12.5	0.031	25	25	1	1.031	ADD
F4	0.781	12.5	0.062	25	25	1	1.062	ADD
F5	1.562	12.5	0.125	25	25	1	1.125	ADD
F6	3.125	12.5	0.25	25	25	1	1.25	ADD

A; Flavonoid extract of *Heracleum nepalense* D. Don, B; Alkaloid extract of *Heracleum nepalense* D. Don, ADD; Additive.

Flavonoid extract (mg/mL)

Alkaloid extract (mg/mL)

1 **A** Growth control	**2** 0.1953	**3** 0.3906	**4** 0.78125	**5** 1.5625	**6** 3.125	**7** 6.25	**8** 12.5 MIC	**9** 25	**10** 50	**11** 100	**12** Sterility control
B 1.5625							ADD				Sterility control
C 3.125						ADD					Sterility control
D 6.25						ADD					Plant extract control A
E 12.5						ADD					Plant extract control A
F 25 MIC	ADD	ADD	ADD	ADD	ADD						Plant extract control B
G 50											Plant extract control B
H 100											Sterility control

Figure 4.41: The checkerboard panel showing the activity of the flavonoid and the alkaloid extracts of *Heracleum nepalense* D. Don in combination against *Escherichia coli.* Sterility control; Muller Hinton Broth, Plant extract control A; Flavonoid extract, Plant extract control B; Alkaloid extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

Table 4.52: FIC Indices for the saponin and the tannin extracts of *Heracleum nepalense* D. Don in combination against *Bacillus cereus*

Wells	MIC of A in combination (mg/mL)	MIC of A alone (mg/mL)	FIC A= MIC A in combination/MIC A alone	MIC of B in combination (mg/mL)	MIC of B alone (mg/mL)	FIC B= MIC B in combination/MIC of B alone	ΣFIC=FICA+FICB	Interpretation
B11	200	200	1	3.125	200	0.015	1.015	ADD
C11	200	200	1	6.25	200	0.031	1.031	ADD
D11	200	200	1	12.5	200	0.062	1.062	ADD
E11	200	200	1	25	200	0.125	1.125	ADD
F11	200	200	1	50	200	0.25	1.25	ADD
G11	200	200	1	100	200	0.5	1.5	ADD
H2	0.390	200	0.001	200	200	1	1.001	ADD
H3	0.782	200	0.003	200	200	1	1.003	ADD
H4	1.562	200	0.007	200	200	1	1.007	ADD
H5	3.125	200	0.015	200	200	1	1.015	ADD
H6	6.25	200	0.031	200	200	1	1.031	ADD
H7	12.5	200	0.062	200	200	1	1.062	ADD
H8	25	200	0.125	200	200	1	1.125	ADD
H9	50	200	0.25	200	200	1	1.25	ADD
H10	100	200	0.5	200	200	1	1.5	ADD

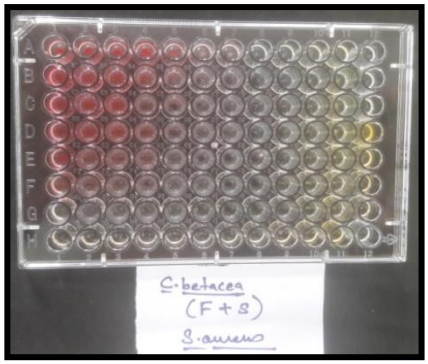
A; Saponin extract of *Heracleum nepalense* D. Don, B; Tannin extract of *Heracleum nepalense* D. Don, ADD; Additive.

Saponin extract (mg/mL)

Tannin extract (mg/mL)

1 **A** Growth control	**2** 0.3906	**3** 0.78125	**4** 1.5625	**5** 3.125	**6** 6.25	**7** 12.5	**8** 25	**9** 50	**10** 100	**11** 200 MIC	**12** Sterility control
B 3.125										ADD	Sterility control
C 6.25										ADD	Sterility control
D 12.5										ADD	Plant extract control A
E 25										ADD	Plant extract control A
F 50										ADD	Plant extract control B
G 100										ADD	Plant extract control B
H 200 MIC	ADD	ADD	ADD	ADD	ADD	ADD	ADD	ADD	ADD		Sterility control

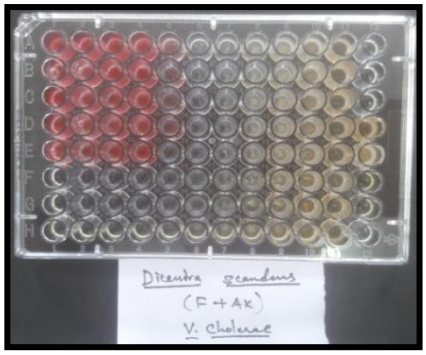
Figure 4.42: The checkerboard panel showing the activity of the saponin and the tannin extracts of *Heracleum nepalense* D. Don in combination against *Bacillus cereus*. Sterility control; Muller Hinton Broth, Plant extract control A; Saponin extract, Plant extract control B; Tannin extract, A-H & 1-12; Well number, ADD; Additive, MIC; Minimum inhibitory concentration. Values in the boxes indicate the concentrations of the extracts in mg/mL. Shaded boxes indicate wells showing growth. All the experiments were performed in duplicate.

a. 

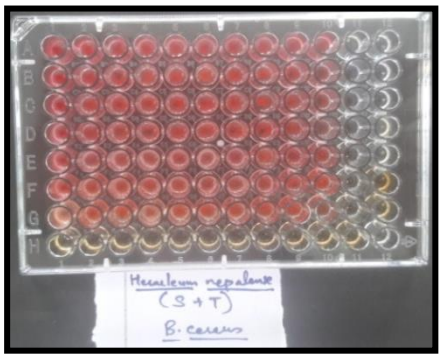
4.5 (a): Combination of flavonoid and saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. against *Staphylococcus aureus*

b. 

4.5 (b): Combination of alkaloid and saponin extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish against *Vibrio cholerae* O139

c. 

4.5 (c): Combination of flavonoid and alkaloid extracts of *Dicentra scandens* (D. Don) Walp. against *Vibrio cholerae* O139

d. 

4.5 (d): Combination of saponin and tannin extracts of *Heracleum nepalense* D. Don against *Bacillus cereus*

Photographic Plates 4.5 (a-d): Microtiter plates showing growth region (pink area) and no growth region after incubation with 2,3,5-triphenyl tetrazolium chloride (TTC) in checkerboard assay.

4.11. Antioxidant properties of various extracts from selected plants

The antioxidant properties of various phytochemical extracts as well as the general extracts (aqueous and methanol) of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don was evaluated using DPPH radical scavenging activity, ferric reducing antioxidant power, hydrogen peroxide scavenging activity, nitric oxide radical scavenging activity and hydroxyl radical scavenging activity.

4.11.1. DPPH radical scavenging activity

4.11.1.1. DPPH radical scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

The DPPH free radical scavenging activity of the various extracts of *Cyphomandra betacea* (Cav.) Sendth. in terms of percentage inhibition of DPPH radical are shown in Table 4.53, Figure 4.43 and Figure 4.44. The activity of the extracts was compared with ascorbic acid as standard. The flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity than other phytochemical extracts. At the concentration of 100 µg/mL, the highest scavenging activity was exhibited by the flavonoid extract with the percentage inhibition of 69.86±0.25. While at the same concentration, the DPPH radical scavenging activity of ascorbic acid was 82.11±0.65 %. The IC_{50} value is defined as the concentration (in µg/mL) of the extract that scavenges the DPPH radicals by 50 %. Among the various phytochemical extracts of*Cyphomandra betacea* (Cav.) Sendth., the lowest IC_{50} value of 30 µg/mL was exhibited by the flavonoid extract. The IC_{50} value of ascorbic acid (standard) was 10 µg/mL. The least DPPH radical

scavenging activity was exhibited by the steroid extract with an IC_{50} value of 100 µg/mL with percentage inhibition of 50.54±0.92 (Table 4.53).

The methanol extract exhibited higher DPPH radical scavenging activity than the aqueous extract at the concentrations of 10 µg/mL to 80 µg/mL. However, at higher concentrations of 90 µg/mL and 100 µg/mL, the aqueous extract exhibited similar DPPH radical scavenging potential as the methanol extract (Figure 4.44).

Table 4.53: DPPH radical scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	26.27±0.39	42.54±0.25*	34.20±0.54	12.04±047	21.78±0.19	26.78±0.92	34.18±1.86#	50.68±0.57
20	35.66±0.17	44.70±0.17*	36.27±1.17	18.37±0.24	24.45±0.85	26.46±0.62	36.80±0.81#	54.14±0.29
30	37.40±0.17	50.88±0.68*	36.35±0.29	22.37±0.71	30.43±1.22	28.74±0.23	40.36±0.23#	55.35±0.20
40	839.98±0.73	53.35±1.18*	36.91±0.37	26.11± 0.16	37.69±1.65	33.43±0.56	42.69±0.32#	57.39±0.66
50	42.81±0.22	58.72±0.47*	38.22±0.75	27.47 ±1.19	39.67±0.59	38.69±0.71	43.21±0.21#	61.15±0.63
60	45.19±0.71	61.81±0.18*	40.23±0.97	29.96± 0.87	42.77±0.49	39.07±0.05	43.67 ±0.27#	67.61±0.64
70	48.00±0.96	63.34±0.69*	43.61±0.46	30.24 ±0.10	50.81±0.25	43.49±0.16	50.10±0.82#	73.20±0.19
80	50.71±0.93	66.83±0.53*	44.93±0.25	35.99±0.32	51.84±0.17	50.47±1.48	53.92±1.31#	74.80±0.12
90	53.16±1.25	67.61±0.56*	50.79±0.56	38.72±0.19	55.94±0.19	54.15 ±0.55	55.97±0.15	77.28±0.98
100	56.29±0.15	69.86±0.25*	54.95±0.76	50.54 ±0.92	60.61±1.05	57.33±0.30	57.52±0.74	82.11±0.65

Each value represents mean±SD. All the experiments were performed in triplicate.*($p<0.05$) significantly different in DPPH radical scavenging activity than other phytochemical extracts. # ($p<0.05$) significantly different in DPPH radical scavenging activity than the aqueous extract.

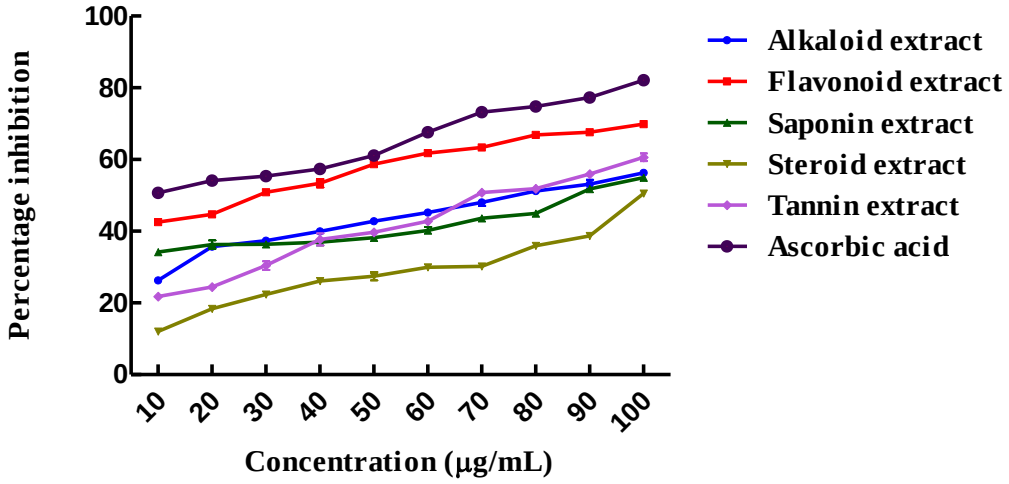


Figure 4.43: DPPH radical scavenging activity of phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth.

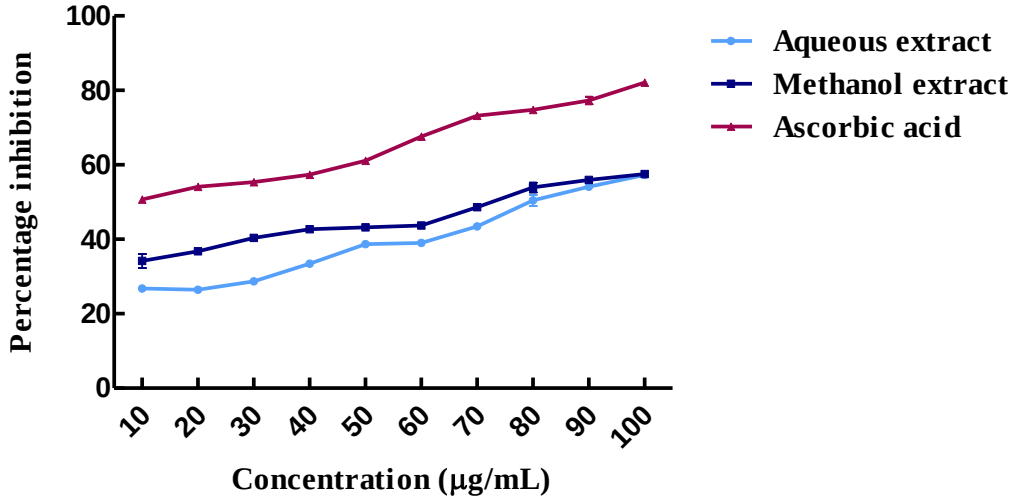


Figure 4.44: DPPH radical scavenging activity of general extracts (aqueous and methanol) of *Cyphomandra betacea* (Cav.) Sendth.

4.11.1.2. DPPH radical scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

The DPPH radical scavenging activity of the various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are shown in Table 4.54, Figure 4.45 and Figure 4.46. The activity of the extracts was compared with ascorbic acid as standard. The flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity as compared to other extracts. At the concentration of 100 µg/mL, the highest scavenging activity in terms of percentage inhibition of DPPH free radical was exhibited by the flavonoid extract with the percentage inhibition of 71.30±0.70. Among the various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, the lowest IC_{50} value of 20 µg/mL was exhibited by the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish. The IC_{50} value of ascorbic acid was 10 µg/mL with percentage inhibition of 50.76±1.28 (Table 4.54).

Among the general extracts, the methanol extract exhibited higher DPPH radical scavenging activity than the aqueous extract up to the concentration of 50 µg/mL. At the concentration of 60 µg/mL and higher, the aqueous extract exhibited similar scavenging potential as the methanol extract (Figure 4.46)

Table 4.54: DPPH radical scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	23.31±0.10	44.43±0.59*	24.08±0.34	19.88±0.29	19.97±0.97	19.30±0.34	26.35±0.99#	50.76±1.28
20	36.50±0.23	50.58±0.92*	29.87±0.70	22.88±0.89	22.76±0.78	20.36±0.87	30.62±0.38#	54.16±0.80
30	41.89±0.42	51.10±0.59*	37.03±0.49	23.57±0.14	23.99±0.61	20.27±1.31	32.88±0.73#	55.74±0.34
40	44.06±0.35	53.89±0.55*	38.14±0.45	29.94±0.42	30.51±0.25	22.06±0.69	33.26±0.68#	57.14±0.56
50	45.49±0.72	58.01±0.57*	39.20±0.35	37.19±0.58	37.07±0.48	27.61±0.51	37.42±0.92#	60.29±0.73
60	46.47±0.28	60.86±0.44*	43.63±1.03	43.84±0.62	41.78±0.10	39.69±0.99	38.82±1.12	67.13±0.62
70	50.15±0.39	64.95±0.97*	46.75±0.26	46.11±0.81	42.50±0.68	40.47±0.26	39.59±0.33	72.80±0.25
80	51.27±0.39	66.07±0.54*	50.77±0.25	51.38±0.48	45.27±0.12	43.46±0.71	44.37±0.10	74.82±1.16
90	55.36±0.31	69.10±0.64*	51.79±0.42	54.69±0.61	51.58±0.22	47.38±1.32	49.10±1.50	77.99±0.42
100	57.41±0.36	71.30±0.70*	57.42±1.08	56.03±0.37	54.78±0.80	50.34±0.78	50.85±0.47	82.63±0.34

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in DPPH radical scavenging activity than other phytochemical extracts. #($p<0.05$) significantly different in DPPH radical scavenging activity than the aqueous extract.

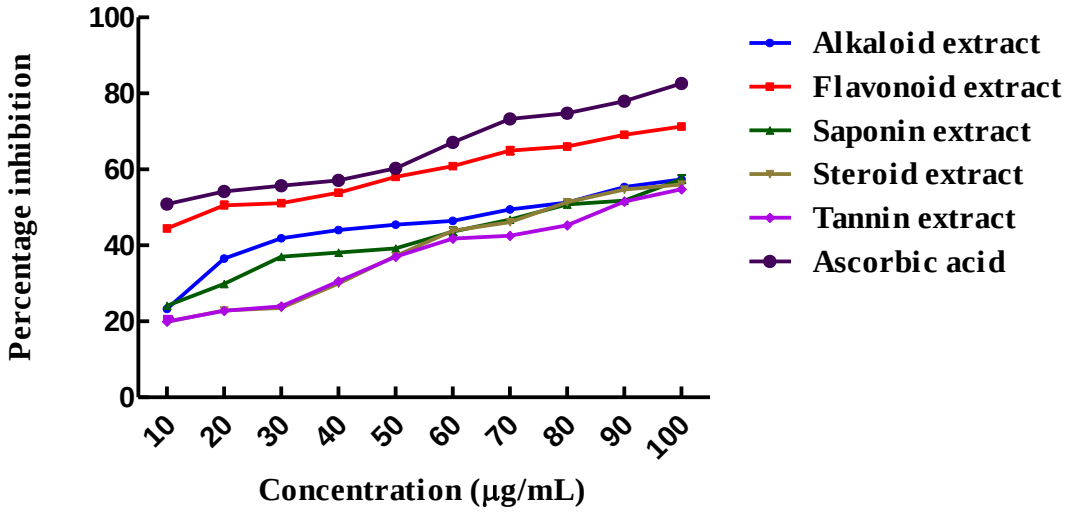


Figure 4.45: DPPH radical scavenging activity of various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

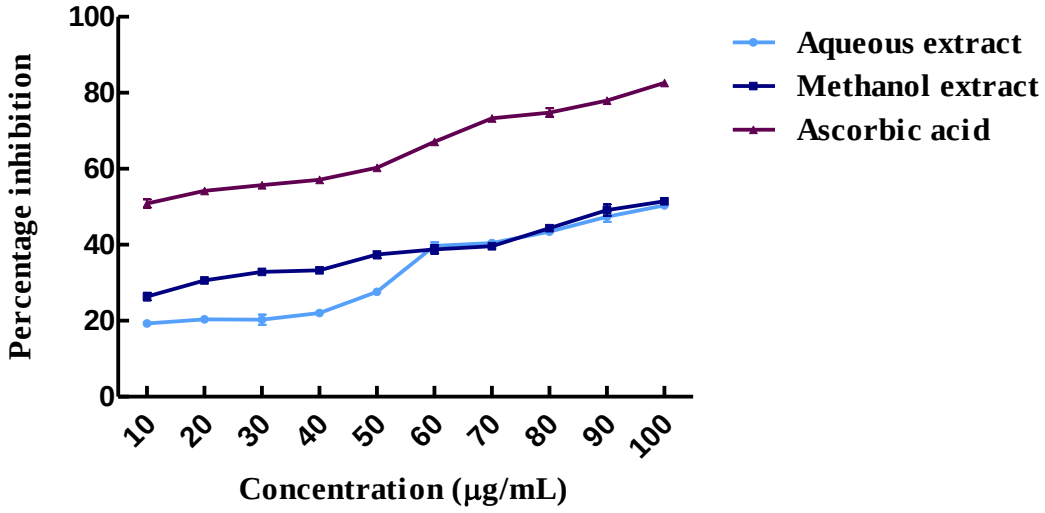


Figure 4.46: DPPH radical scavenging activity of general extracts (aqueous and methanol) of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

4.11.1.3. DPPH radical scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp.

The DPPH free radical scavenging activity of the various extracts of *Dicentra scandens* (D. Don) Walp.are shown in Table 4.55, Figure 4.47 and Figure 4.48. The activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the flavonoid extract of *Dicentra scandens* (D. Don) Walp. exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity (Figure 4.47). The lowest IC_{50} value of 50 µg/mL was exhibited by the flavonoid extract of *Dicentra scandens* (D. Don) Walp. with the percentage inhibition of 50.59±0.83. The IC_{50} value of ascorbic acid was 10 µg/mL with percentage inhibition of 50.87±0.99 (Table 4.55).

Among all the extracts of *Dicentra scandens* (D. Don) Walp., at the concentration of 100 µg/mL, the highest DPPH radical scavenging activity was exhibited by the aqueous extract with the percentage inhibition of 64.78±0.56 (Table 4.55). Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity at the concentrations of 10 µg/mL to 50 µg/mL. However, at the concentrations of 60 µg/mL to 100 µg/mL the aqueous extract exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity than the methanol extract (Figure 4.48).

Table 4.55: DPPH radical scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	35.53±0.52	35.18±1.28	39.13±0.19	24.20±0.32	21.61±0.44	10.39±0.49	29.21±0.84$^{\#}$	50.87±0.99
20	37.04±1.19	46.74±1.33*	39.48±0.06	28.74±0.46	23.89±0.46	16.13±0.94	35.57±0.21$^{\#}$	54.24±1.19
30	38.30±1.51	47.85±0.31*	39.38±0.60	31.11±0.10	27.28±0.12	26.32±0.51	36.40±0.26$^{\#}$	55.11±0.25
40	42.74±0.21	48.07±0.90*	41.51±0.01	33.51±0.05	27.98±0.14	33.86±0.07	36.73±0.33$^{\#}$	57.54±0.42
50	43.13±0.13	50.59±0.83*	42.75±0.23	34.56±0.21	31.13±0.47	37.32±0.15	43.06±0.31$^{\#}$	61.33±0.42
60	44.63±0.03	52.04±0.13*	42.64±0.44	36.81±0.21	33.50±1.00	48.49±0.89$^{\delta}$	43.98±0.40	67.37±0.75
70	45.79±0.44	52.51±0.23*	42.91±0.30	41.10±0.16	47.55±1.31	50.67±0.27$^{\delta}$	46.42±0.15	73.97±0.60
80	50.94±0.65	54.15±1.31*	43.80±1.29	44.60±0.53	49.87±0.41	54.24±0.85$^{\delta}$	50.07±0.60	75.02±0.41
90	54.54±0.62	56.98±0.56*	46.69±0.40	48.37±0.18	50.20±0.05	59.64±0.07$^{\delta}$	52.18±0.11	77.70±0.78
100	60.66±0.59	62.42±0.44*	50.18±0.23	50.62±0.40	53.43±0.87	64.78±0.56$^{\delta}$	61.12±1.08	82.86±0.56

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in DPPH radical scavenging activity than other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in DPPH radical scavenging activity than the aqueous extract. $^{\delta}$ ($p<0.05$) significantly different in DPPH radical scavenging activity than the methanol extract.

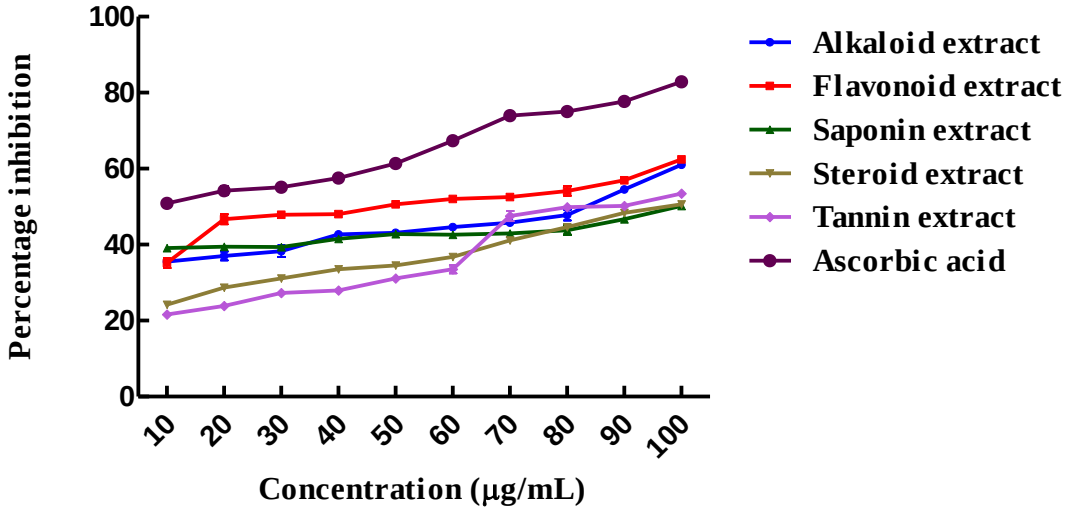


Figure 4.47: DPPH radical scavenging activity of various phytochemical extracts of *Dicentra scandens* (D. Don) Walp.

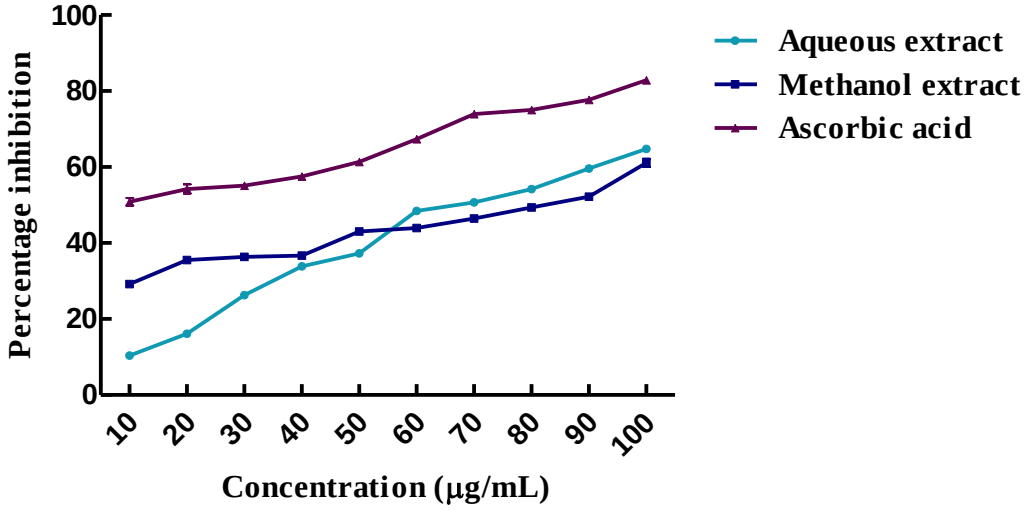


Figure 4.48: DPPH radical scavenging activity of general extracts (aqueous and methanol) of *Dicentra scandens* (D. Don) Walp.

4.11.1.4. DPPH radical scavenging activity of various extracts of *Heracleum nepalense* D. Don

The DPPH free radical scavenging activity of the various extracts of *Heracleum nepalense* D. Don are shown in Table 4.56, Figure 4.49 and Figure 4.50. The activity of the extracts was compared with ascorbic acid as standard. The saponin extract of *Heracleum nepalense* D. Don exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity than the other phytochemical extracts (Figure 4.49). The lowest IC_{50} value of 20 µg/mL was exhibited by the saponin extract of *Heracleum nepalense* D. Don with the percentage inhibition of 50.19±0.47. The IC_{50} value of ascorbic acid was 10 µg/mL with percentage inhibition of 50.58±0.81 (Table 4.56).

The aqueous extract exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity than the methanol extract (Figure 4.50). Among all the extracts of *Heracleum nepalense* D. Don, at the concentration of 100 µg/mL, the highest scavenging activity was exhibited by the aqueous extract with the percentage inhibition of 66.08±0.03 (Table 4.56).

Table 4.56: DPPH radical scavenging activity of various extracts of *Heracleum nepalense* D. Don

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	23.78±0.74	32.39±0.11	47.90±0.67*	19.22±0.83	19.10±0.46	42.45±0.31#	32.12±0.12	50.58±0.81
20	27.93±0.03	33.23±0.34	50.19±0.47*	22.91±0.74	26.04±0.35	44.52±1.03#	39.21±0.46	54.28±0.47
30	32.14±0.24	35.15±0.81	54.37±0.83*	25.42±0.33	29.09±0.68	47.17±0.78#	44.99±0.33	55.93±0.54
40	33.72±0.10	36.49±0.10	54.58±0.89*	41.29±0.68	40.23±0.10	50.64±0.96#	48.44±0.21	57.01±0.89
50	39.70±0.79	38.92±0.58	56.14±0.35*	45.05±0.22	41.26±0.74	52.15±0.69#	50.37±0.68	61.21±0.65
60	42.98±0.65	39.41±0.10	56.81±0.87*	48.75±0.12	43.01±0.63	54.79±0.56#	52.61±0.25	67.79±0.96
70	46.67±1.34	39.82±0.29	57.24±0.73*	50.93±0.33	46.52±0.84	59.25±0.91#	53.71±1.20	73.95±0.85
80	49.62±0.11	43.77±0.09	57.37±0.92*	52.08±0.98	50.46±0.71	59.44±0.50#	55.79±0.07	75.26±0.82
90	50.64±0.67	47.32±0.17	59.92±0.08*	52.99±0.71	51.69±0.39	62.36±0.79#	56.91±0.22	77.49±0.68
100	54.51±0.74	50.30±0.34	60.51±0.11*	55.13±0.24	53.00±0.68	66.08±0.03#	57.25±0.07	82.98±0.42

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in DPPH radical scavenging activity than other phytochemical extracts. # ($p<0.05$) significantly different in DPPH radical scavenging activity than the methanol extract.

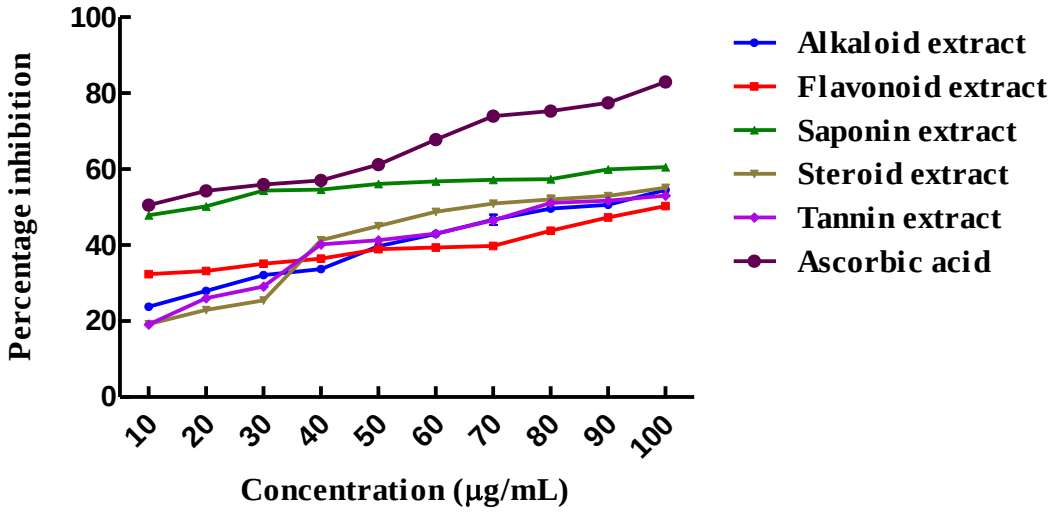


Figure 4.49: DPPH radical scavenging activity of various phytochemical extracts of *Heracleum nepalense* D. Don

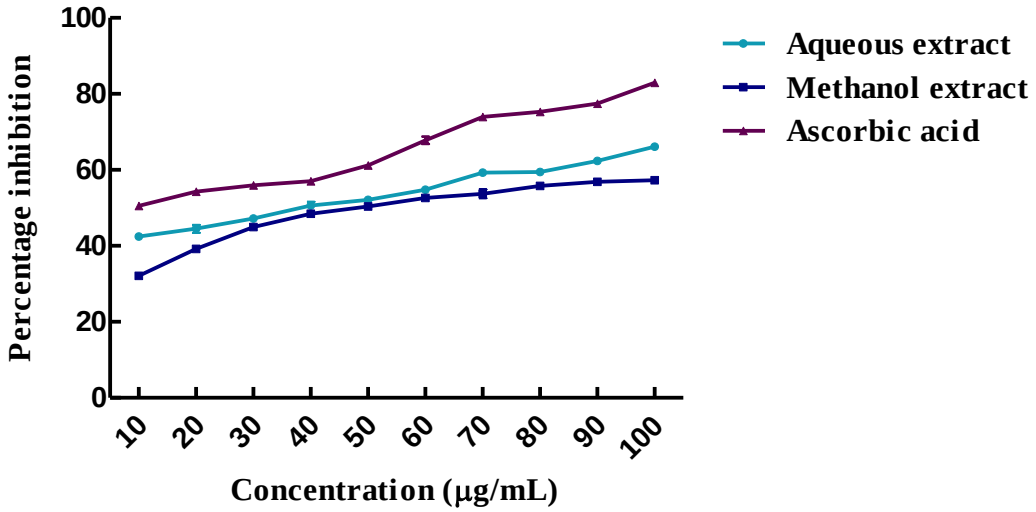


Figure 4.50: DPPH radical scavenging activity of general extracts (aqueous and methanol) of *Heracleum nepalense* D. Don

4.11.2. Ferric reducing antioxidant power

The ferric reducing antioxidant power of the various phytochemical extracts, as well as the aqueous and the methanol extracts, were compared with the ascorbic acid as standard. The reducing capability of the extracts was found to increase with an increase in the concentration of extract.

4.11.2.1. Ferric reducing antioxidant power of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

The ferric reducing antioxidant power of the various extracts of *Cyphomandra betacea* (Cav.) Sendth. are shown in (Table 4.57, Figure 4.51 and Figure 4.52). It is interesting to observe that, the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth., exhibited significantly ($p<0.05$) higher ferric reducing ability than the standard ascorbic acid (Figure 4.51). Other extracts also exhibited concentration-dependent ferric reducing ability.

Similarly, the methanol extract at the concentrations of 60 µg/mL to 100 µg/mL also exhibited significantly ($p<0.05$) higher ferric reducing ability than the ascorbic acid (Figure 4.52). The aqueous extract exhibited concentration-dependent increase in ferric reducing antioxidant power.

Table 4.57: Ferric reducing antioxidant power of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

Concentration (µg/mL)	Absorbance at 700 nm							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	0.0611±0.0001	0.5023±0.0013*	0.1291±0.0005	0.0885±0.0004	0.0601±0.0008	0.1036±0.0003	0.1983±0.0008$^{\#}$	0.2669±0.0011
20	0.0734±0.0003	0.8477±0.0014*	0.1783±0.0005	0.0895±0.0004	0.0733±0.0004	0.1170±0.0002	0.2969±0.0006$^{\#}$	0.3948±0.0005
30	0.0878±0.0004	1.3224±0.0009*	0.2005±0.0007	0.0913±0.0007	0.1091±0.0003	0.1326±0.0001	0.4473±0.0002$^{\#}$	0.4684±0.0009
40	0.0924±0.0002	1.3858±0.0016*	0.2628±0.0009	0.0949±0.0005	0.1261±0.0015	0.2087±0.0012	0.5394±0.0005$^{\#}$	0.5865±0.0003
50	0.0927±0.0001	1.5812±0.0019*	0.2881±0.0022	0.0952±0.0004	0.1623±0.0006	0.3228±0.0008	0.6392±0.0012$^{\#}$	0.6573±0.0005
60	0.0980±0.0002	1.6888±0.0019*	0.2996±0.0030	0.0964±0.0003	0.1636±0.0012	0.4201±0.0008	0.7752±0.0024$^{\#\varepsilon}$	0.7461±0.0005
70	0.1063±0.0003	1.9766±0.0046*	0.4066±0.0018	0.0983±0.0002	0.1825±0.0010	0.4940±0.0007	0.8672±0.0006$^{\#\varepsilon}$	0.8136±0.0006
80	0.1125±0.0001	2.3334±0.0067*	0.4641±0.0012	0.0997±0.0005	0.2119±0.0010	0.5387±0.0003	1.0182±0.0006$^{\#\varepsilon}$	0.9571±0.0001
90	0.1365±0.0003	2.5434±0.0124*	0.5273±0.0003	0.1258±0.0007	0.2645±0.0002	0.6212±0.0007	1.0704±0.0002$^{\#\varepsilon}$	1.0278±0.0004
100	0.1377±0.0004	2.9552±0.0227*	0.5559±0.0016	0.1558±0.0004	0.2881±0.0012	0.7118±0.0011	1.1376±0.0018$^{\#\varepsilon}$	1.1185±0.0011

Each value represents mean ± SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in ferric reducing antioxidant power than the other phytochemical extracts and the ascorbic acid. $^{\#}$ ($p<0.05$) significantly different in ferric reducing antioxidant power than the aqueous extract. $^{\varepsilon}$($p<0.05$) significantly different in ferric reducing antioxidant power than the acorbic acid.

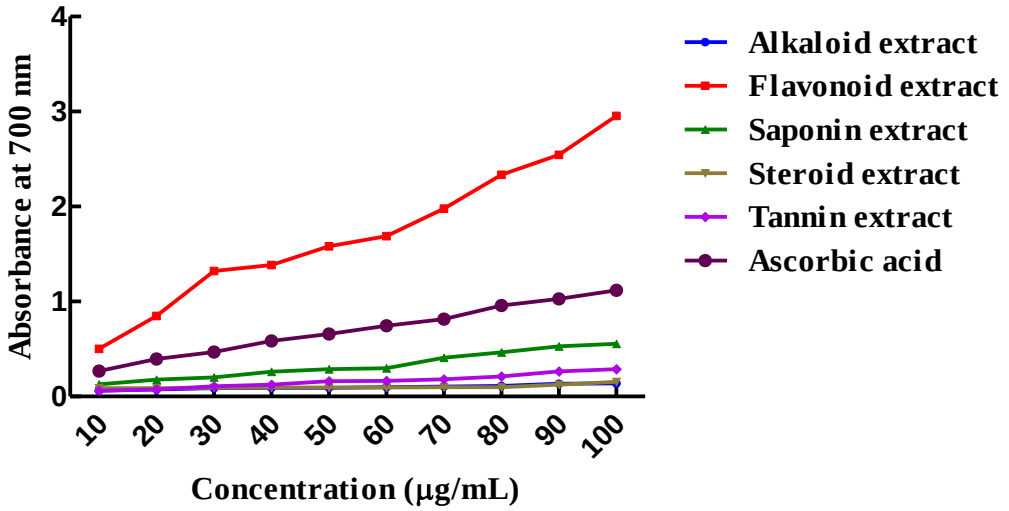


Figure 4.51: Ferric reducing antioxidant power of various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth.

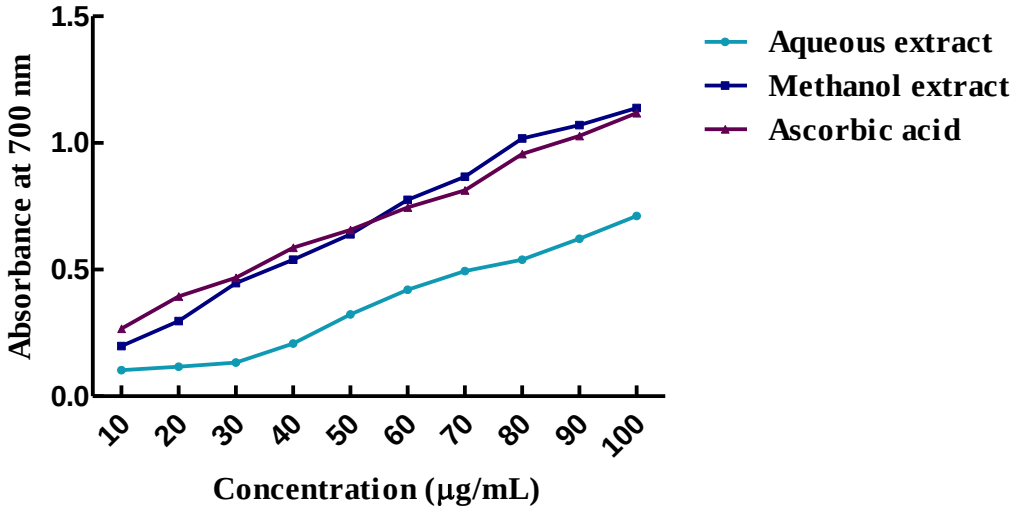


Figure 4.52: Ferric reducing antioxidant power of general extracts (aqueous and methanol) of *Cyphomandra betacea* (Cav.) Sendth.

4.11.2.2. Ferric reducing antioxidant power of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

The ferric reducing ability of the *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are shown in Table 4.58, Figure 4.53 and Figure 4.54. Saponin extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited significantly ($p<0.05$) higher ferric reducing power than the other extracts (Figure 4.53). In the case of general extracts, the methanol extract exhibited significantly ($p<0.05$) higher ferric reducing power than the aqueous extract at higher concentrations of 70 µg/mL to 100 µg/mL. However, at lower concentrations, the aqueous extract showed higher ferric reducing ability (Figure 4.54).

Table 4.58: Ferric reducing antioxidant power of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Concentration (µg/mL)	Absorbance at 700 nm							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	0.1136±0.0003	0.0915±0.0002	0.1174±0.0014	0.0847±0.0001	0.0685±0.0002	0.1233±0.0004 $^{\delta}$	0.0382±0.0001	0.2639±0.0018
20	0.1218±0.0004	0.1161±0.0009	0.1438±0.0019*	0.0862±0.0006	0.0758±0.0050	0.1255±0.0006 $^{\delta}$	0.0528±0.0012	0.3910±0.0007
30	0.1557±0.0005	0.1301±0.0006	0.2179±0.0012*	0.0890±0.0004	0.0943±0.0017	0.1378±0.0006 $^{\delta}$	0.0691±0.0014	0.4627±0.0015
40	0.1636±0.0006	0.1970±0.0004	0.3115±0.0019*	0.0913±0.0008	0.1073±0.0024	0.1537±0.0004$^{\delta}$	0.1198±0.0009	0.5843±0.0008
50	0.1738±0.0006	0.1946±0.0045	0.3611±0.0076*	0.0950±0.0002	0.1357±0.0035	0.1612±0.0006	0.1528±0.0027	0.6426±0.0007
60	0.1978±0.0004	0.2095±0.0007	0.3718±0.0016*	0.0977±0.0003	0.1676±0.0016	0.1723±0.0004	0.1660±0.0018	0.7437±0.0016
70	0.2324±0.0142	0.2773±0.0008	0.4099±0.0052*	0.1022±0.0010	0.1724±0.0013	0.1953±0.0009	0.2234±0.0043$^{\#}$	0.8065±0.0009
80	0.2589±0.0006	0.3053±0.0012	0.4459±0.0036*	0.1115±0.0010	0.2134±0.0011	0.2293±0.0010	0.2533±0.0027$^{\#}$	0.9523±0.0012
90	0.3114±0.0014	0.3086±0.0009	0.4660±0.0008*	0.1229±0.0016	0.2281±0.0011	0.2422±0.0003	0.2859±0.0010$^{\#}$	1.0255±0.0012
100	0.3361±0.0002	0.3363±0.0012	0.5479±0.0038*	0.1264±0.0004	0.2742±0.0012	0.2552±0.0008	0.3385±0.0012$^{\#}$	1.1145±0.0016

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in ferric reducing antioxidant power than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in ferric reducing antioxidant power than the aqueous extract.$^{\delta}$ ($p<0.05$) significantly different in ferric reducing antioxidant power than the methanol extract.

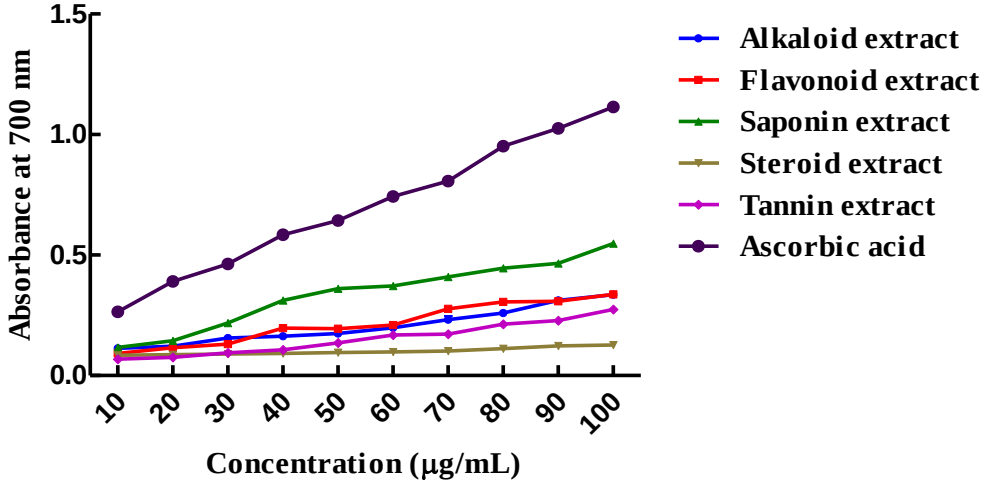


Figure 4.53: Ferric reducing antioxidant power of various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

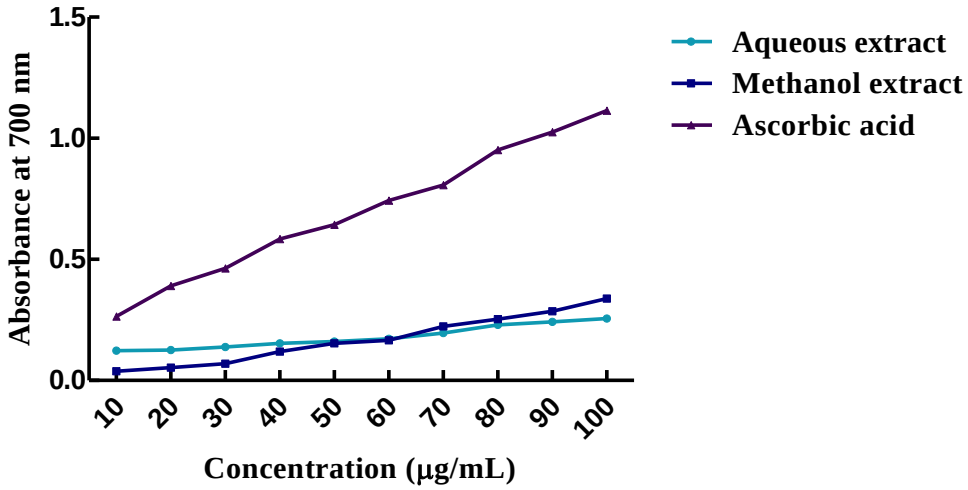


Figure 4.54: Ferric reducing antioxidant power of general extracts (aqueous and methanol) of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

4.11.2.3. Ferric reducing antioxidant power of various extracts of *Dicentra scandens* (D. Don) Walp.

The ferric reducing antioxidant power of the various extracts of *Dicentra scandens* (D. Don) Walp. are shown in Table 4.59, Figure 4.55 and Figure 4.56. Among the phytochemical extracts the saponin extract of *Dicentra scandens* (D. Don) Walp. exhibited significantly ($p<0.05$) higher ferric reducing power than the other extracts (Figure 4.55).

Similarly, the methanol extract exhibited significantly ($p<0.05$) higher ferric reducing antioxidant power than the aqueous extract (Figure 4.56).

Table 4.59: Ferric reducing antioxidant power of various extracts of *Dicentra scandens* (D. Don) Walp.

Concentration (µg/mL)	Absorbance at 700 nm							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	0.0664±0.0002	0.0523±0.0004	0.1021±0.0001	0.0331±0.0003	0.0300±0.0002	0.0395±0.0002	0.1271±0.0006$^{\#}$	0.2645±0.0009
20	0.1044±0.0005	0.0729±0.0005	0.1190±0.0018	0.0382±0.0003	0.0322±0.0002	0.0520±0.0007	0.1858±0.0001$^{\#}$	0.3973±0.0014
30	0.1057±0.0003	0.0892±0.0003	0.1413±0.0002	0.0416±0.0002	0.0341±0.0001	0.0538±0.0003	0.2245±0.0005$^{\#}$	0.4697±0.0024
40	0.1621±0.0001	0.0938±0.0004	0.1699±0.0003	0.0431±0.0016	0.0386±0.0002	0.0557±0.0005	0.2904±0.0005$^{\#}$	0.5837±0.0022
50	0.1741±0.0003	0.1214±0.0004	0.2046±0.0005	0.0479±0.0006	0.0434±0.0006	0.0572±0.0004	0.3471±0.0010$^{\#}$	0.6557±0.0016
60	0.1805±0.0005	0.1349±0.0004	0.2848±0.0011*	0.0504±0.0001	0.0482±0.0054	0.0622±0.0009	0.3889±0.0005$^{\#}$	0.7443±0.0009
70	0.2052±0.0010	0.1523±0.0008	0.3611±0.0004*	0.0511±0.0002	0.0546±0.0002	0.0634±0.0003	0.4646±0.0002$^{\#}$	0.8178±0.0020
80	0.2061±0.0003	0.1761±0.0001	0.4327±0.0016*	0.0521±0.0002	0.0621±0.0002	0.0655±0.0002	0.5480±0.0004$^{\#}$	0.9529±0.0015
90	0.2508±0.0002	0.1941±0.0044	0.5016±0.0004*	0.06100±0.0003	0.0767±0.0007	0.0667±0.0010	0.5781±0.0002$^{\#}$	1.0251±0.0021
100	0.2785±0.00053	0.2006±0.0007	0.5323±0.0016*	0.0735±0.0010	0.0873±0.0010	0.0917±0.0004	0.6225±0.0016$^{\#}$	1.1159±0.0069

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in ferric reducing antioxidant power than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in ferric reducing antioxidant power than the aqueous extract.

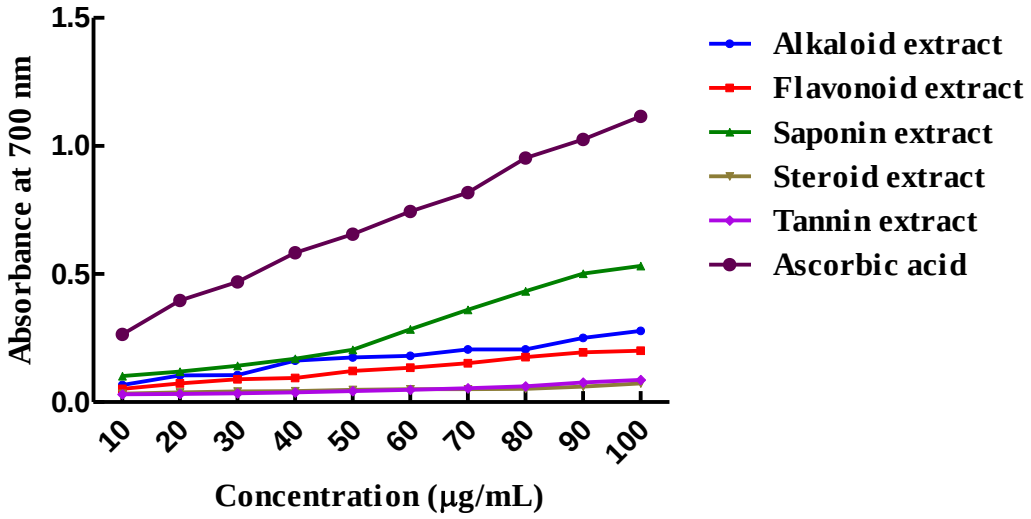


Figure 4.55: Ferric reducing antioxidant power of various phytochemical extracts of *Dicentra scandens* (D. Don) Walp.

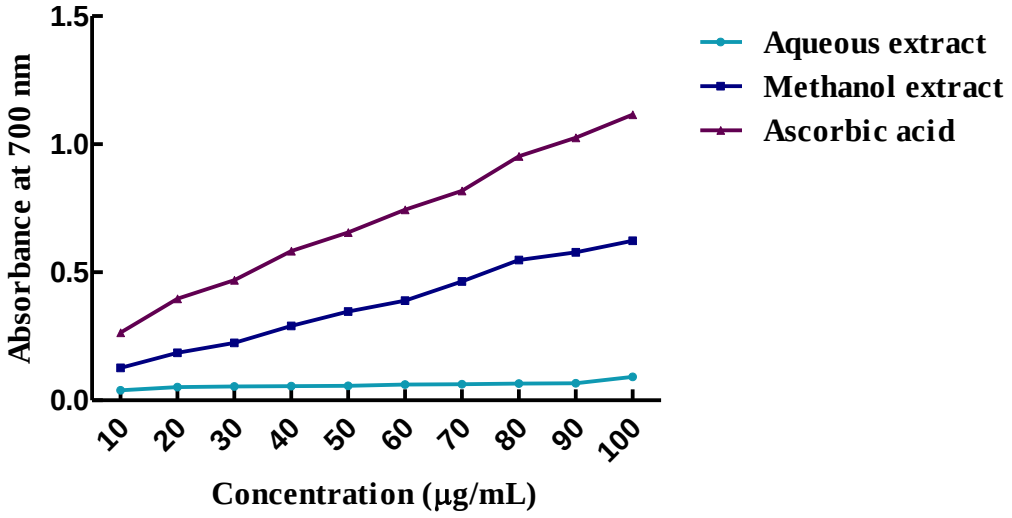


Figure 4.56: Ferric reducing antioxidant power of general extracts (aqueous and methanol) of *Dicentra scandens* (D. Don) Walp.

4.11.2.4. Ferric reducing antioxidant power of various extracts of *Heracleum nepalense* D. Don

The ferric reducing ability of the various extracts of *Heracleum nepalense* D. Don are shown in Table 4.60, Figure 4.57 and Figure 4.58. Tannin extract of *Heracleum nepalense* D. Don exhibited significantly ($p<0.05$) higher ferric reducing power than the other phytochemical extracts (Figure 4.57).

Among the general extracts, the aqueous extract exhibited significantly ($p<0.05$) higher ferric reducing power than the methanol extract (Figure 4.58).

Table 4.60: Ferric reducing antioxidant power of various extracts of *Heracleum nepalense* D. Don

Concentration (µg/mL)	Absorbance at 700 nm							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	0.0357±0.0003	0.0612±0.0001	0.0493±0.0004	0.0778±0.0016	0.0879±0.0007	0.0966±0.0003 $^{\delta}$	0.0482±0.0003	0.2690±0.0008
20	0.0438±0.0001	0.0633±0.0003	0.0499±0.0007	0.0870±0.0006	0.1050±0.0017*	0.1196±0.0003 $^{\delta}$	0.0566±0.0004	0.3948±0.0005
30	0.0521±0.0005	0.0690±0.0006	0.0497±0.0059	0.0912±0.0007	0.1140±0.0007*	0.1366±0.0008 $^{\delta}$	0.0580±0.0002	0.4713±0.0009
40	0.0545±0.0003	0.0729±0.0001	0.0541±0.0002	0.0951±0.0018	0.1471±0.0006*	0.1541±0.0038 $^{\delta}$	0.0644±0.0002	0.5854±0.0011
50	0.0615±0.0003	0.0771±0.0019	0.0617±0.0010	0.0979±0.0012	0.1953±0.0001*	0.1638±0.0008 $^{\delta}$	0.0702±0.0002	0.6570±0.0059
60	0.0705±0.0002	0.0811±0.0008	0.0754±0.0004	0.1071±0.0003	0.2211±0.0003*	0.1813±0.0018 $^{\delta}$	0.0719±0.0002	0.7429±0.0015
70	0.0746±0.0001	0.0838±0.0003	0.0870±0.0039	0.1145±0.0001	0.2530±0.0011*	0.1921±0.0007 $^{\delta}$	0.0743±0.0010	0.8154±0.0011
80	0.0760±0.0004	0.0986±0.0012	0.0904±0.0001	0.1143±0.0024	0.2660±0.0004*	0.2169±0.0005 $^{\delta}$	0.0869±0.0044	0.9518±0.0015
90	0.0874±0.0006	0.1076±0.0008	0.0931±0.0006	0.1254±0.0012	0.2875±0.0004*	0.2380±0.0010 $^{\delta}$	0.0978±0.0007	1.0240±0.0016
100	0.0941±0.0001	0.1104±0.0013	0.0996±0.0003	0.1301±0.0004	0.3027±0.0010*	0.2546±0.0010 $^{\delta}$	0.1026±0.0003	1.1197±0.0022

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in ferric reducing antioxidant power than the other phytochemical extracts. $^{\delta}$ ($p<0.05$) significantly different in ferric reducing antioxidant power than the methanol extract.

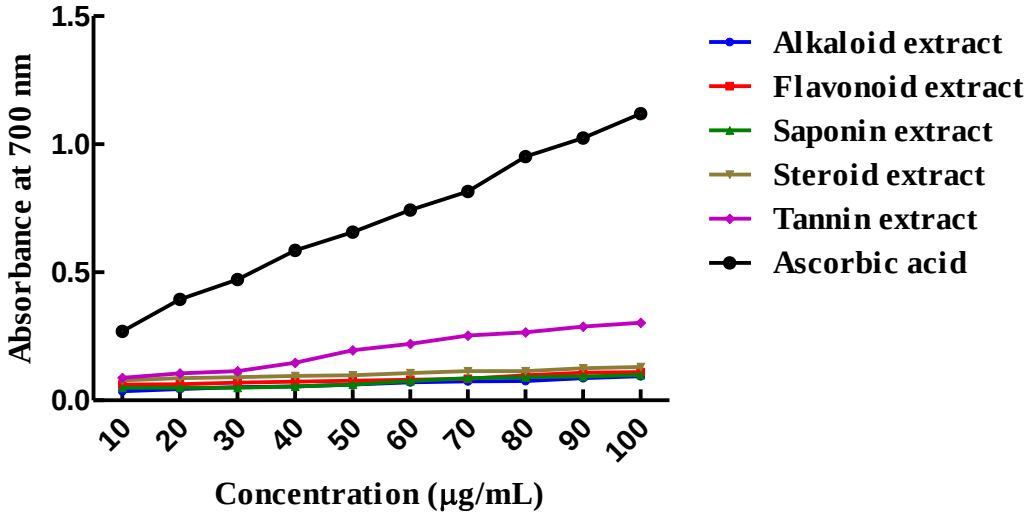


Figure 4.57: Ferric reducing antioxidant power of various phytochemical extracts of *Heracleum nepalense* D. Don

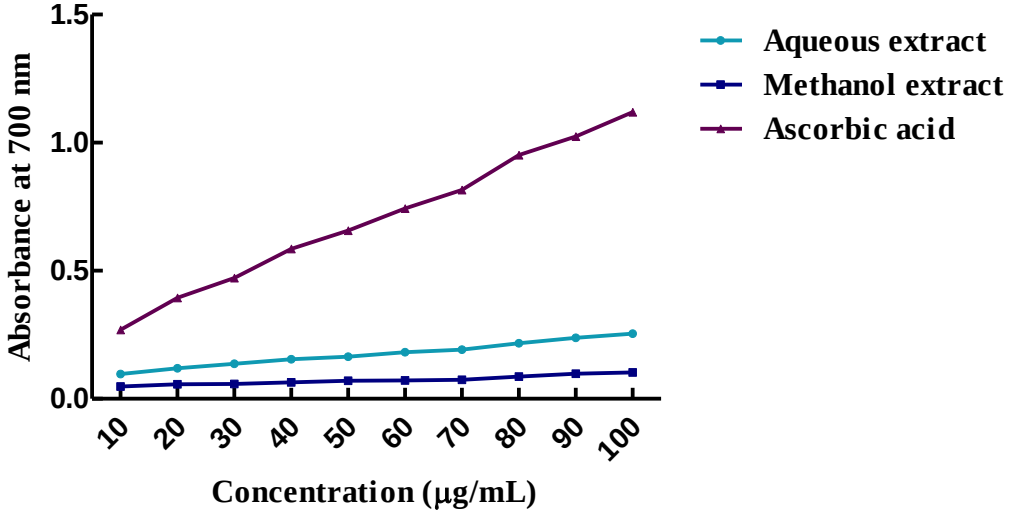


Figure 4.58: Ferric reducing antioxidant power of general extracts (aqueous and methanol) of *Heracleum nepalense* D. Don

4.11.3. Hydrogen peroxide scavenging activity

4.11.3.1 Hydrogen peroxide scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

The hydrogen peroxide scavenging activity of the various extracts of *Cyphomandra betacea* (Cav.) Sendth. are shown in Table 4.61, Figure 4.59 and Figure 4.60. The hydrogen peroxide scavenging activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity followed by the alkaloid extract (Figure 4.59). At the concentration of 100 µg/mL, the highest scavenging activity was exhibited by the flavonoid extract with the percentage inhibition of 82.20±0.40, while at the same concentration, the hydrogen peroxide scavenging activity of ascorbic acid was 84.03±0.78 %. Among the extracts, the lowest IC_{50} value of 30 µg/mL was exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. with percentage inhibition of 50.69±0.10. The IC_{50} value of 10 µg/mL was exhibited by ascorbic acid with the percentage inhibition of 50.75±0.81 (Table 4.61).

Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity than the aqueous extract (Figure 4.60).

Table 4.61: Hydrogen peroxide scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	33.44±0.33	34.47±0.71	10.62±0.23	5.88±0.44	25.41±0.56	7.55±0.98	25.41±0.56 #	50.75±0.81
20	40.48±0.96	43.50±0.57*	13.4±0.14	12.92±0.15	34.38±1.68	10.93±0.91	34.22±0.89 #	54.16±0.54
30	44.07±0.72	50.69±0.10*	18.4±0.17	17.29±0.81	37.34±0.97	13.62±0.79	40.64±0.83 #	59.91±0.23
40	47.80±0.71	54.20±0.971*	33.33±0.53	20.08±0.03	40.19±1.09	23.41±0.55	44.09±0.48 #	67.45±0.59
50	50.70±0.73	60.95±0.26*	39.05±0.77	23.43±0.23	45.87±0.45	32.65±0.82	50.85±0.48 #	72.51±0.55
60	57.67±0.55	69.04±0.22*	47.07±0.92	33.33±0.14	50.47±0.64	46.74±0.89	55.09±0.96 #	73.12±0.12
70	68.80±0.37	71.79±0.13*	51.11±0.91	43.54±0.12	57.62±0.01	50.85±0.71	62.79±0.50 #	75.67±0.49
80	73.20±0.15	76.13±0.25*	58.30±0.16	50.86±0.88	68.24±1.55	54.64±0.12	69.92±0.51 #	79.98±0.20
90	76.87±0.62	78.21±0.50*	71.20±0.14	52.82±0.20	70.73±0.08	64.60±1.20	75.71±0.01 #	81.09±0.65
100	80.13±1.64	82.20±0.40*	77.54±0.22	58.12±0.92	76.34±0.36	72.09±0.83	81.40±0.57 #	84.03±0.78

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in hydrogen peroxide scavenging activity than the other phytochemical extracts. #($p<0.05$) significantly different in hydrogen peroxide scavenging activity than the aqueous extract.

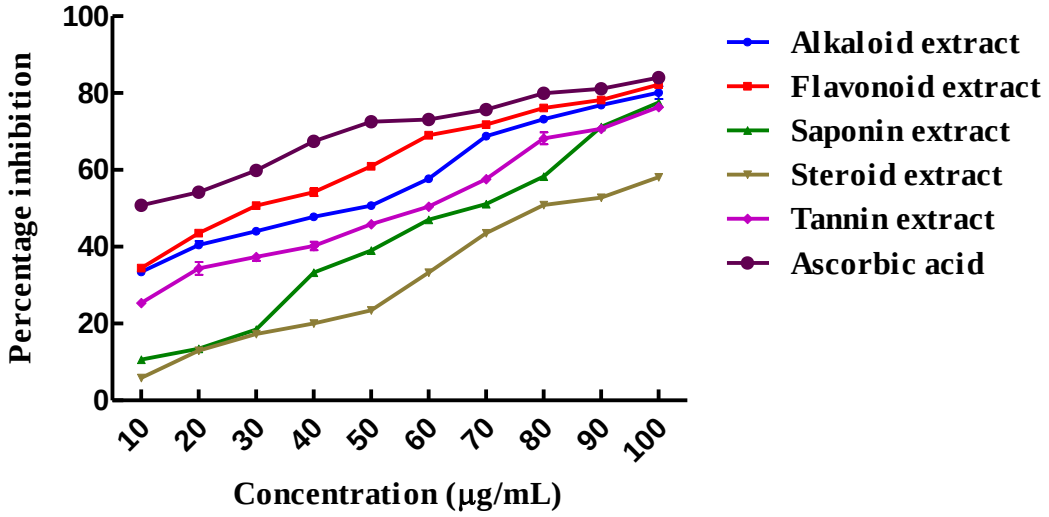


Figure 4.59: Hydrogen peroxide scavenging activity of various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth.

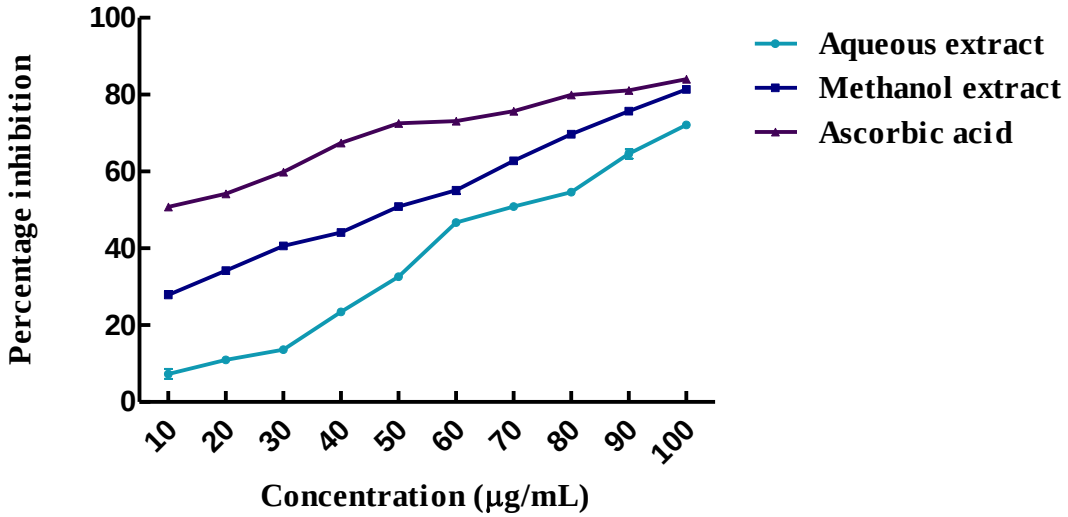


Figure 4.60: Hydrogen peroxide scavenging activity of general extracts (aqueous and methanol) of *Cyphomandra betacea* (Cav.) Sendth.

4.11.3.2. Hydrogen peroxide scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

The hydrogen peroxide scavenging activity of the various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are shown in Table 4.62, Figure 4.61 and Figure 4.62. The hydrogen peroxide scavenging activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity (Figure 4.61). At the concentration of 100 µg/mL, the highest scavenging activity in terms of percentage inhibition of hydrogen peroxide was exhibited by the flavonoid extract with the percentage inhibition of 80.49±1.13. Among the extracts, the lowest IC_{50} value of 40 µg/mL was exhibited by the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish with percentage inhibition of 50.79±0.75. The IC_{50} value of 10 µg/mL was exhibited by ascorbic acid with the percentage inhibition of 50.77±0.82 (Table 4.62).

Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity than the aqueous extract (Figure 4.62).

Table 4.62: Hydrogen peroxide scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	13.06±0.50	32.07±0.38*	13.47±0.30	15.32±0.57	10.75±0.08	14.49±0.51	24.21±0.84$^{\#}$	50.77±0.82
20	18.40±0.30	39.08±1.27*	15.49±0.44	17.42±1.054	13.43±0.92	19.38±0.76	30.38±0.18	54.51±0.96
30	22.97±0.73	45.29±0.55*	20.83±0.88	20.23±0.35	14.73±0.70	25.14±0.30	34.24±0.42$^{\#}$	59.63±0.50
40	29.28±0.91	50.79±0.75*	26.76±1.01	22.72±0.50	21.95±0.28	34.20±0.66	43.39±0.57	67.20±0.54
50	32.99±0.51	53.75±0.67*	34.05±0.91	31.83±0.43	25.52±0.33	47.14±0.38	50.64±0.08$^{\#}$	72.10±0.63
60	37.01±0.90	56.52±0.52*	39.64±0.69	33.56±0.58	30.85±0.97	51.83±0.03	54.42±1.01$^{\#}$	73.82±0.64
70	38.11±0.42	63.50±0.33*	44.00±0.81	40.12±1.02	37.27±0.94	58.04±0.79	61.56±0.18$^{\#}$	75.30±0.95
80	42.66±0.71	70.91±0.22*	50.45±1.07	50.45±0.89	42.66±0.71	61.23±0.91	68.99±0.10$^{\#}$	79.93±0.29
90	50.77±0.78	74.71±0.62*	55.05±0.88	55.05±0.78	50.87±0.51	67.34±0.06	71.81±0.64$^{\#}$	82.26±0.47
100	57.86±0.33	80.49±1.13*	61.34±0.39	59.25±0.18	53.14±0.95	70.18±0.77	77.83±0.71$^{\#}$	84.47±0.90

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in hydrogen peroxide scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in hydrogen peroxide scavenging activity than the aqueous extract.

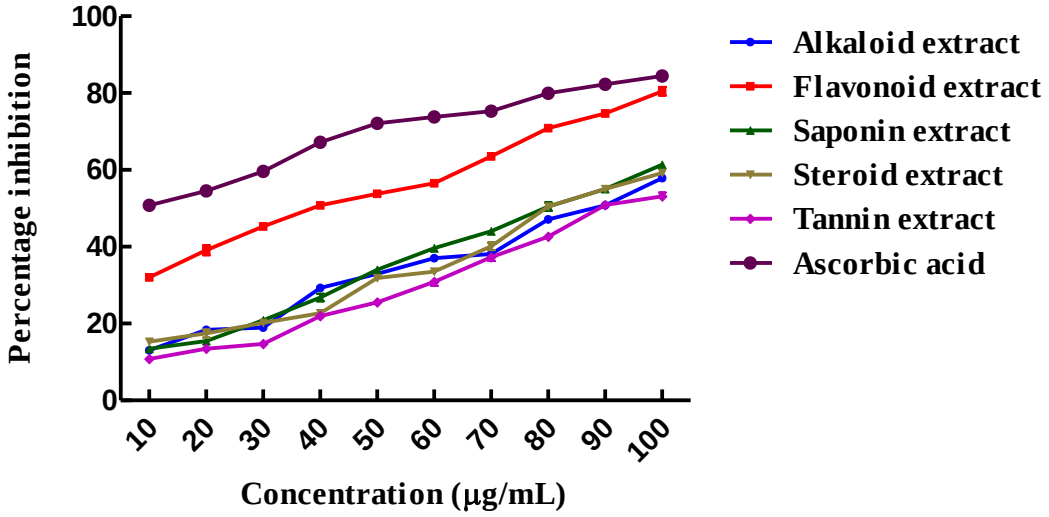


Figure 4.61: Hydrogen peroxide scavenging activity of various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

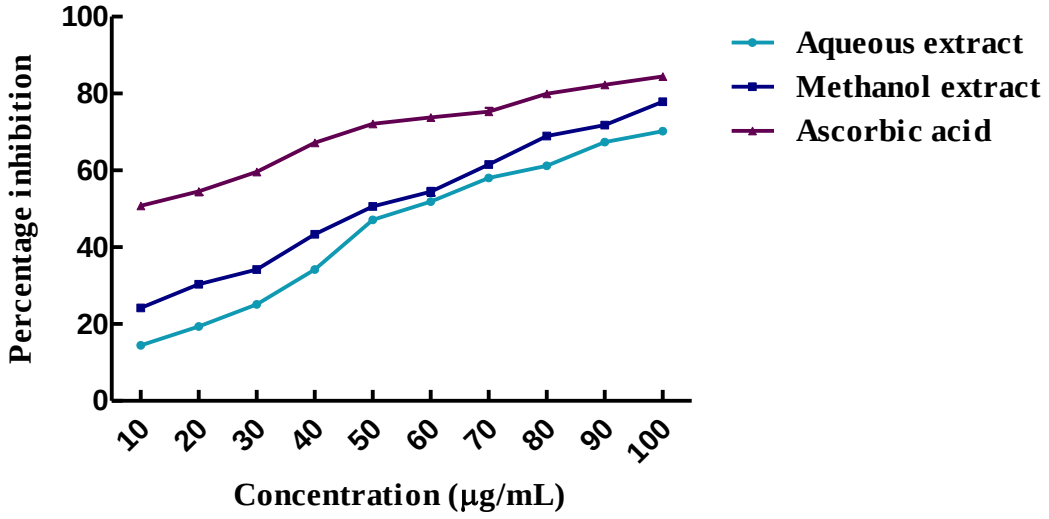


Figure 4.62: Hydrogen peroxide scavenging activity of general extracts (aqueous and methanol) of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

4.11.3.3. Hydrogen peroxide scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp.

The hydrogen peroxide scavenging activity of the various extracts of *Dicentra scandens* (D. Don) Walp. are shown in Table 4.63, Figure 4.63 and Figure 4.64. The hydrogen peroxide scavenging activity of the extracts was compared with ascorbic acid as standard. The tannin extract followed by the steroid extract of *Dicentra scandens* (D. Don) Walp. exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity than the other phytochemical extracts (Figure 4.63). However, among all the extracts, the highest scavenging activity was exhibited by the aqueous extract with the percentage inhibition of 78.12±0.81. The lowest IC_{50} value of 30 µg/mL was exhibited by the aqueous extract of *Dicentra scandens* (D. Don) Walp. with the percentage inhibition of 51.74±0.58. The IC_{50} value of 10 µg/mL was exhibited by ascorbic acid with the percentage inhibition of 50.61±0.55 (Table 4.63). The aqueous extract of *Dicentra scandens* (D. Don) Walp. exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity than the methanol extract (Figure 4.64).

Table 4.63: Hydrogen peroxide scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp.

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	7.34±0.91	8.89±0.169	15.47±0.49	23.86±0.16	33.13±0.20*	42.77±1.36 $^{\delta}$	16.76±0.57	50.61±0.55
20	9.61±0.11	9.35±0.05	17.28±0.15	30.83±1.39	34.51±0.96*	48.16±1.06 $^{\delta}$	18.44±1.03	54.38±0.81
30	11.44±0.15	10.03±0.17	18.96±1.12	33.27±0.79	35.46±0.06*	51.74±0.58 $^{\delta}$	23.23±1.18	59.74±0.79
40	11.73±0.26	14.08±0.30	19.96±0.15	37.49±1.00	39.01±0.08	59.15±0.21 $^{\delta}$	27.55±0.84	67.53±1.06
50	16.08±0.06	15.91±0.20	25.48±0.26	43.76±1.17	44.80±0.35	61.33±0.35 $^{\delta}$	34.66±1.22	71.19±0.97
60	17.13±0.50	18.68±0.65	28.47±0.37	47.99±1.12	46.84±0.92	65.29±0.57 $^{\delta}$	37.80±0.69	73.84±0.61
70	20.93±0.42	21.01±0.23	38.31±0.10	50.79±0.77	50.76±0.51	68.01±0.07 $^{\delta}$	41.27±1.11	75.35±0.53
80	28.85±0.17	31.99±0.08	46.28±0.40	53.45±0.57	57.99±0.87*	68.52±0.30 $^{\delta}$	48.55±0.60	79.50±0.79
90	41.43±0.19	45.74±0.20	50.48±0.45	60.84±0.91	61.56±0.52	76.15±0.19 $^{\delta}$	50.95±0.66	81.44±0.74
100	50.15±1.30	50.23±0.85	52.42±0.61	66.70±0.53	68.51±0.14*	78.12±0.81 $^{\delta}$	53.19±0.71	84.55±0.06

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in hydrogen peroxide scavenging activity than the other phytochemical extracts. $^{\delta}$($p<0.05$) significantly different in hydrogen peroxide scavenging activity than the methanol extract.

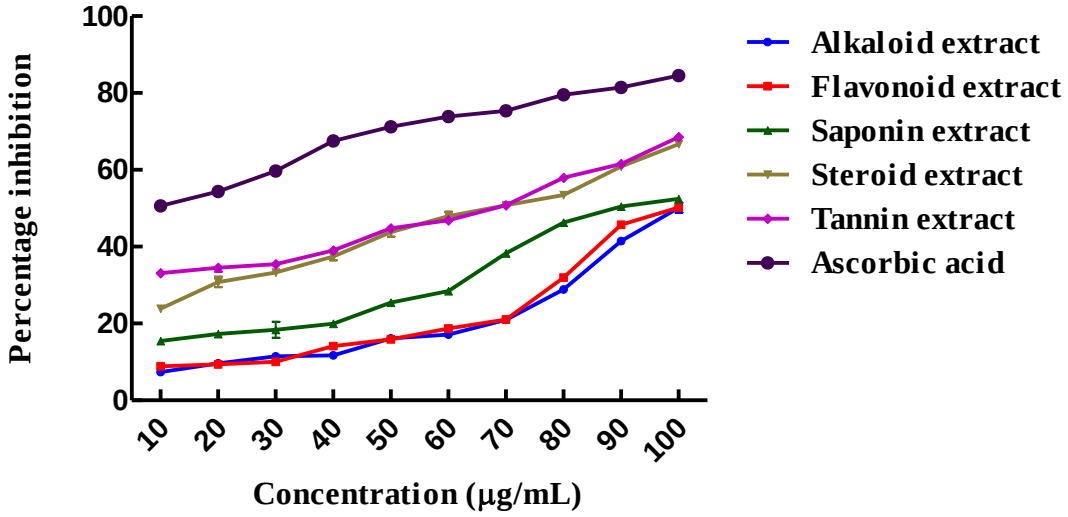


Figure 4.63: Hydrogen peroxide scavenging activity of various phytochemical extracts of *Dicentra scandens* (D. Don) Walp.

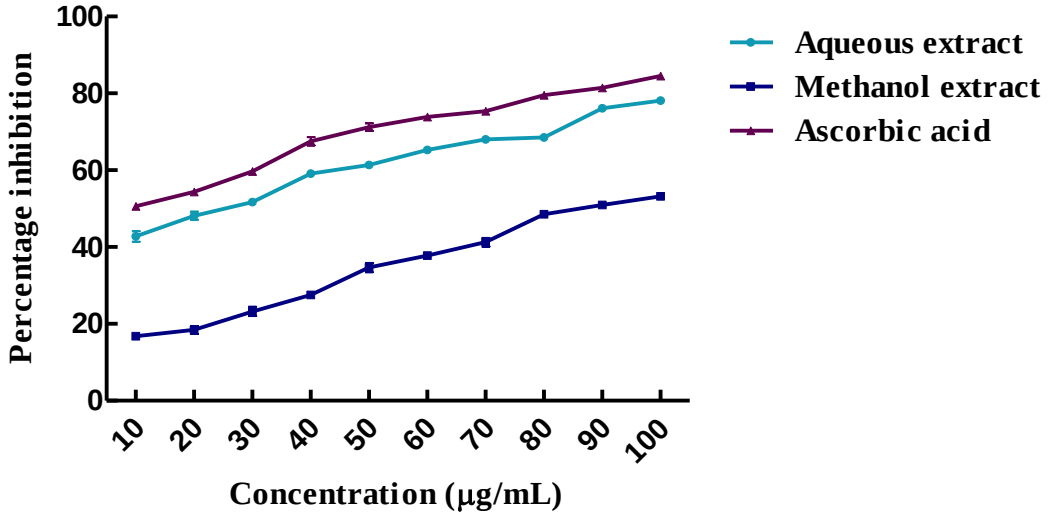


Figure 4.64: Hydrogen peroxide scavenging activity of general (aqueous and methanol) extracts of *Dicentra scandens* (D. Don) Walp.

4.11.3.4. Hydrogen peroxide scavenging activity of various extracts of *Heracleum nepalense* D. Don

The hydrogen peroxide scavenging activity of the various extracts of *Heracleum nepalense* D. Don are shown in Table 4.64, Figure 4.65 and Figure 4.66. The Hydrogen peroxide scavenging activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the flavonoid extract followed by the alkaloid extract of *Heracleum nepalense* D. Don exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity than the other phytochemical extracts (Figure 4.65). At the concentration of 100 µg/mL, the highest scavenging activity was exhibited by the flavonoid extract with the percentage inhibition of 81.98±0.27. Among the extracts, the lowest IC_{50} value (10 µg/mL) was achieved by the flavonoid extract of *Heracleum nepalense* D. Don which was similar to that of ascorbic acid (Table 4.64).

Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity than the aqueous extract (Figure 4.66).

Table 4.64: Hydrogen peroxide scavenging activity of various extracts of *Heracleum nepalense* D. Don

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	27.04±0.53	50.16±0.37*	12.20±0.88	5.48±0.324	9.02±0.13	6.66±0.10	24.78±0.57$^{\#}$	50.92±0.44
20	37.69±0.88	52.28±0.87*	15.14±1.56	7.54±0.51	14.68±0.53	11.70±0.30	32.09±0.44$^{\#}$	54.50±0.50
30	38.69±0.54	55.68±0.85*	18.96±0.22	10.61±0.39	18.31±0.92	14.08±0.32	35.42±0.67$^{\#}$	59.16±0.96
40	43.41±0.33	59.83±0.33*	27.51±0.51	12.88±0.88	27.20±0.43	22.97±0.29	38.52±0.88$^{\#}$	67.15±0.28
50	50.88±0.30	64.31±0.36*	35.28±0.92	22.67±0.43	30.75±0.94	24.23±0.18	41.09±1.16$^{\#}$	72.44±0.62
60	55.32±0.22	68.68±0.70*	39.24±0.95	36.94±0.44	33.04±0.31	28.16±0.47	43.20±0.28$^{\#}$	74.13±0.63
70	61.49±0.46	71.49±0.22*	50.01±0.77	46.12±0.08	39.64±0.98	35.04±0.96	50.15±0.50$^{\#}$	75.39±0.61
80	61.94±0.50	76.19±0.68*	53.70±0.18	50.95±0.60	47.60±1.11	40.82±0.47	54.50±1.02$^{\#}$	79.50±0.82
90	64.51±0.42	79.51±0.41*	57.97±0.89	53.07±0.41	50.82±0.80	47.04±0.18	60.53±0.08$^{\#}$	81.80±0.84
100	70.91±0.30	81.98±0.27*	63.20±0.68	58.83±0.56	57.54±0.23	51.04±0.15	65.30±0.31$^{\#}$	84.76±0.74

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in hydrogen peroxide radical scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in hydrogen peroxide radical scavenging activity than the aqueous extract.

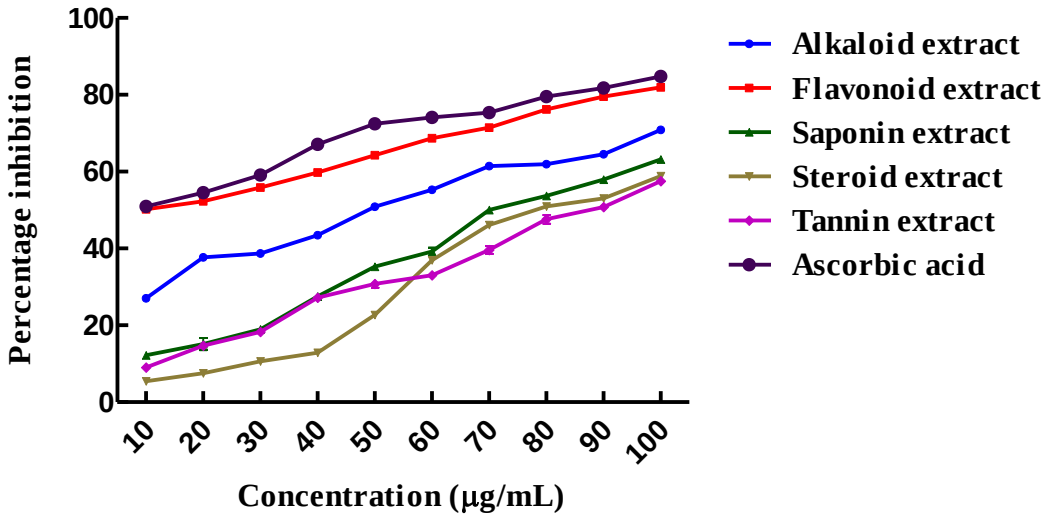


Figure 4.65: Hydrogen peroxide scavenging activity of various phytochemical extracts of *Heracleum nepalense* D. Don

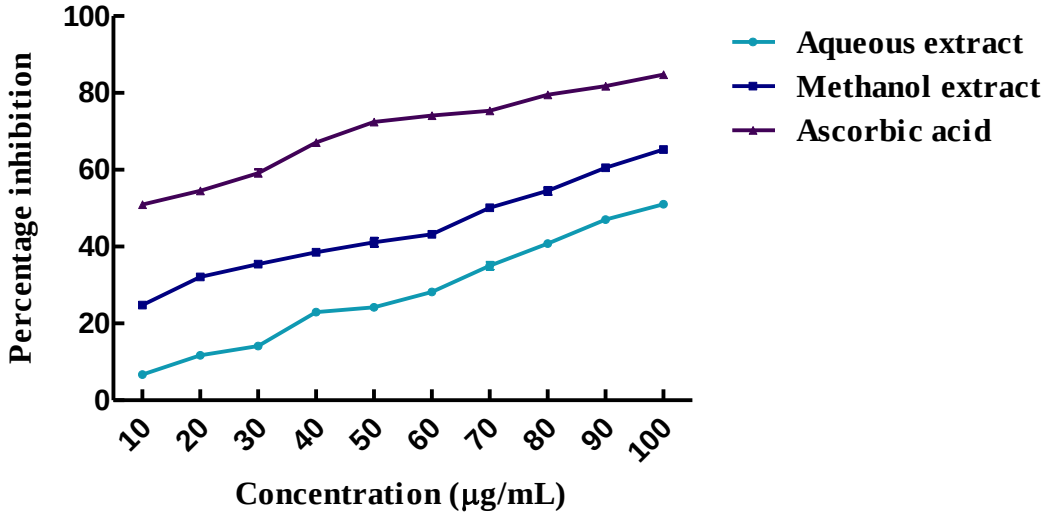


Figure 4.66: Hydrogen peroxide scavenging activity of general extracts (aqueous and methanol) of *Heracleum nepalense* D. Don

4.11.4. Nitric oxide radical scavenging activity

4.11.4.1. Nitric oxide radical scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

The nitric oxide radical scavenging activity of the various extracts of *Cyphomandra betacea* (Cav.) Sendth. are shown in Table 4.65, Figure 4.67 and Figure 4.68. The nitric oxide radical scavenging activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity (Figure 4.67). At the concentration of 100 µg/mL, the highest scavenging activity was exhibited by the flavonoid extract with the percentage inhibition of 65.86±0.67. The lowest IC_{50} value of 40 µg/mL was exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth.with the percentage inhibition of 50.21±0.20. The IC_{50} value of 20 µg/mL was exhibited by ascorbic acid with the percentage inhibition of 50.90±0.82 (Table 4.65).

Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity than the aqueous extract (Figure 4.68).

Table 4.65: Nitric oxide radical scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	24.80±0.33	43.00±0.85*	13.68±0.30	20.67±0.19	11.49±0.52	14.60±0.69	28.06±0.06$^{\#}$	48.78±0.16
20	32.30±1.05	45.54±0.52*	19.02±0.43	28.13±1.411	27.59±0.85	22.01±0.21	34.65±1.24$^{\#}$	50.90±0.82
30	36.45±1.09	47.19±0.48*	27.90±0.47	35.07±0.56	30.18±0.27	31.01±1.46	36.92±0.13$^{\#}$	54.27±0.03
40	42.48±0.73	50.21±0.20*	32.48±1.17	38.37±0.20	37.62±0.13	36.50±0.06	42.04±1.01$^{\#}$	58.33±0.81
50	44.74±0.48	53.47±0.13*	36.21±1.23	41.90±1.03	38.28±0.20	41.61±1.34	45.90±0.10$^{\#}$	61.15±0.17
60	46.52±0.40	55.70±0.07*	41.12±0.87	44.05±0.77	41.10±0.38	45.38±0.54	49.29±0.63$^{\#}$	63.25±0.95
70	48.64±1.27	57.33±0.30*	44.36±0.20	46.99±0.89	43.29±0.27	49.51±0.95	50.90±0.06	66.51±0.81
80	50.25±0.87	59.34±0.50*	50.23±0.17	48.62±0.58	50.39±0.77	50.90±1.04	53.51±0.94$^{\#}$	70.93±1.34
90	52.02±0.10	61.79±1.39*	52.22±0.33	50.97±1.31	52.57±1.01	51.68±1.28	57.04±0.61$^{\#}$	73.47±0.58
100	54.05±0.50	65.86±0.67*	54.38±0.06	56.30±0.64	56.70±1.54	54.29±0.10	59.38±1.08$^{\#}$	77.33±0.89

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in nitric oxide radical scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in nitric oxide radical scavenging activity than the aqueous extract.

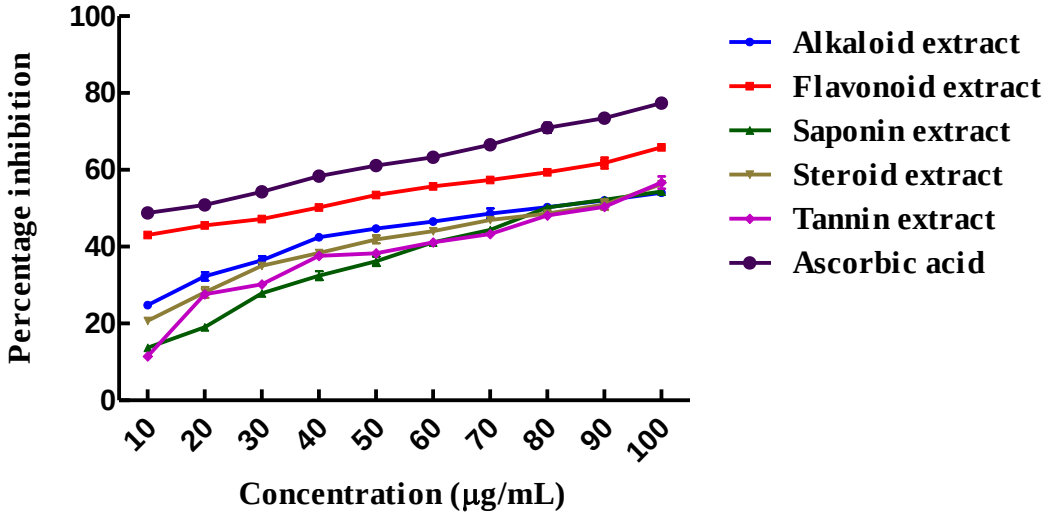


Figure 4.67: Nitric oxide radical scavenging activity of various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth.

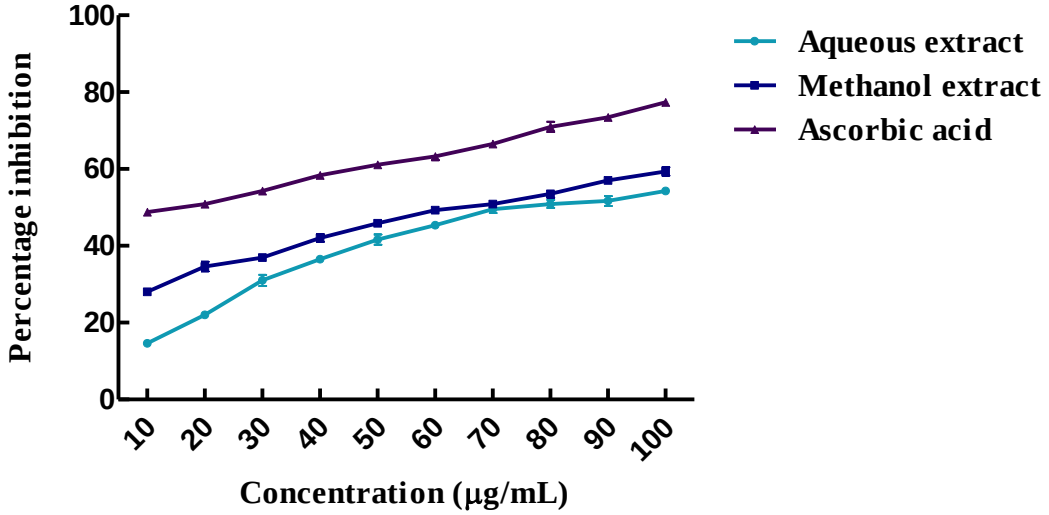


Figure 4.68: Nitric oxide radical scavenging activity of general extracts (aqueous and methanol) of *Cyphomandra betacea* (Cav.) Sendth.

4.11.4.2. Nitric oxide radical scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

The nitric oxide radical scavenging activity of the various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are shown in Table 4.66, Figure 4.69 and Figure 4.70. The nitric oxide scavenging activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the flavonoid extract followed by the tannin extract exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity than the other phytochemical extracts (Figure 4.69). However, among all the extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish the methanol extract exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity. At the concentration of 100 µg/mL the methanol extract showed highest nitric oxide radical scavenging activity with the percentage inhibition of 62.65±0.39. The lowest IC_{50} value of 60 µg/mL was also exhibited by the methanol extract with the percentage inhibition of 51.78±0.15. The IC_{50} value of 20 µg/mL was exhibited by ascorbic acid with the percentage inhibition of 50.21±0.87 (Table 4.66). The methanol extract also exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity than the aqueous extract (Figure 4.70).

Table 4.66: Nitric oxide radical scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	8.68±0.17	25.89±0.19*	15.89±0.66	8.79±0.19	18.53±1.84	15.09±0.71	32.82±0.30#	48.11±0.75
20	11.48±0.67	28.49±1.00*	19.74±0.89	9.50±1.27	24.05±0.48	20.37±0.85	37.99±0.18#	50.21±0.87
30	17.89±0.92	35.18±0.19*	24.64±0.17	11.35±0.55	26.88±0.72	25.44±0.12	41.18±0.10#	53.56±0.45
40	29.09±0.38	38.64±0.22*	27.11±1.37	17.79±0.56	30.14±0.37	28.60±0.17	46.18±0.29#	58.31±0.47
50	32.04±0.29	42.96±0.38*	32.90±0.82	20.75±0.22	33.25±0.15	34.59±0.21	47.62±0.10#	61.30±0.97
60	35.57±0.74	44.25±0.90*	38.61±1.52	26.02±0.15	38.43±0.96	35.18±0.22	51.78±0.15#	63.47±1.00
70	38.10±0.24	47.10±0.67*	43.16±0.14	28.19±0.25	42.37±0.84	40.75±0.80	54.84±0.25#	66.01±0.80
80	43.50±0.84	50.04±0.46	46.12±0.29	39.81±0.27	50.29±0.54	42.21±0.66	56.37±0.12#	70.28±0.65
90	50.16±0.46	52.58±0.14	50.16±0.27	41.59±0.61	54.17±0.12*	45.04±0.62	59.45±0.84#	73.18±0.13
100	52.66±0.38	57.88±1.10	52.46±0.15	50.09±0.35	59.14±0.31*	50.03±1.00	62.65±0.39#	77.18±0.87

Each value represents mean±SD. All the experiments were performed in triplicate.*($p<0.05$) significantly different in nitric oxide radical scavenging activity than the other phytochemical extracts. #($p<0.05$) significantly different in nitric oxide radical scavenging activity than the aqueous extract.

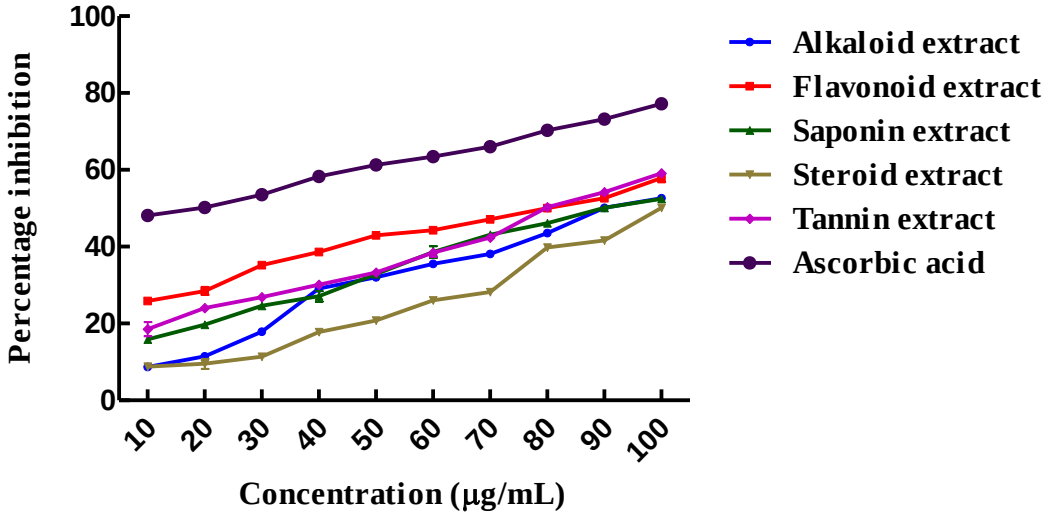


Figure 4.69: Nitric oxide radical scavenging activity of various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

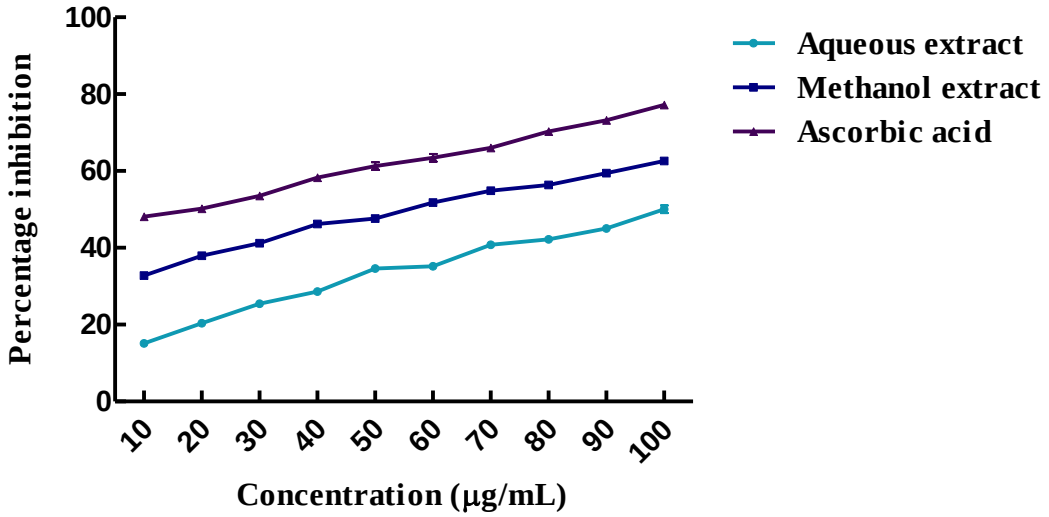


Figure 4.70: Nitric oxide radical scavenging activity of general extracts (aqueous and methanol) of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

4.11.4.3. Nitric oxide radical scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp.

The nitric oxide radical scavenging activity of the various extracts of *Dicentra scandens* (D. Don) Walp. are shown in Table 4.67, Figure 4.71 and Figure 4.72. The nitric oxide radical scavenging activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the flavonoid extract of *Dicentra scandens* (D. Don) Walp. exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity than the other phytochemical extracts (Figure 4.71). At the concentration of 100 µg/mL, the highest scavenging activity in terms of percentage inhibition of nitric oxide radical was exhibited by the flavonoid extract with the percentage inhibition of 63.69±0.81. The lowest IC_{50} value of 40 µg/mL was exhibited by the flavonoid extract of *Dicentra scandens* (D. Don) Walp. with the percentage inhibition 50.35±0.05. The standard ascorbic acid exhibited an IC_{50} value of 20 µg/mL with the percentage inhibition of 50.41±0.81 (Table 4.67).

Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity than the aqueous extract (Figure 4.72).

Table 4.67: Nitric oxide radical scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp.

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	30.00±0.39	41.74±0.17*	17.60±0.02	17.54±0.48	19.30±0.73	15.00±0.29	33.92±0.75$^{\#}$	48.71±0.70
20	35.58±0.52	47.75±0.36*	20.82±0.80	21.01±0.52	21.63±0.69	19.95±0.20	38.24±0.65$^{\#}$	50.41±0.81
30	36.25±0.097	49.69±0.05*	22.99±0.34	25.44±0.90	27.25±0.58	26.21±1.04	42.89±2.37$^{\#}$	54.87±0.86
40	37.90±0.07	50.35±0.05*	25.67±0.68	30.99±0.80	30.86±0.25	28.20±0.17	44.76±0.15$^{\#}$	57.84±0.87
50	45.77±0.27	51.08±0.10*	28.79±1.00	37.57±0.53	35.44±0.83	31.11±0.08	46.07±1.37$^{\#}$	61.39±0.90
60	47.16±1.29	54.41±0.07*	39.34±1.10	41.17±0.56	42.20±0.59	36.27±0.07	48.99±0.34$^{\#}$	63.67±0.74
70	48.09±0.028	56.63±0.48*	45.22±0.75	43.58±0.84	48.26±0.67	41.43±0.25	50.08±0.36$^{\#}$	66.42±1.67
80	51.29±1.21	58.25±0.04*	47.91±0.85	45.82±0.62	50.79±0.68	44.52±0.96	52.50±0.12$^{\#}$	70.84±0.82
90	53.87±0.05	59.59±0.07*	50.20±0.29	47.50±0.78	54.14±0.78	47.16±0.65	53.16±0.04$^{\#}$	73.36±0.68
100	57.65±0.15	63.69±0.81*	54.07±1.35	51.06±0.56	56.55±1.39	50.07±0.69	59.85±1.50$^{\#}$	77.00±0.97

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in nitric oxide radical scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in nitric oxide radical scavenging activity than the aqueous extract.

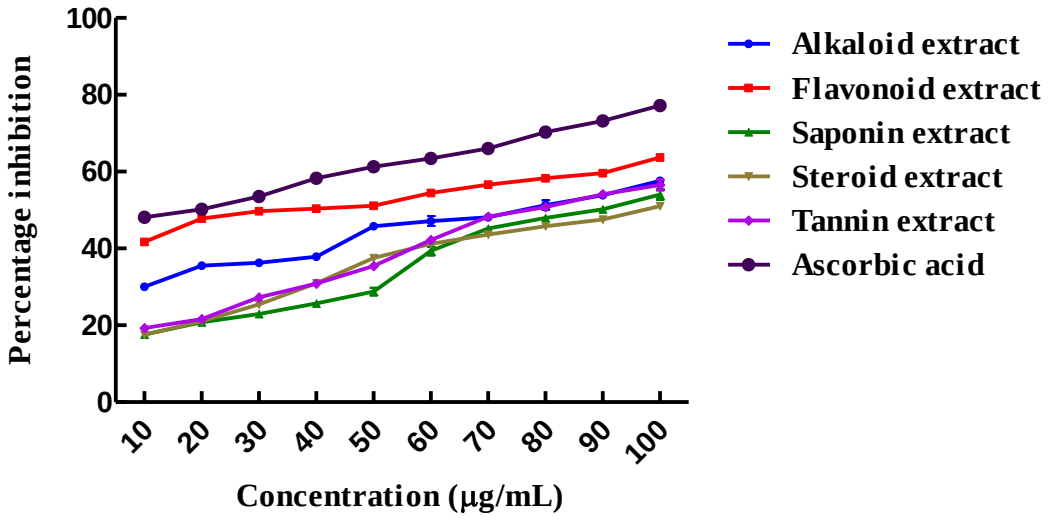


Figure 4.71: Nitric oxide radical scavenging activity of various phytochemical extracts of *Dicentra scandens* (D. Don) Walp.

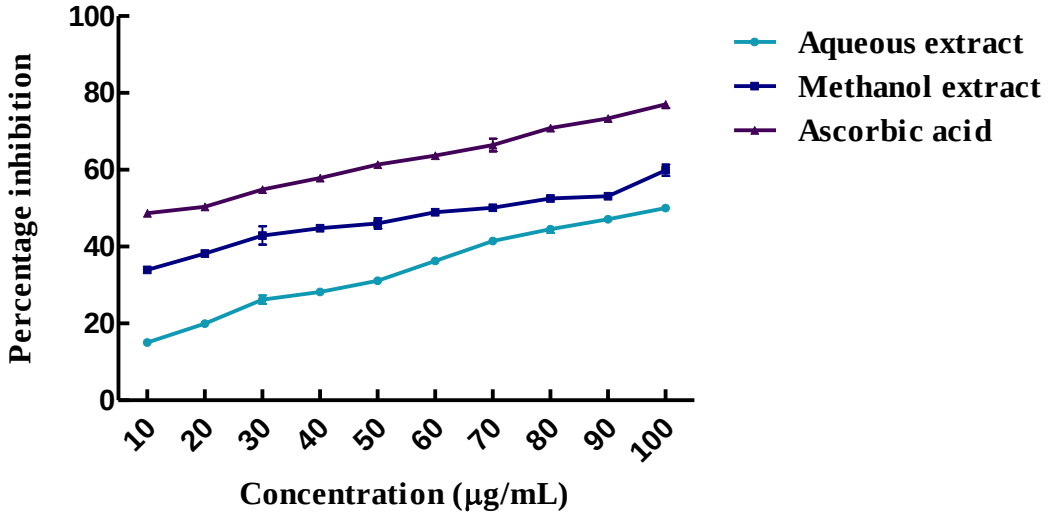


Figure 4.72: Nitric oxide radical scavenging activity of general extracts (aqueous and methanol) of *Dicentra scandens* (D. Don) Walp.

4.11.4.4. Nitric oxide radical scavenging activity of various extracts of *Heracleum nepalense* D. Don

The nitric oxide radical scavenging activity of the various extracts of *Heracleum nepalense* D. Don are shown in Table 4.68, Figure 4.73 and Figure 4.74. The nitric oxide radical scavenging activity of the extracts was compared with ascorbic acid as standard. Among the phytochemical extracts, the tannin extract of *Heracleum nepalense* D. Don exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity than the other phytochemical extracts (Figure 4.73). At the concentration of 100 µg/mL, the highest scavenging activity was exhibited by the tannin extract with the percentage inhibition of 70.08±0.71. The lowest IC_{50} value of 60 µg/mL was exhibited by the tannin extract of *Heracleum nepalense* D. Don with the percentage inhibition 50.84±0.29. The IC_{50} value of 20 µg/mL was exhibited by ascorbic acid with the percentage inhibition of 50.12±0.78 (Table 4.68).

Similarly, the methanol extract exhibited significantly ($p<0.05$) higher nitric oxide radical scavenging activity than the aqueous extract (Figure 4.74).

Table 4.68: Nitric oxide radical scavenging activity of various extracts of *Heracleum nepalense* D. Don

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	Ascorbic acid
10	13.25±0.56	14.24±0.140	11.67±0.56	12.67±0.46	27.60±0.10*	9.29±0.07	25.87±0.56$^{\#}$	48.04±0.67
20	21.87±0.35	20.15±0.93	16.07±0.95	14.26±0.66	35.25±0.51*	13.82±0.94	31.42±0.22$^{\#}$	50.12±0.78
30	25.87±0.42	24.23±0.79	25.14±0.41	22.72±0.35	37.36±0.05*	17.12±0.05	33.94±0.80$^{\#}$	54.14±0.88
40	36.53±0.26	25.62±0.98	31.01±0.05	24.46±1.04	42.54±0.77*	21.69±2.03	37.89±0.95$^{\#}$	58.24±0.63
50	38.07±0.99	28.07±0.73	34.36±0.70	33.80±0.86	45.52±0.64*	25.46±0.77	43.09±0.75$^{\#}$	61.59±0.84
60	42.22±0.29	38.15±1.07	36.34±0.14	41.18±0.35	50.84±0.29*	30.37±0.85	49.08±1.29$^{\#}$	64.12±1.83
70	45.02±1.05	49.81±0.94	42.20±0.33	48.92±1.02	55.01±0.83*	34.61±0.96	50.60±0.70$^{\#}$	66.13±0.27
80	50.44±0.87	51.54±0.45	50.07±0.36	50.42±0.75	59.73±0.67*	41.31±0.78	55.56±0.58$^{\#}$	70.48±0.83
90	51.93±0.76	54.23±0.22	52.79±0.92	53.78±0.22	65.08±0.09*	47.22±1.06	59.73±0.77$^{\#}$	73.69±0.96
100	55.36±0.84	58.76±0.97	58.62±1.03	55.67±0.40	70.08±0.71*	50.18±0.51	64.41±0.68$^{\#}$	77.02±0.96

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in nitric oxide radical scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in nitric oxide radical scavenging activity than the aqueous extract.

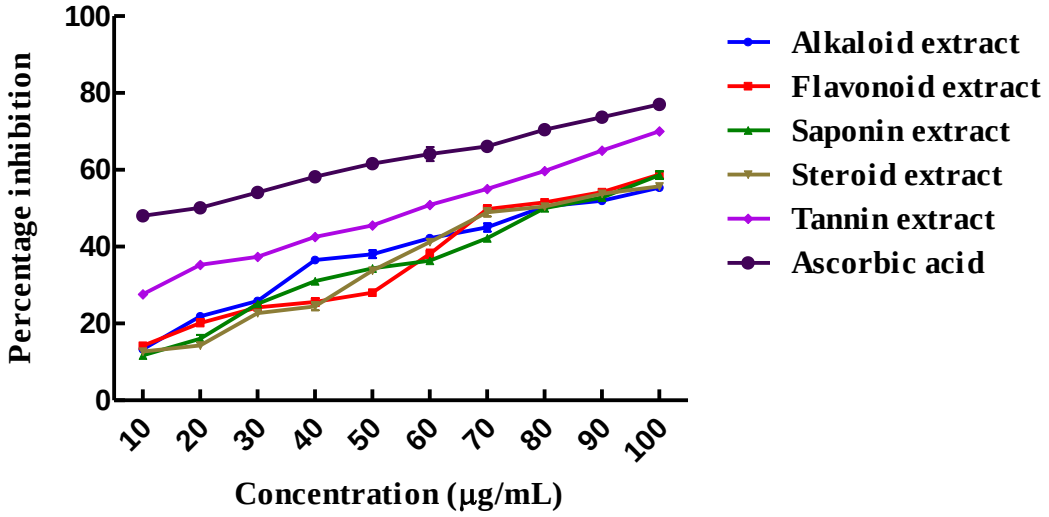


Figure 4.73: Nitric oxide radical scavenging activity of various phytochemical extracts of *Heracleum nepalense* D. Don

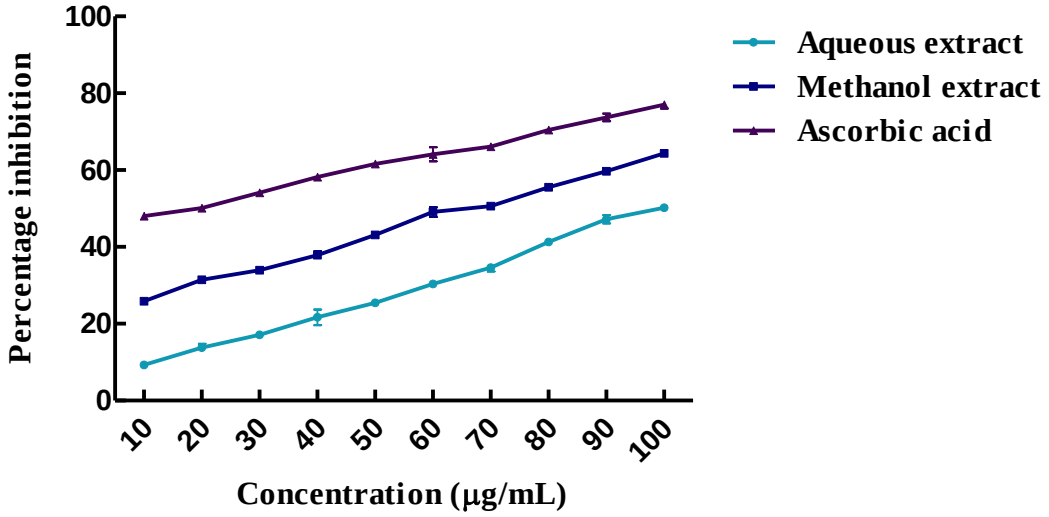


Figure 4.74: Nitric oxide radical scavenging activity of general extracts (aqueous and methanol) of *Heracleum nepalense* D. Don

4.11.5. Hydroxyl radical scavenging activity

4.11.5.1. Hydroxyl radical scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

Hydroxyl radical scavenging activity of the various extracts of *Cyphomandra betacea* (Cav.) Sendth. are shown in Table 4.69, Figure 4.75 and Figure 4.76. The hydroxyl radical scavenging activity of the extracts was compared with butylated hydroxytoluene (BHT) as standard. Among the phytochemical extracts, the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. exhibited significantly ($p<0.05$) higher hydroxyl radical scavenging activity than the other phytochemical extracts (Figure 4.75). At the concentration of 100 µg/mL, the highest scavenging activity in terms of percentage inhibition of hydroxyl radical was exhibited by the flavonoid extract with the percentage inhibition of 67.39±0.55. The flavonoid extract exhibited the lowest IC_{50} value of 50 µg/mLwith the percentage inhibition of 51.04±0.58. The IC_{50} value exhibited by standard BHT was 40 µg/mL with percentage inhibition of 50.45±0.86 (Table 4.69).

Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher hydroxyl radical scavenging activity than the aqueous extract (Figure 4.76).

Table 4.69: Hydroxyl radical scavenging activity of various extracts of *Cyphomandra betacea* (Cav.) Sendth.

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	BHT
10	20.75±0.17	33.11±0.40*	22.17±0.65	16.10±0.63	20.95±0.15	17.19±0.25	22.27±0.10$^{\#}$	38.52±0.34
20	22.57±0.39	37.69±0.59*	24.53±0.02	18.28±0.19	22.88±0.19	21.41±0.22	26.92±0.16$^{\#}$	42.96±0.14
30	25.03±0.47	41.13±0.16*	26.20±0.06	20.97±0.19	28.76±0.50	25.35±0.20	32.27±0.19$^{\#}$	47.40±0.06
40	26.27±0.66	48.04±0.47*	28.15±0.38	25.22±0.22	31.55±0.20	32.05±0.52	37.02±0.20$^{\#}$	50.45±0.86
50	29.06±0.46	51.04±0.58*	31.06±0.40	29.88±0.19	35.40±0.24	34.03±0.56	38.72±0.27$^{\#}$	53.61±0.36
60	32.18±1.25	53.89±0.14*	35.88±0.45	34.05±0.42	42.81±0.68	36.08±0.970	42.53±0.79$^{\#}$	56.04±0.47
70	35.68±0.38	57.40±0.27*	42.36±0.83	37.99±0.61	44.73±0.37	38.26±0.536	47.77±0.98$^{\#}$	61.61±0.77
80	42.80±0.54	60.89±0.46*	47.12±0.81	42.44±0.53	47.57±0.48	42.11±0.26	50.14±0.30$^{\#}$	65.76±0.56
90	47.60±1.21	65.87±0.64*	50.77±0.37	47.28±0.34	50.48±0.47	48.37±0.26	54.99±1.02$^{\#}$	68.17±0.49
100	51.06±0.24	67.39±0.55*	52.28±0.41	50.05±0.56	53.39±0.67	50.34±0.42	57.57±0.56$^{\#}$	71.67±0.35

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in hydroxyl radical scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in hydroxyl radical scavenging activity than the aqueous extract.

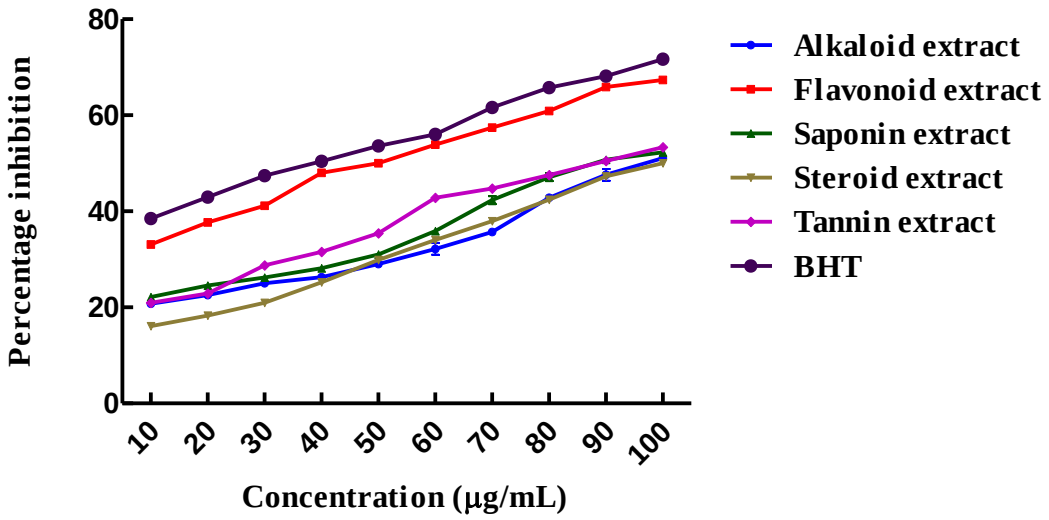


Figure 4.75: Hydroxyl radical scavenging activity of various phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth.

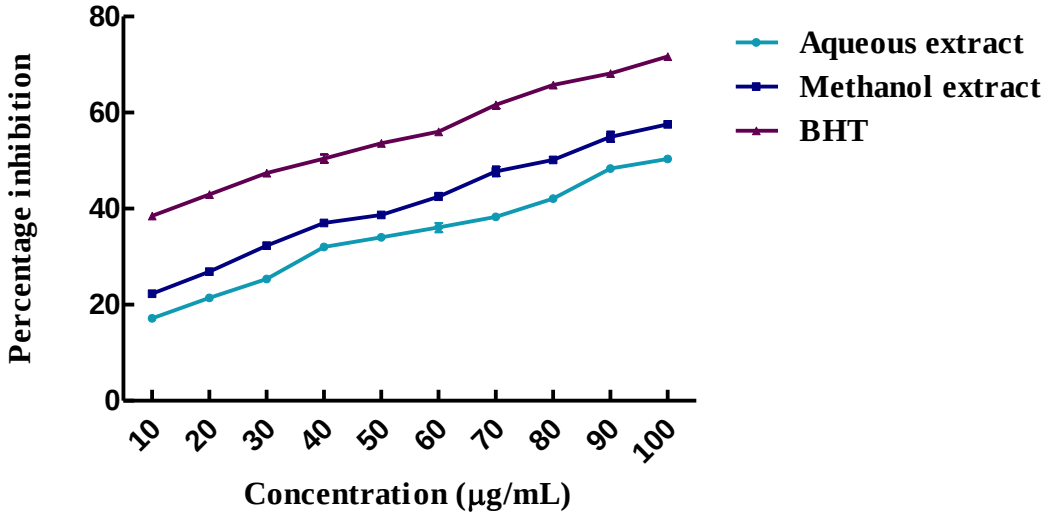


Figure 4.76: Hydroxyl radical scavenging activity of general extracts (aqueous and methanol) of *Cyphomandra betacea* (Cav.) Sendth.

4.11.5.2. Hydroxyl radical scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Hydroxyl radical scavenging activity of the various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are shown in Table 4.70, Figure 4.77 and Figure 4.78. The hydroxyl radical scavenging activity of the extracts was compared with BHT as standard. The saponin extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited significantly ($p<0.05$) higher hydroxyl radical scavenging activity than the other phytochemical extracts (Figure 4.77). The lowest IC_{50} value of 60 µg/was exhibited by the saponin and the methanol extracts with the percentage inhibition of 50.25±0.24 and 51.04±0.56 respectively. The IC_{50} value exhibited by standard BHT was 40 µg/ml with percentage inhibition of 50.04±0.41. At the concentration of 100 µg/mL, the highest scavenging activity in terms of percentage inhibition of hydroxyl radical was exhibited by the methanol extract with the percentage inhibition of 64.97±0.28 (Table 4.70). The hydroxyl radical scavenging activity of methanol extract was higher than the aqueous extract (Figure 4.78).

Table 4.70: Hydroxyl radical scavenging activity of various extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	BHT
10	16.87±1.09	23.46±0.59	25.53±0.03*	14.26±0.32	16.34±1.32	17.73±0.26	31.52±0.17$^{\#}$	38.44±0.24
20	19.55±0.49	26.97±0.75	32.46±0.46*	19.85±0.66	18.88±0.15	19.07±0.20	37.68±0.13$^{\#}$	42.77±0.44
30	23.73±0.18	31.95±0.59	37.03±0.25*	25.70±0.38	26.64±0.32	23.27±0.12	40.06±0.08$^{\#}$	47.64±0.82
40	29.10±0.21	34.92±0.64	42.95±0.25*	29.20±0.41	31.02±0.14	29.80±0.73	44.14±0.52$^{\#}$	50.04±0.41
50	34.76±0.498	37.49±0.79	45.91±0.25*	32.20±0.55	34.96±0.75	31.10±0.12	47.48±0.43$^{\#}$	53.19±0.33
60	36.23±0.65	42.71±0.51	50.25±0.24*	35.71±0.15	40.04±0.66	37.40±0.56	51.04±0.56$^{\#}$	55.48±0.58
70	40.35±0.41	46.90±1.03	52.03±0.34*	39.57±0.25	44.67±0.61	42.36±0.44	54.38±0.48$^{\#}$	61.10±0.49
80	42.67±0.51	49.24±0.27	54.59±0.28*	44.56±0.38	47.89±0.46	47.58±0.64	57.88±0.56$^{\#}$	65.56±0.72
90	50.21±0.99	51.12±0.33	59.42±0.57*	47.87±0.47	51.10±0.12	50.58±0.43	61.01±0.39$^{\#}$	68.03±0.27
100	54.11±0.82	53.08±0.49	61.05±0.60*	50.11±0.74	53.70±0.59	52.67±0.74	64.97±0.28	71.12±0.43

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in hydroxyl radical scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in hydroxyl radical scavenging activity than the aqueous extract.

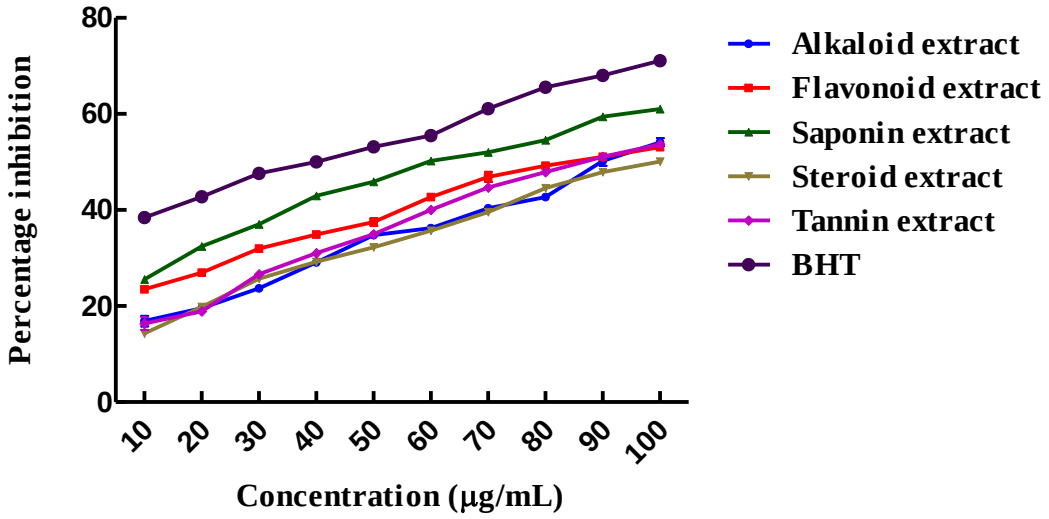


Figure 4.77: Hydroxyl radical scavenging activity of various phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

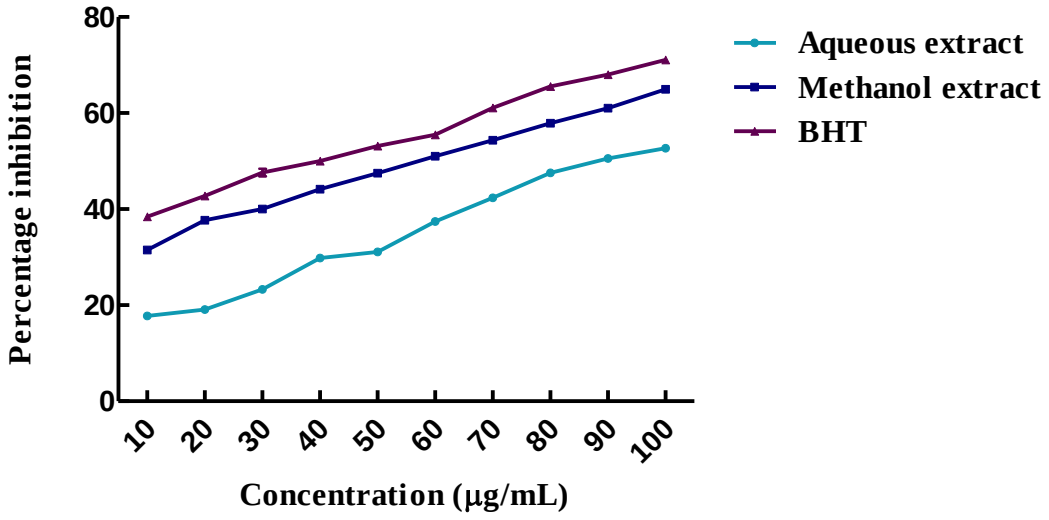


Figure 4.78: Hydroxyl radical scavenging activity of general extracts (aqueous and methanol) of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

4.11.5.3. Hydroxyl radical scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp.

Hydroxyl radical scavenging activity of the various extracts of *Dicentra scandens* (D. Don) Walp. are shown in Table 4.71, Figure 4.79 and Figure 4.80. The hydroxyl radical scavenging activity of the extracts was compared with BHT as standard. Among the phytochemical extracts, the saponin, flavonoid and tannin extracts showed similar hydroxyl radical scavenging activity (Figure 4.79).

Among all the extracts of *Dicentra scandens* (D. Don) Walp. the methanol extract exhibited significantly ($p<0.05$) higher hydroxyl radical scavenging activity. At the concentration of 100 µg/mL, the highest scavenging activity in terms of percentage inhibition of hydroxyl radical was exhibited by the methanol extract with the percentage inhibition of 57.86±0.71. The lowest IC_{50} value of 70 µg/mL was exhibited by the methanol extract of *Dicentra scandens* (D. Don) Walp. with the percentage inhibition 50.27±0.29. The IC_{50} value exhibited by standard BHT was 40 µg/mL with percentage inhibition of 50.59±0.76 (Table 4.71).

Table 4.71: Hydroxyl radical scavenging activity of various extracts of *Dicentra scandens* (D. Don) Walp.

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	BHT
10	16.96±0.59	26.09±1.98	24.04±0.10	22.20±0.56	31.68±0.12	18.37±0.12	32.89±0.53$^{\#}$	38.44±0.57
20	19.00±0.71	29.33±0.19	27.51±0.60	25.01±0.59	33.04±0.37	22.23±0.20	35.14±0.70$^{\#}$	42.04±0.96
30	21.92±0.13	34.50±1.55	32.23±0.56	27.66±0.81	35.48±0.27	29.35±0.131	37.17±0.48$^{\#}$	47.07±0.17
40	24.94±0.71	38.92±0.15	36.37±0.90	30.87±0.14	37.58±1.13	34.62±0.15	40.09±0.47$^{\#}$	50.59±0.76
50	33.49±0.25	40.24±0.23	42.02±0.40	34.90±0.19	39.77±0.42	39.75±0.16	43.25±0.37$^{\#}$	53.36±0.59
60	35.36±0.58	42.41±0.66	44.53±0.49	37.27±0.48	42.23±0.86	43.25±0.20	47.74±0.73$^{\#}$	56.07±0.67
70	40.29±0.60	45.00±1.07	48.41±0.29	40.79±0.36	48.03±0.50	44.74±0.28	50.27±0.29$^{\#}$	61.10±0.63
80	45.34±0.44	50.15±0.47	51.08±0.22	45.03±0.72	50.05±0.34	50.19±0.80	52.77±0.62$^{\#}$	64.54±0.26
90	47.41±0.21	52.22±0.33	53.16±0.34	47.68±0.55	52.03±0.45	53.09±0.16	54.23±0.34	68.93±0.28
100	50.25±0.41	54.65±0.36	57.43±0.54	50.08±0.70	55.76±0.34	55.28±0.44	57.86±0.71$^{\#}$	71.79±0.45

Each value represents mean±SD. All the experiments were performed in triplicate. $^{\#}(p<0.05)$ significantly different in hydroxyl radical scavenging activity than the aqueous extract.

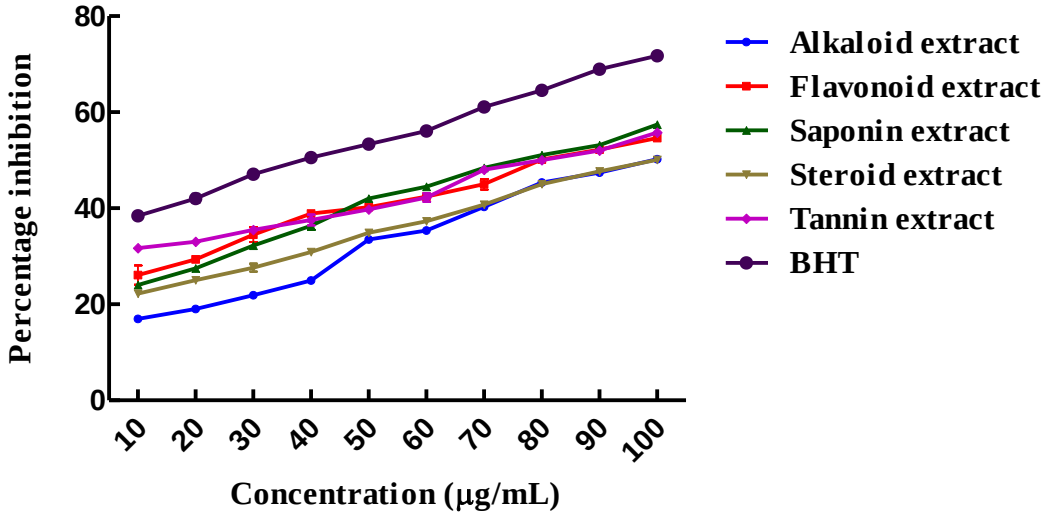


Figure 4.79: Hydroxyl radical scavenging activity of various phytochemical extracts of *Dicentra scandens* (D. Don) Walp.

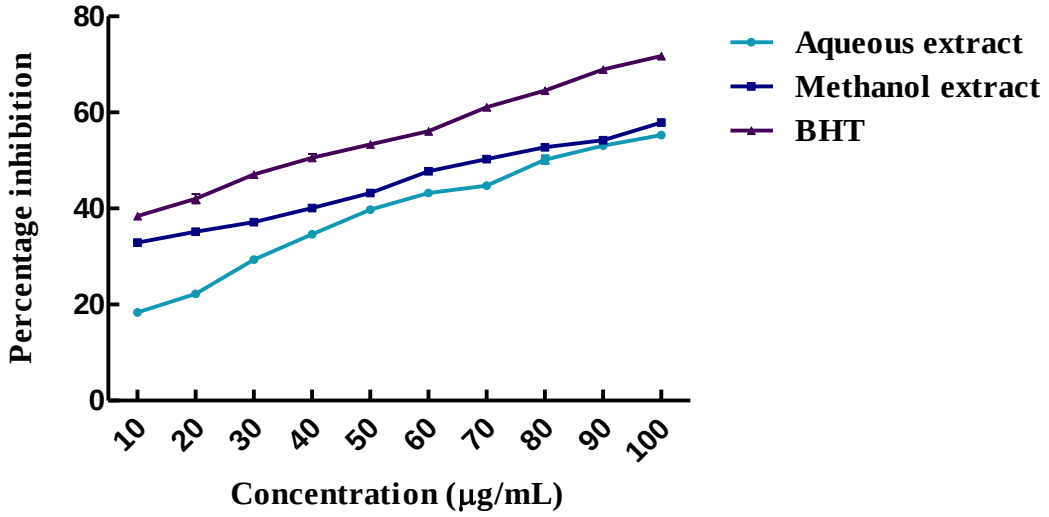


Figure 4.80: Hydroxyl radical scavenging activity of general extracts (aqueous and methanol) of *Dicentra scandens* (D. Don) Walp.

4.11.5.4. Hydroxyl radical scavenging activity of various extracts of *Heracleum nepalense* D. Don

Hydroxyl radical scavenging activity of the various extracts of *Heracleum nepalense* D. Don are shown in Table 4.72, Figure 4.81and Figure 4.82. The hydroxyl radical scavenging activity of the extracts was compared with BHT as standard. Among the phytochemical extracts, the tannin extract exhibited significantly ($p<0.05$) higher hydroxyl radical scavenging activity than the other phytochemical extracts (Figure 4.81).

The methanol extract of *Heracleum nepalense* D. Don exhibited significantly ($p<0.05$) higher hydroxyl radical scavenging activity among all the extracts. At the concentration of 100 µg/mL, the highest scavenging activity in terms of percentage inhibition of hydroxyl radical was exhibited by the methanol extract with the percentage inhibition of 60.98±0.71. Among the extracts, the lowest IC_{50} value of 60 µg/mL was exhibited by the methanol extract with the percentage inhibition 50.73±0.68. The IC_{50} value exhibited by standard BHT was 40 µg/mL with percentage inhibition of 50.19±0.59. The least scavenging activity in terms of IC_{50} value was exhibited by the steroid extract, since the 50 % inhibition of hydroxyl radical was not observed up to the concentration of 100 µg/mL of the steroid extract (Table 4.72).

Among the general extracts, the methanol extract exhibited significantly ($p<0.05$) higher hydroxyl radical scavenging activity than the aqueous extract (Figure 4.82).

Table 4.72: Hydroxyl radical scavenging activity of various extracts of *Heracleum nepalense* D. Don

Concentration (µg/mL)	Percentage inhibition							
	Phytochemical extracts					General extracts		Standard
	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Aqueous	Methanol	BHT
10	15.32±0.92	22.37±0.44	17.42±0.88	15.16±0.63	22.35±0.62	14.92±0.87	22.72±0.60$^{\#}$	38.15±0.16
20	19.59±0.75	24.93±0.58	25.09±0.54	20.36±0.52	27.09±0.32*	22.77±0.63	28.50±0.65$^{\#}$	42.87±0.43
30	23.00±0.50	27.74±0.76	30.23±0.72	22.40±0.33	30.99±0.38*	25.09±0.84	34.44±0.80$^{\#}$	47.67±0.86
40	27.26±0.86	32.72±0.78	34.93±1.04	26.52±0.58	37.63±0.64*	30.19±0.96	40.29±0.62$^{\#}$	50.19±0.59
50	34.06±0.23	34.74±0.48	38.16±0.69	30.02±0.54	41.98±0.43*	35.51±0.66	43.82±0.11$^{\#}$	53.16±0.68
60	38.01±0.62	40.44±0.74	42.98±0.57	35.06±1.01	48.44±0.69*	38.07±0.19	50.73±0.68$^{\#}$	55.14±0.62
70	43.21±0.36	44.94±0.53	45.09±0.56	37.75±0.61	50.40±0.40*	42.71±0.20	53.92±0.11$^{\#}$	61.23±0.36
80	47.65±0.35	51.27±0.37*	48.06±0.71	40.49±0.67	51.46±0.99*	48.29±0.51	56.06±0.66$^{\#}$	65.70±0.45
90	51.24±0.14	55.35±0.58*	50.27±0.70	43.12±0.49	56.23±0.25*	51.81±0.24	58.17±0.59$^{\#}$	68.27±0.86
100	53.39±0.61	58.11±0.38*	52.44±0.68	47.09±0.42	57.91±0.33*	55.14±0.44	60.98±0.71$^{\#}$	71.41±0.66

Each value represents mean±SD. All the experiments were performed in triplicate. *($p<0.05$) significantly different in hydroxyl radical scavenging activity than the other phytochemical extracts. $^{\#}$($p<0.05$) significantly different in hydroxyl radical scavenging activity than the aqueous extract.

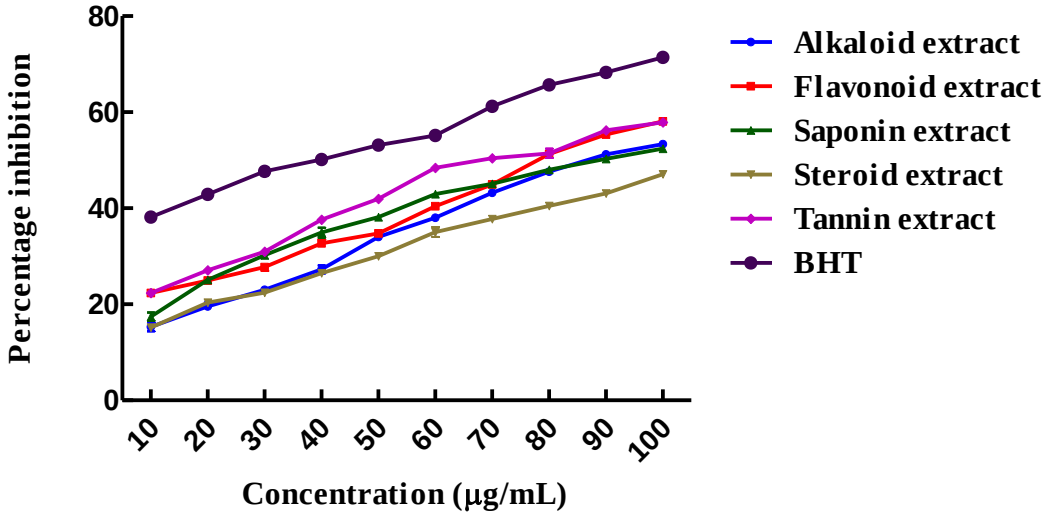


Figure 4.81: Hydroxyl radical scavenging activity of various phytochemical extracts of *Heracleum nepalense* D. Don

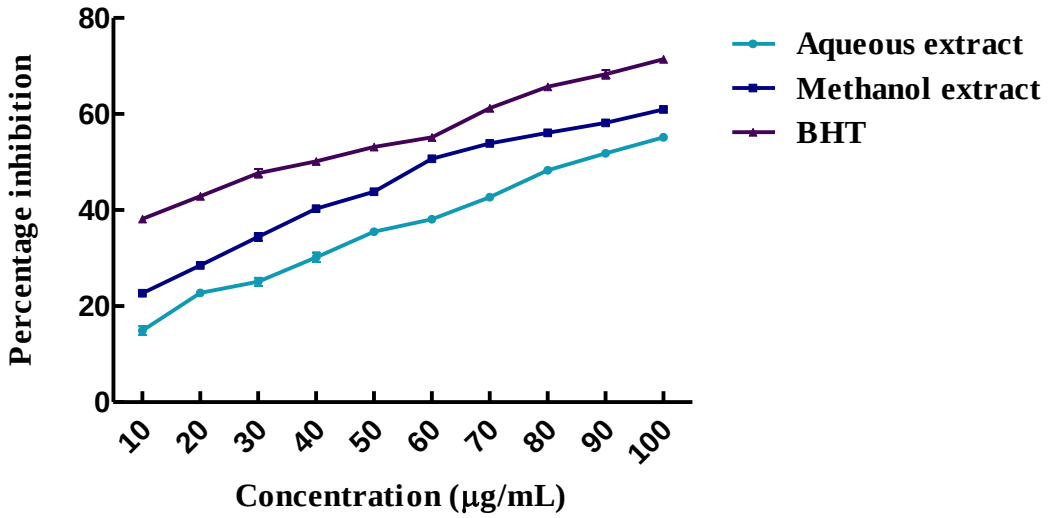


Figure 4.82: Hydroxyl radical scavenging activity of general extracts (aqueous and methanol) of *Heracleum nepalense* D. Don

4.12. Characterization of extracts by Gas Chromatography and Mass Spectrometry (GC-MS) analysis

Some selected plant extracts namely the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, methanol and alkaloid extracts of *Dicentra scandens* (D. Don) Walp. were characterized by GC-MS analysis.

4.12.1. GC-MS analysis of the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

GC-MS analysis of the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish detected 38 compounds. The GC-MS chromatogram of the extract is shown in Figure 4.83. Some of the compounds showing more than 1% peak area with the reported biological activities are presented in Table 4.73. Capsaicin (24.64 %) and Dihydrocapsaicin (16.57 %) are the major compounds detected in the extract.

4.12.2. GC-MS analysis of the methanol extract of *Dicentra scandens* (D. Don) Walp.

GC-MS analysis of the methanol extract of *Dicentra scandens* (D. Don) Walp. detected 29 compounds. The GC-MS chromatogram of the extract is shown in Figure 4.84. Some of the compounds showing more than 1 % peak area are shown in Table 4. 74. The characterization of the extract revealed the presence of two major alkaloids namely Protopine (53.78 %) and Corydine (18.20 %) in the extract (Table 4.74).

4.12.3. GC-MS analysis of the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

GC-MS analysis of the alkaloid extract of *Dicentra scandens* (D. Don) Walp. detected 23 compounds. The GC-MS chromatogram of the extract is shown in Figure 4.85.

Some of the compounds showing more than 1 % peak area are shown in Table 4.75. In the alkaloid extract, Protopine (45.24 %) and Corydine (38.49 %) were detected. The total peak area of the alkaloid extract was found to increase in the extract as compared to the methanol extract.

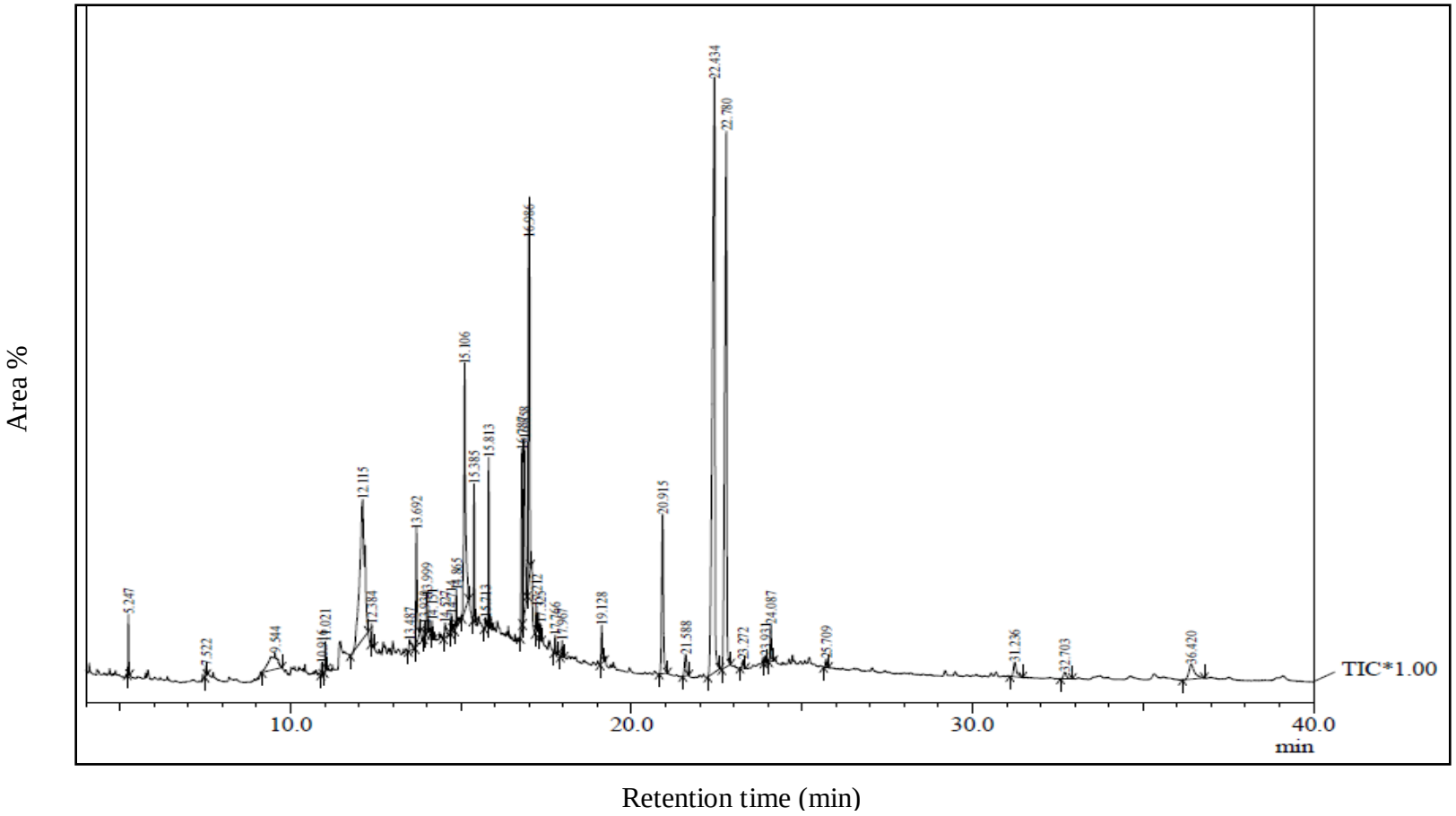


Figure 4.83: Gas chromatography-mass spectrometry chromatogram of the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Table 4.73: Some of the compounds (more than 1 % peak area) possessing various biological activities as detected by the GC-MS analysis of the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish

Sl. No.	Retention time	Area %	Compound Name	Formula	Molecular weight	Compound nature	Biological activities
1	9.544	2.11	Guanosine	$C_{10}H_{13}N_5O_5$	283	Purine nucleoside	Antiprotozoal (Avila *et al*., 1987)
2	15.106	6.84	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	Lauric acid	Antimicrobial Antioxidant [(#)] (Mujeeb *et al*., 2014)
3	15.385	1.62	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	Palmitic acid ester	Antimicrobial, antioxidant [(#)]
4	15.813	2.05	Geranyl linalool isomer	$C_{20}H_{34}O$	290	Essential oil	antimicrobial, antioxidant (Chen and Viljoen, 2010)
5	16.858	6.07	9,12-Octadecadienoic acid (z,z)-	$C_{18}H_{32}O_2$	280	Linoleic acid ester	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective (Mohanambal and Murugaiah, 2015)
6	22.434	24.64	Capsaicin	$C_{18}H_{27}NO_3$	305	Alkaloid	Antimicrobial (Stephen and Kumar, 2014) Antioxidant (Okada and Okajima, 2001; Rosa *et al*., 2002)
7	22.780	16.57	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	307	Alkaloid	Antimicrobial (Nascimento *et al*., 2014) Antioxidant (Rosa *et al*., 2002)
8	36.420	1.35	Stigmast-5-en-3-OL, (3.beta.)-	$C_{29}H_{50}O$	414	Sterol	Antimicrobial (Odiba *et al*., 2014)

[#] Source: ("Dr. Duke's phytochemical and ethnobotanical databases,")

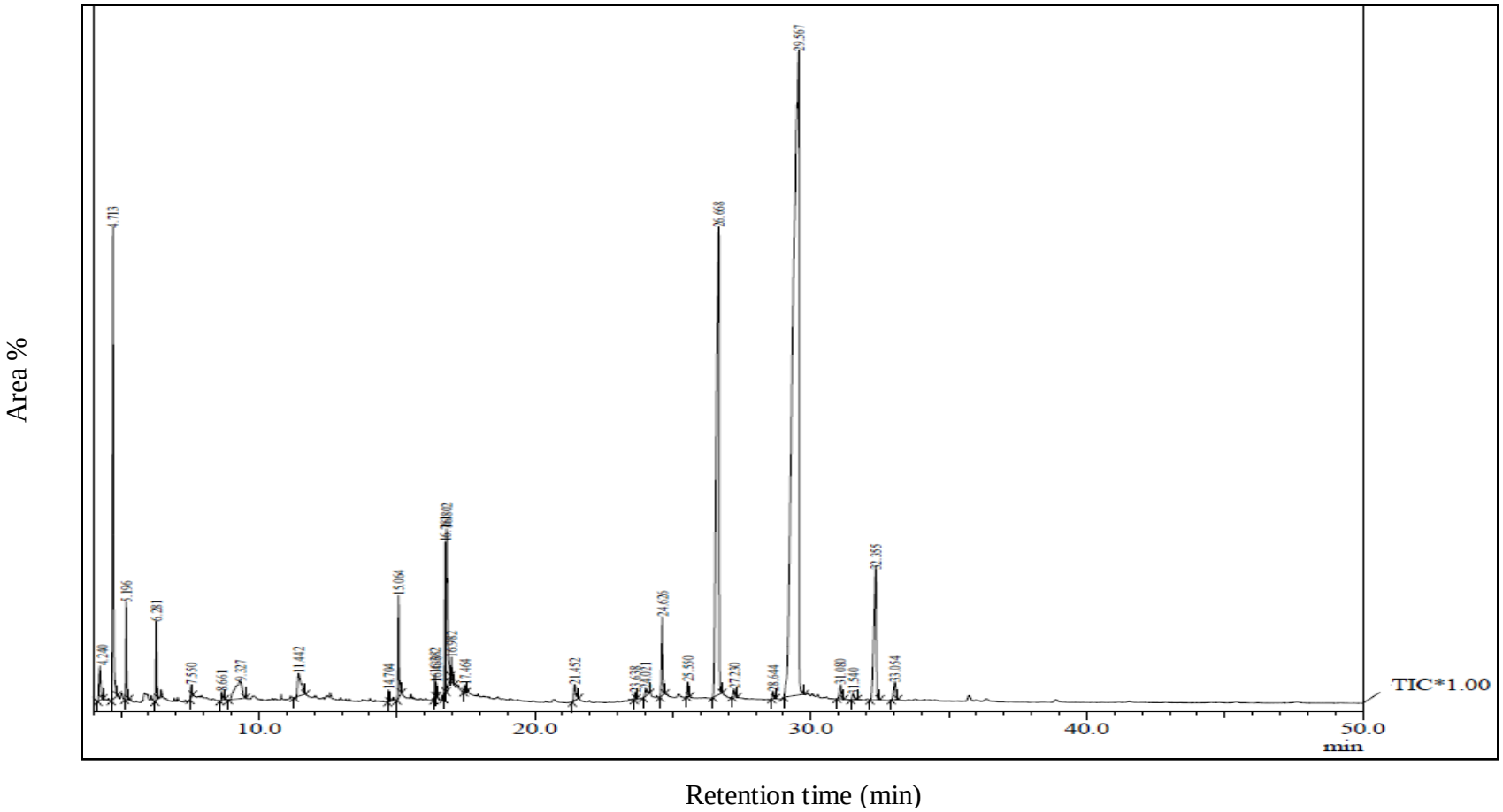


Figure 4.84: Gas chromatography-mass spectrometry chromatogram of the methanol extract of *Dicentra scandens* (D. Don) Walp.

Table 4.74: Some of the compounds (more than 1 % peak area) possessing various biological activities as detected by the GC-MS analysis of the methanol extract of *Dicentra scandens* (D. Don) Walp.

Sl. No.	Retention time	Area %	Compound Name	Formula	Molecular weight	Compound Nature	Biological activities
1	4.713	6.21	2,5-Dihydro-1H-pyrrole	C_4H_7N	69	Pyrole	Antioxidant (Balabani *et al*., 2011)
2	5.196	1.13	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One	$C_6H_8O_4$	144	Flavonoid	Antimicrobial, anti-inflammatory (Mohanambal and Murugaiah, 2015)
3	11.442	1.09	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192	Quinic acid	Antimicrobial, anti-inflammatory, antioxidant (Senthilkumar *et al*., 2015)
4	15.064	1.34	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	Lauric acid	Antimicrobial Antioxidant [(#)](Mujeeb *et al*., 2014)
5	16.761	1.99	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	Linoleic acid ester	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective (Mohanambal and Murugaiah, 2015),
6	24.626	1.51	Hordenine	$C_{10}H_{15}NO$	165	Alkaloid	Anti-cholinesterase Activity (Schweitzer and Wright, 1938)
7	26.668	18.20	Corydine	$C_{20}H_{23}NO_4$	341	Alkaloid	DNA damaging activity (Goren *et al*., 2003)
8	29.567	53.78	Protopine	$C_{20}H_{19}NO_5$	353	Alkaloid	Antibacterial, antiviral, antifungal, antiparasitic, antithrombotic, anti-inflammatory, anti-spasmodic, neuroprotective (Vacek *et al*., 2010) Antioxidant (Xiao *et al*., 2008)

Source: ("Dr. Duke's phytochemical and ethnobotanical databases,")

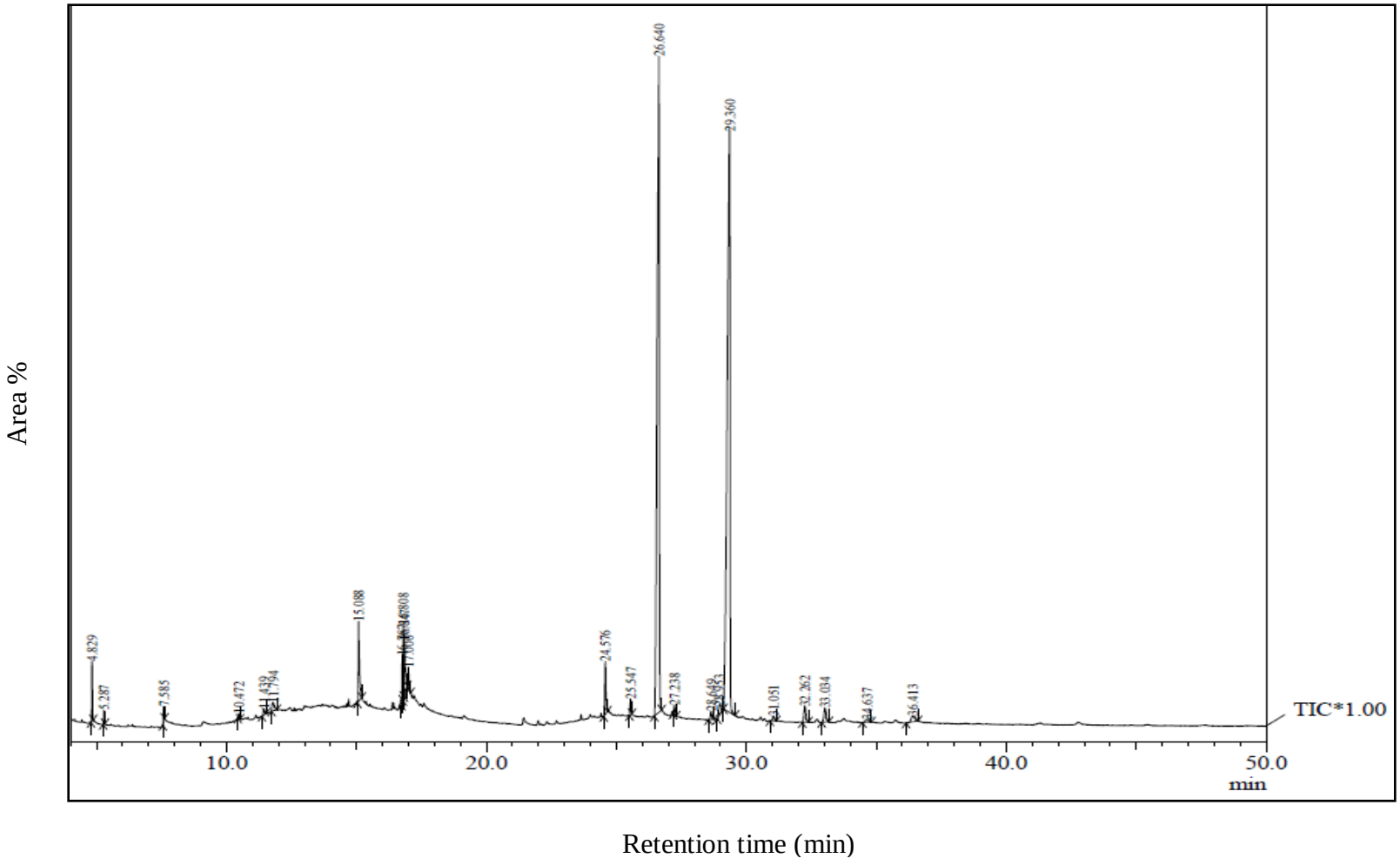


Figure 4.85: Gas chromatography-mass spectrometry chromatogram of the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

Table 4.75: Some of the compounds (more than 1 % peak area) possessing various biological activities as detected by the GC-MS analysis of the alkaloid extract of *Dicentra scandens* (D. Don) Walp.

Sl. No.	Retention time	Area %	Compound Name	Formula	Molecular weight	Compound Nature	Biological activities
1	15.088	2.65	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	Lauric acid	Antioxidant Antimicrobial [(#)](Mujeeb *et al*., 2014)
2	16.847	1.83	cis-9-Hexadecenal	$C_{16}H_{30}O$	238	Aldehyde	Antimicrobial [(#)](Mujeeb *et al*., 2014)
3	24.576	1.70	Hordenine	$C_{10}H_{15}NO$	165	Alkaloid	Anti-cholinesterase Activity (Schweitzer and Wright, 1938)
4	26.640	38.49	Corydine	$C_{20}H_{23}NO_4$	341	Alkaloid	DNA damaging activity (Goren *et al*., 2003)
5	29.360	45.24	Protopine	$C_{20}H_{19}NO_5$	353	Alkaloid	Antibacterial, antiviral, antifungal, antiparasitic, antithrombotic, anti-inflammatory, anti-spasmodic, neuroprotective (Vacek *et al*., 2010) Antioxidant (Xiao *et al*., 2008)

[#] Source: ("Dr. Duke's phytochemical and ethnobotanical databases,")

5. DISCUSSION

Phytochemicals from medicinal plants have an immense scope in drug development today (Mohanraj *et al*., 2018). Ancient civilizations, in particular, the Babylonians, Egyptians, Chinese and Indians had always made use of medicinal plants to heal diseases (Roca, 2003). Particularly in India, plants have been used from centuries to treat diseases (Mohanraj *et al*., 2018). Sikkim, a North Eastern state of India is rich in plant diversity and also has a rich culture of folk medicine. Ethnobotanical study of some medicinal plants has been documented from Sikkim (Idrisi *et al*., 2010). However not sufficient literature have been found relating to the study of the antimicrobial and antioxidant properties of the various phytochemicals present in the medicinal and herbal plants from Sikkim.

In the past decades medicinal plants, fruits and vegetables have been studied across the globe for many biological activities including antimicrobial and antioxidant activities (Reyes-Munguia *et al*., 2016). The biological activity of the plant extracts is attributed to the presence of phytochemicals in the extracts. Majority of the study on antimicrobial and antioxidant activities of the plant extracts focus on the study of the aqueous, methanol, ethanol, hexane or ethyl acetate extracts of the plants. Some other studies relate to the isolated compounds. The present study evaluated the antimicrobial and antioxidant properties of various phytochemicals namely alkaloid, flavonoid, saponin, steroid and tannin extracted from *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don. Water is used in various traditional medicine preparations and methanol is the solvent of choice for extraction because of its extraction efficiency (Abbasi *et al*., 2010;

Kumoro *et al*., 2009; Parvez and Yadav, 2010). Therefore, in the present study, the antimicrobial and antioxidant properties of the aqueous and the methanol extracts of the test plants were also investigated.

5.1. Extraction yield and phytochemical analysis

Various phytochemicals were extracted from the test plants and the percentage yield of the extracts was determined. Among all the extracts of the test plants, the highest yield (29.09±1.40 %) was obtained from tannin extract of *Dicentra scandens* (D. Don) Walp. (Table 4.3). In the present study, the tannin was extracted using the microwave. It has been reported that the microwave-assisted extraction procedure resulted in the high extraction efficiency of tannin as compared to reflux extraction, sonication and maceration (Cobzac *et al*., 2005; Naima *et al*., 2015). Among all the test plants, the highest tannin content of 36.24±0.03 mg TAE/g of extract was estimated in the tannin extract of *Dicentra scandens* (D. Don) Walp (Table 4.10). Among the phytochemical extract of the test plants, the highest yield (17.35±0.98 %) of alkaloid was obtained from *Dicentra scandens* (D. Don) Walp. (Table 4.3). The GC-MS analysis also detected the presence of alkaloid as the major component in the alkaloid extract *Dicentra scandens* (D. Don) Walp. (Table 4.75). Among the flavonoid extracts of four test plants, the highest yield (16.66±0.47 %) of flavonoid was obtained from *Cyphomandra betacea* (Cav.) Sendth. (Table 4.3). In the quantitative determination of flavonoid, the total flavonoid content of 10.39±0.60 mg RE/g of extract was estimated in the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth (Table 4.8). It has been reported that the fruits of *Cyphomandra betacea* have high contents of phenolic compounds (Schmeda-Hirschmann *et al*., 2005), flavonoid and anthocyanins (Bobbio *et al*., 1983). In the

present study, ethyl acetate is used as one of the solvents for extraction of the flavonoid. Other studies also reported maximum content of flavonoid in ethyl acetate fraction (Assefa *et al.*, 2016). Among the phytochemical extracts of the test plants, the highest yield of 19.91±0.51 % of saponin was obtained from *Cyphomandra betacea* (Cav.) Sendth. (Table 4.3). The aqueous and the methanol extracts of all the test plants also exhibited better extraction yield. The highest extraction yield in methanol has also been reported from other studies (Ibrahim *et al*., 2017). However, the yield of steroid was the least among all the phytochemicals from all the test plants. Percentage yield of extract, chemical composition as well as the biological activity is largely dependent on the solvent used and methods of extraction (Dhanani *et al*., 2017). In the qualitative phytochemical analysis, in the general extracts (methanol and aqueous) majority of the phytochemicals including alkaloid, flavonoid, tannin, phenol, saponin and steroid were detected. However in the specific phytochemical extracts, the particular phytochemical and some other related phytochemicals were detected (Table 4.4-Table 4.7). In the present study specific extraction procedures and solvents were used for the extraction of phytochemicals, which may have resulted in the extraction of these phytochemicals exclusively. It has been reported that various pre-extraction and extraction procedures affect the phytochemical constituents in the final extract. As no universal method of extraction is ideal for all plants, the previously optimized method can be used for extraction based on the plant sample and the objective of the study (Azwanida, 2015).

5.2. Antimicrobial activity

In the present study the phytochemical extracts namely alkaloid, flavonoid, saponin, steroid, tannin as well as the general extracts (aqueous and methanol) of fruits of

Cyphomandra betacea (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Heracleum nepalense* D. Don and roots of *Dicentra scandens* (D. Don) Walp. were evaluated for antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella enterica* ser. *typhi, Shigella flexneri* and *Vibrio cholerae* O139. The extracts exhibited varying levels of antimicrobial activity in terms of the diameter of inhibition zone.

In the case of *Cyphomandra betacea* (Cav.) Sendth. among the phytochemical extracts the alkaloid, flavonoid and saponin extracts inhibited the growth of all the test microorganisms (Figure 4.1). Among all the phytochemical extracts, at the concentration of 400 mg/mL, the alkaloid extract exhibited the largest zone of inhibition (25.66±0.57 mm) against *Pseudomonas aeruginosa* and was found to be effective against both Gram positive and Gram-negative test microorganisms (Table 4.12). Alkaloids are the nitrogenous compound which interferes with cell division thereby exerting antimicrobial effect (Chabner and Horwitz, 1990; Tolulope, 2007). The flavonoid extract showed the largest zone of inhibition (24.66±0.57 mm) against *Staphylococcus aureus* (Table 4.13). Among all the extracts the lowest MIC value of 3.125 mg/mL was also exhibited by the flavonoid extract against *Staphylococcus aureus* (Table 4.26). Several reports are available on flavonoid groups which exhibited potential biological activities such as antimicrobial, antioxidant, anti-inflammatory and anticancer (Anyasor *et al*., 2010; Chao *et al*., 2002; Igbinosa *et al*., 2009; Thitilertdecha *et al*., 2008). The saponin extract exhibited the largest zone of inhibition (20.66±0.57 mm) against *Staphylococcus aureus* and was found to be effective against both Gram positive and Gram-negative test

microorganisms (Table 4.14). Saponins are also bioactive constituent which is involved in the plant defense system because of their antimicrobial activity (Barile *et al*., 2007). In the alkaloid extract of *Cyphomandra betacea* (Cav.) Sendth. alkaloid, anthocyanin and glycoside were detected. In the flavonoid extract apart from the flavonoid component glycoside, anthocyanin and phenol were detected. In saponin extract, anthocyanin, glycoside and saponin were detected (Table 4.4). Hence the antimicrobial activity of the extracts could be attributed to the presence of these phytochemicals in the extracts. The tannin extract inhibited the growth of *Vibrio cholerae* O139 and *Shigella flexneri* at relatively higher concentrations of 200 mg/mL (Figure 4.2) and 400 mg/mL (Figure 4.1). Tannin was not detected in qualitative phytochemical tests of aqueous and methanol extracts (Table 4.4). In the quantitative determination, total tannin content of 5.72±0.03 mg TAE/g of extract was estimated in the tannin extract. Percentage yield of tannin (15.35±0.66) was comparatively less than the other plant extracts. Hence, the relatively less content of tannin in the tannin extract may possibly be related to the lesser antimicrobial activity in terms of zone of inhibition.

The aqueous extract inhibited the growth of *Bacillus cereus, Escherichia coli, Proteus vulgaris, Salmonella enterica* ser. *typhi*, *Shigella flexneri* and *Vibrio cholerae* O139 at higher concentration of 400 mg/mL. However, the methanol extract inhibited the growth of all the test microorganisms and exhibited significantly ($p<0.05$) higher antimicrobial activity as compared to the aqueous extract (Figure 4.5). At the concentration of 400 mg/mL, the largest zone of inhibition (26.33±0.57 mm) was exhibited by the methanol extract against *Proteus vulgaris*. The methanol extract was found to be effective against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* at the

concentration of 25 mg/mL (Table 4.15) which was the lowest concentration of the extract used for antimicrobial assay in our study. In the phytochemical analysis, except for the presence of glycoside in methanol extract, both methanol and the aqueous extracts revealed the presence of alkaloid, anthocyanin, flavonoid, phenol, saponin and steroid (Table 4.4). Among all test plants, the highest phenolic content of 17.43±0.01 mg GAE/g of extract was estimated in the methanol extract. The flavonoid content of 5.28±0.24 mg RE/g of extract was also estimated in the extract (Table 4.8). Methanol has been reported to exhibit better phytochemical extraction efficiency (Cowan, 1999; Lawrence *et al*., 2009). Hence the antimicrobial activity of the methanol extract may possibly be related to the effective extraction of the phytochemicals in the solvent. Numerous studies have been performed to characterize anthocyanin from *Cyphomandra betacea*. Ordonez *et al*., (2010) reported that the content of anthocyanin, flavones and flavanone in ethanolic maceration was higher than in the aqueous extract. The results of the present study also correspond with these findings as methanolic extract revealed the higher amount of phenolic and flavonoid contents (Table 4.8) than aqueous extract and thus exhibited significantly higher antimicrobial activity.

As reported by Swamy *et al.*, (2015), various solvent extracts of *Lantana camara* were effective against both tests Gram positive and Gram-negative bacteria. The results of the present study revealed that the various extracts of *Cyphomandra betacea* (Cav.) Sendth. effectively inhibited the growth of both Gram positive and Gram-negative test bacteria with a varying zone of inhibition. Usually, the antimicrobial activity of plant extracts depends on the dose and the type of bacterial strains employed (Parekh and Chanda, 2007; Taylor *et al*., 2001). This variation in antibacterial actions could be related to the

chemical components in the crude extracts and also due to the ability of the extracts to inactivate microbial activity, enzymes and cell envelope transport proteins (Sekar *et al*., 2012; Vadlapudi and Kaladhar, 2012).

In the case of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish at the concentration of 400 mg/mL the alkaloid extract inhibited the growth of all the nine test microorganisms with the largest zone of inhibition (29.33±0.57 mm) observed against *Staphylococcus aureus* (Table 4.16). The saponin extract showed antimicrobial activity against *Vibrio cholerae* O139 and *Bacillus cereus.* The zone of inhibition formed by alkaloid extract was significantly higher ($p<0.05$) than the saponin extract (Figure 4.6). Other phytochemical extracts namely flavonoid, steroid and tannin did not exhibit antimicrobial activity. In the phytochemical analysis of the extracts, respective phytochemicals were detected whereas alkaloid was not detected in the flavonoid, steroid and tannin extracts (Table 4.5). Hence the absence of alkaloid in these extracts could be related to the absence of antimicrobial activity.

The methanol extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish inhibited the growth of *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella enterica* ser. *typhi, Shigella flexneri* and *Vibrio cholerae* O139 while the aqueous extract did not inhibit the growth of all the test microorganisms (Figure 4.10). The result of the present was in accordance with the findings of Karaman *et al*., (2003) who reported that methanol extracts of *Juniperus oxycedrus* L. exhibited antimicrobial activity against different test microorganisms while the aqueous extract did not exhibit antimicrobial activity against any of the test microorganisms. The qualitative phytochemical analysis of the aqueous and the methanol extracts revealed the presence of alkaloid, anthocyanin,

flavonoid, glycoside, phenol, saponin, steroid and tannin (Table 4.5). Though, the phytochemical constituents were similar in both the extracts only methanol extract exhibited antimicrobial activity. It has been reported that the aromatic and saturated organic compounds which are active antimicrobial constituents are extracted better in methanol than in water (Cowan, 1999; Lawrence *et al*., 2009).

In the present study the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited higher antimicrobial activity than other phytochemical extracts. The extract inhibited the growth of *Escherichia coli*, *Shigella flexneri*, *Vibrio cholerae* O139 which are related to enteric diseases. In Sikkim *Capsicum annuum* var. *cerasiforme* locally called as '*Dalle Khorsani*' and generally termed as red cherry pepper is regularly consumed. It is believed to be useful in gastritis. It is considered as one of the hottest chilli in terms of Scoville rating of 100,000 to 350,000 Scoville Heat Units (SHU) (Bhutia *et al*., 2016). Capsaicin, a pungent principle of *Capsicum annuum* is also reported to have many biological activities. Its beneficial effects include gastrointestinal modulation by stimulating mucus secretion, inhibiting acid secretion and increasing gastric mucosal blood flow thereby preventing and curing gastric ulcer (Satyanarayana, 2006). Further, commercially available capsaicin has been investigated against *Helicobacter pylori*. The capsaicin inhibited the growth of *Helicobacter pylori*, a causative microorganism of peptic ulcer (Zeyrek and Oguz, 2005). In the present study, the GC-MS analysis of the alkaloid extract of *Capsicum annuum* var. *cerasiforme* detected the presence of Capsaicinoids including capsaicin (24.64 %) and dihydrocapsaicin (16.57 %) as two major alkaloids in the extract (Table 4.73). Dihydrocapsaicin also has reported antimicrobial activity (Nascimento *et al*., 2014).

Hence the observed antimicrobial activity in the extract could be due to the presence of high amount of capsaicin and dihydrocapsaicin in the extract.

In the case of *Dicentra scandens* (D. Don) Walp. the alkaloid extract inhibited the growth of all the test microorganisms. At the concentration of 400 mg/mL, the zone of inhibition formed by the alkaloid extract against most of the test microorganisms was significantly ($p<0.05$) larger than the zone of inhibition exhibited by the flavonoid, saponin and tannin extracts (Figure 4.11). The alkaloid extract inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enterica* ser. *typhi* at the lower concentration of 50 mg/mL. At the concentration of 50 mg/mL, the flavonoid extract inhibited the growth of *Bacillus cereus* and *Staphylococcus aureus* (Figure 4.14). Alkaloid extract revealed the presence of alkaloid, anthocyanin and glycoside. In the flavonoid, saponin, steroid and tannin extracts, the alkaloid was not detected (Table 4.6). Among all the test plants, the highest extraction yield of alkaloid (17.35±0.98 %) was obtained from *Dicentra scandens* (D. Don) Walp. Hence the presence of a high amount of alkaloid in the extract may have contributed to the higher antimicrobial activity than the other phytochemical extracts.

At the concentration of 400 mg/mL, the aqueous extract inhibited the growth of *Staphylococcus aureus* and *Proteus vulgaris* only. However, the methanol extract inhibited the growth of all the test microorganisms (Figure 4.15). The phytochemical analysis of the extracts revealed a similar phytochemical profile (Table 4.6). However, the difference in the antimicrobial activity could be related to the extraction efficiency of the solvents. In the present study the GC-MS analysis of the methanol extract of *Dicentra scandens* (D. Don) Walp. revealed the presence of two major alkaloids namely protopine

(53.78 %) and corydine (18.20 %) in the extract (Table 4.74). The total percentage of the alkaloid compounds was increased in the alkaloid extract (Table 4.75). Protopine is reported to have many biological activities including antibacterial activity (Vacek *et al*., 2010). Similarly, corydine has DNA damaging activity (Goren *et al*., 2003). Hence the presence of these alkaloid compounds along with other components in the methanol and the alkaloid extracts may have attributed for antimicrobial activity. Other compounds with a reported antimicrobial activity which were detected in the methanol extract were Pentadecanoic acid (1. 34 %), 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One (1.13 %) and 1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic acid (1.09 %) (Table 4.74). Pentadecanoic acid (2.65 %) and cis-9-Hexadecenal (1.83 %) were detected in the alkaloid extract (Table 4.75).

In Sikkim the root of *Dicentra scandens* (D. Don) Walp. is used by the traditional healers to treat various enteric infections including gastritis (Sharma and Sharma, 2010). In the present study, it was found that phytochemical extracts and the methanol extract of *Dicentra scandens* (D. Don) Walp. exhibited antimicrobial activity against *Escherichia coli, Klebsiella pneumoniae, Salmonella enterica* ser. *typhi* and *Shigella flexneri* which are related to enteric infections. Antimicrobial activity of the crude alkaloid extract of *Dicentra scandens* (D. Don) Walp. against *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Salmonella enterica typhi* has been reported. However, the growth of *Escherichia coli* was not inhibited by the extract (Nakhuru *et al*., 2014). In the present study, the alkaloid extract exhibited antimicrobial activity against *Escherichia coli*. The variation in the antimicrobial activity could be due to variation in the

geographical location of the plant and the strains of the test microorganisms used in the study (Jaradat *et al*., 2017; Kumar *et al*., 2015)

In the case of *Heracleum nepalense* D. Don at the concentration of 400 mg/mL, the alkaloid extract exhibited antimicrobial activity against most of the test microorganisms. The zone of inhibition formed by the extract was significantly ($p<0.05$) larger than the other phytochemical extracts (Figure 4.16). The alkaloid extract exhibited the zone of inhibition against *Bacillus cereus* and *Escherichia coli* even at the lower concentration of 50 mg/mL (Figure 4.19). The flavonoid extract of *Heracleum nepalense* D. Don inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*. The saponin extract showed zone of inhibition against *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio cholerae* O139. Tannin extract inhibited the growth of *Bacillus cereus* only. In the qualitative phytochemical analysis of the alkaloid extract, alkaloid, anthocyanin, glycoside and steroid were detected. In the flavonoid extract apart from flavonoid component, anthocyanin and phenol were detected. Saponin extract revealed the presence of anthocyanin, glycoside saponin and steroid. In steroid extract, anthocyanin and steroid were detected. In the tannin extract anthocyanin, phenol, saponin and tannin were detected (Table 4.7). Since alkaloid extract exhibited higher antimicrobial activity and inhibited the growth of most of the test microorganisms hence, it can be speculated that the antimicrobial activity of *Heracleum nepalense* D. Don could possibly be attributed to the alkaloid followed by the flavonoid and saponin.

At the concentration of 400 mg/mL, the methanol extract of *Heracleum nepalense* D. Don inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Vibrio cholerae* O139 (Figure 4.20). The methanol extract inhibited the growth of

Bacillus cereus even at the concentration of 25 mg/mL (Table 4.25). The aqueous extract did not exhibit antimicrobial activity against the test microorganisms. Bose *et al*., (2007) reported the antibacterial activity of methanol extract of roots of *Heracleum nepalense* D. Don against bacteria causing diarrhea namely *Shigella dysenteriae, Escherichia coli, Shigella boydii, Vibrio cholerae, Salmonella typhimurium*. In the present study the methanol extract of fruits of *Heracleum nepalense* D. Don. exhibited antimicrobial activity against four test microorganisms namely *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio cholerae* O139 (Table 4.25). *Vibrio cholerae* O139 causes cholera characterized by life-threatening secretory diarrhea (Finkelstein, 1996). This strain was associated with the emergence of epidemics of cholera in India and Bangladesh during 1992–1993 (Faruque *et al*., 2003). Since the extract inhibited the growth of these microorganisms hence it can be explored further as a source of potent antimicrobial agent.

The steroid extract of all the four test plants did not exhibit zone of inhibition against any of the test microorganisms under study. The percentage yield of steroid extract of all the four test plants was relatively low as compared to the other phytochemical extracts (Table 4.3). In the qualitative phytochemical analysis of the steroid extract, phytochemicals such as flavonoid, alkaloid and saponin which possibly could contribute to the microbial growth inhibiting property were not detected in the extract. Thus it can be speculated that absence of these major phytochemicals in the steroid extract could be related to the absence of antimicrobial activity in the extract.

In most of the test plants, the alkaloid extract inhibited the growth of most of the test microorganisms. Studies on antimicrobial activity of alkaloids from different plants have

been reported. Alkaloid extracts from *Callistemon citrinus* and *Vernonia adoensis* have been reported to exhibit antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Mabhiza *et al*., 2016).

The aqueous extract of all the test plants exhibited comparatively lesser antimicrobial activity. In some cases, the zone of inhibition was not observed with the extract against any of the test microorganisms. Similar results were reported by Ekwenye and Elegalam, (2005) where the aqueous extract of *Allium sativum* L. had no inhibitory effect on the test microorganism namely *Escherichia coli* and *Salmonella typhi* whereas the aqueous extract of *Zingiber officinale* Roscoe inhibited the growth of *Salmonella typhi* (Ekwenye and Elegalam, 2005).

5.2.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Various extracts of all the four plants exhibited different MIC and MBC values. Among all the extracts of all the test plants, the lowest MIC value of 3.125 mg/mL was exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. against *Staphylococcus aureus*. However, the extracts of all the test plants exhibiting antimicrobial activity were found to exhibit bactericidal activity as indicated by the MIC index which was greater than 4 (Table 4.26 to Table 4.29). The value of MIC index determines whether the plant extract is bactericidal or bacteriostatic in nature. When the MIC index is ≤ 4, the extract is bactericidal and when the MIC index is >4, the extract is bacteriostatic (Kone *et al*., 2007). For some test microorganisms the MBC values of some test extracts were same as the MIC values. Similar results were also reported by Kim *et al*., (1995) where the MBC

values of the test compounds were similar to the MIC values. This indicated that the test compounds at these concentrations showed inhibitory effect against bacteria by killing them (Kim *et al*., 1995).

5.3. Bacterial killing property by time kill assay

Based on the antimicrobial activity assay, the extracts of *Cyphomandra betacea* (Cav.) Sendth. showed potential antimicrobial activity followed by *Dicentra scandens* (D. Don) Walp., *Capasicum annuum* var. *cerasiforme* and *Heracleum nepalense* D. Don. Among the four test plants under study, two test plants namely *Cyphomandra betacea* (Cav.) Sendth. and *Dicentra scandens* (D. Don) Walp. were chosen for further study. The flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. were investigated further for time kill assay against *Escherichia coli* and *Staphylococcus aureus*. Among all the test phytochemical extracts, the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. exhibited the largest zone of inhibition (24.66±0.57 mm) against *Staphylococcus aureus* among the Gram positive test bacteria (Figure 4.1). The lowest value of MIC (3.125 mg/mL) against *Staphylococcus aureus* was also exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. (Table 4.26). Although the alkaloid extract of *Cyphomandra betacea* (Cav.) Sendth. also exhibited higher antimicrobial activity than the other phytochemical extracts, the zone of inhibition formed by the flavonoid extract was larger than that of the alkaloid extract against most of the test microorganisms including *Escherichia coli* and *Staphylococcus aureus* (Figure 4.1).

Similarly, in the case of *Dicentra scandens* (D. Don) Walp., alkaloid extract exhibited significantly ($p<0.05$) higher antimicrobial activity against all the test microorganisms than the other phytochemical extracts. At the concentration of 400 mg/mL, the extract exhibited largest zone of inhibition against *Escherichia coli* among the Gram negative test microorganism and against *Staphylococcus aureus* among the Gram positive test microorganism (Figure 4.11). Hence the time kill assay was performed with these extracts against the two test microorganisms.

With the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. the complete killing of *Escherichia coli* with more than two log reduction in bacterial count was observed at the concentrations of 2×MIC, MIC and 1/2×MIC at 30 min, 90 min and 120 min respectively (Figure 4.21 and Table 4.30). In the case of *Staphylococcus aureus* complete bacterial killing was observed at 60 min at the concentration of 2×MIC (Figure 4.22). However, more than two log reduction in the bacterial count was observed at 60 min and 120 min at the concentrations of 2×MIC and MIC respectively (Table 4.31). It was observed from the result that the extract was more effective against *Escherichia coli* than *Staphylococcus aureus.* The differences in the activity could be related to the MIC value of extracts against each test microorganism. The MIC value for *Escherichia coli* (25 mg/mL) was higher than that of *Staphylococcus aureus* (3.125 mg/mL).

With the alkaloid extract of *Dicentra scandens* (D. Don) Walp. the complete killing of *Escherichia coli* was observed at 90 min at the concentration of 2×MIC (Figure 4.23). With the alkaloid extract more than two log reduction in the bacterial count of *Escherichia coli* was observed at 90 min at the concentration of 2×MIC only (Table 4.32). In the case of *Staphylococcus aureus* with 2×MIC, complete bacterial killing was

observed at 120 min (Figure 4.24). At 2×MIC more than two log reduction in bacterial count was observed at 90 min (Table 4.33).

Saritha *et al*., (2015) have reported that the antibacterial activity is considered to be potent if there is more than two log reduction in the bacterial count. In the present study more than two log reduction implies to a value of log reduction ≥ 2.5. Hence, the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. exhibited potential antimicrobial activity at all the three concentrations that is 2×MIC, MIC and 1/2×MIC against *Escherichia coli* and at 2×MIC and MIC against *Staphylococcus aureus* causing more than two log reduction in the bacterial count. On the other hand, the alkaloid extract of *Dicentra scandens* (D. Don) Walp. exhibited two log reduction in bacterial count at the concentration of 2×MIC against *Escherichia coli* and *Staphylococcus aureus* thereby exhibiting potential antimicrobial activity at 2×MIC. At the concentration of MIC, 99.51 % reduction in bacterial count of *Escherichia coli* was observed at 120 min (Table 4.32). In case of *Staphylococcus aureus* treated at the concentration of MIC 97.58 % reduction in bacterial count was observed at 120 min (Table 4.33). Hence the extracts exhibited the time dependent killing of test microorganisms at concentration of MIC. The results of the time kill assay revealed that the extracts exhibited varying antimicrobial potential against the test microorganisms. It has been reported that the activity of the plant extract depends largely upon dose, type of microorganism, solvent and the plant extract used (Goyal *et al*., 2008).

Based on the time kill assay the mode of antimicrobial action of a compound can be speculated in two ways; the compounds affecting the membrane integrity (instantaneous action) or it can be affecting the cellular processes (time dependent) (Saritha *et al*., 2015).

In the present study the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. exhibited time and concentration-dependent killing of the test microorganisms possibly suggesting that the extracts may have affected the membrane integrity and thereby affecting the cellular processes. The untreated growth control showed a gradual increase in the bacterial count as time increased.

5.4. Cellular leakage property and scanning electron microscope study

Antimicrobial compounds act by different mechanisms which include interfering with cell wall synthesis, plasma membrane integrity, nucleic acid synthesis, ribosomal function and folate synthesis (Neu and Gootz, 1996). Many antimicrobial compounds that act on the bacterial cytoplasmic membrane induce the leakage of cellular materials absorbing at 260 nm (nucleic acid) and cellular materials absorbing at 280 nm (protein) (Sri *et al*., 2008). Hence to examine the possible mechanisms of antimicrobial action of the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. the cellular leakage assay was performed at MIC and 400 mg/mL concentration which was the highest concentration of the extract used in the present study. The present study revealed the leakage of cellular materials absorbing at 260 nm and 280 nm in the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspensions treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. (Figure 4.25 and Figure 4.26). The result possibly suggests that the nucleic acids and protein were lost through a damaged cytoplasmic membrane. It was observed that leakage of cellular materials absorbing at 280 nm was higher than that of the leakage of cellular materials absorbing at 260 nm in the supernatant of *Escherichia coli* and

Staphylococcus aureus suspensions treated with the extract (Figure 4.25 and Figure 4.26). The protein content in the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspensions treated with the extract was significantly ($p<0.05$) higher than that of untreated cells (Figure 4.27). The result indicated possible damage in the cell envelope of the bacterial cells treated with the plant extract which may have resulted in the leakage of protein.

In the case of *Dicentra scandens* (D.Don) Walp., there was a significant ($p<0.05$) time-dependent increase in the absorbance at 260 nm and 280 nm. The absorbance of the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspensions were increased after 30 min of incubation with the alkaloid extract of *Dicentra scandens* (D. Don) at MIC and at the concentration of 400 mg/mL (Figure 4.28 and Figure 4.29). Leakage of cellular materials absorbing at 280 nm was higher as compared to the leakage of cellular materials absorbing at 260 nm from both the test bacteria (Figure 4.28 and Figure 4.29). There was significant ($p<0.05$) increase in protein content over time. The protein content in the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspensions treated with the extract was significantly ($p<0.05$) higher than that of untreated cells (Figure 4.30).

Cellular leakage assay revealed the leakage of 260 nm and 280 nm absorbing material from the test microorganisms treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. Hence to further validate the cellular leakage property of the extracts scanning electron microscope study was performed. The scanning electron microscope study revealed that the bacterial cells treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the

alkaloid extract of *Dicentra scandens* (D. Don) Walp. at MIC and at the concentration of 400 mg/mL exhibited the adverse morphological changes including deformation of cell structures, cell wall breakage, swelling of cells and leakage of cellular contents from the treated bacterial cells as compared to the untreated control cells which exhibited normal cell morphology. An increased extent of membrane damage and leakage of cellular contents was observed at 400 mg/mL as compared to the bacterial cells treated at MIC (Figure 4.31 and Figure 4.32).

The cellular leakage property of the flavonoid extract could be due to the detergent properties of phenols and flavonoids (Saritha *et al*., 2015). In the present study, the qualitative phytochemical analysis of the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. revealed the presence of anthocyanin, flavonoid, glycoside and phenol (Table 4.4). Two major anthocyanins namely pelargonidin rutinoside and delphinidin rutinoside were reported in the red variety of Tamarillo (Mertz *et al*., 2009). Anthocyanin belongs to class flavonoid and has been reported to interact with other phytochemicals to potentiate the biological effects of the extract (Lila, 2004). It has been reported that the antimicrobial activity of anthocyanin-containing fruits and berries could be due to various mechanisms and synergies among the compounds such as anthocyanins, weak organic acids and phenolic acids. The antimicrobial action of the anthocyanin could be attributed to the membrane and intracellular interactions (Cisowska *et al*., 2011). In the case of *Dicentra scandens* (D. Don) Walp. the high amount of alkaloids were detected. Alkaloids such as corydine are known to possess DNA damaging activity (Goren *et al*., 2003). Hence the presence of these components in the extract may have damaged the bacterial cell thereby causing the leakage of cellular materials.

5.5. Synergistic antimicrobial effect of extracted phytochemicals against different test microorganisms

Development of antibiotic resistance during the monotherapy treatment of infected patients has led to the use of multiple combinations of antibacterial agents (El Solh and Alhajhusain, 2009; Jayaraman *et al*., 2010). Since decades natural products are used for the development of novel drugs to treat various bacterial infections. There are a number of reports suggesting that the plant extracts in combination with an antibiotic can potentiate the activity of antibiotics and can serve as a new strategy for an alternative therapy for treatment of various infections (Jayaraman *et al*., 2010). It has been reported that the secondary metabolites such as alkaloid, flavonoid, tannin, saponin, steroid, glycoside and terpenoid exist as a complex mixture in the crude extracts. Each of these compounds has different chemical functional groups and act on pathogens by binding to the specific target site (Enyiukwu *et al*., 2014). Hence the interaction among the phytochemicals and the combined actions of secondary metabolites determine their antimicrobial activity and thus the efficiency (Basri and Sandra, 2016; Efferth and Koch, 2011). Various studies reported that the whole or partially purified extract of a plant is more effective than the single isolated compound (Rasoanaivo *et al*., 2011; Williamson, 2001). Instead of isolated compounds, whole plant or plant parts or the mixtures of different plants are used in traditional medicine. There are reports suggesting that crude plant extracts exhibited greater *in vitro* or *in vivo* antiplasmodial activity than isolated constituents at the equivalent dose (Rasoanaivo *et al*., 2011).

In the present study possible synergistic interaction for antimicrobial activity was studied among the different phytochemicals of the test plants extracts using the checkerboard

assay. The phytochemical extracts exhibiting antimicrobial activity for almost all the test microorganisms in the agar well diffusion assay were selected for the checkerboard assay. The test microorganisms for which the largest zone of inhibition was observed at the concentration of 400 mg/mL were considered for the study.

In the case of *Cyphomandra betacea* (Cav.) Sendth. the combination of the flavonoid and the saponin extracts exhibited synergistic activity against *Staphylococcus aureus.* The extracts in combination at two concentrations (flavonoid extract at 3.125 mg/mL and saponin extract at 6.25 mg/mL) and (flavonoid extract at 3.125 mg/mL and saponin extract 12.5 at mg/mL) exhibited synergism ($\Sigma FIC \leq 0.5$) (Table 4.45 and Figure 4.35). The synergistic interactions suggest that the two compounds are acting possibly by different mechanisms on the target (Basri and Sandra, 2016; Wang *et al*., 2012). The flavonoid and saponin can be considered as a potential combination of phytochemicals with synergistic antimicrobial action against *Staphylococcus aureus. Staphylococcus aureus* is a pathogen of great concern because of its intrinsic virulence, ability to cause various life-threatening infections and development of resistance mechanism to various antibiotics including Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) (Lowy, 2003; Pantosti *et al*., 2007). Hence the combinations of the phytochemicals can be studied further against resistant strains of *Staphylococcus aureus*. All other combinations of the phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth. exhibited additive interaction against the test microorganisms ($0.5 < \Sigma FIC \leq 2$) (Table 4.43, Table 4.44, Figure 4.33 and Figure 4.34).

In the case of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish the alkaloid and the saponin extract in combination exhibited additive interaction against *Vibrio cholerae* O139 and *Bacillus cereus* ($0.5 < \Sigma FIC \leq 2$) (Table 4.46, Table 4.47 and Figure 4.36, Figure 4.37).

In the case of *Dicentra scandens* (D. Don) Walp. the combination of the flavonoid and the alkaloid extract exhibited additive interaction against *Staphylococcus aureus* at some of the concentrations ($0.5 < \Sigma FIC \leq 2$). However, at other concentrations the FIC index was in the range of indifference ($2 < \Sigma FIC \leq 4$). All other combinations of the phytochemical extracts exhibited additive effect against test microorganisms. (Table 4.48 - Table 4.50 and Figure 4.38 - Figure 4.40). The indifference interaction indicates that the combined effect of the two compounds or drugs is the same as the individual effect (Renneberg, 1993).

In the case of *Heracleum nepalense* D. Don all the combinations of the phytochemicals tested exhibited additive interaction (Table 4.51, Table 4.52 and Figure 4.41, Figure 4.42). In the present study most of the combinations of the phytochemical extracts of the test plants revealed that the interaction among the phytochemicals tested were additive. The antagonistic effect was not observed with the phytochemical extracts tested. However, further studies are required to identify the mechanism of interaction among the phytochemicals exhibiting antimicrobial activity.

There are a number of studies reporting the antimicrobial activity of the plant extracts in combination with antibiotics. Ofokansi *et al*., (2012) studied antibacterial activities of the combination of the leaf extract of *Phyllanthus muellerianus* and Ciprofloxacin against

Staphylococcus aureus a urogenital isolates and reported that some of the combinations revealed synergistic interaction. However the interaction was mostly indifferent and antagonist (Ofokansi *et al*., 2012). Some of the studies have reported the combinatorial effects of two test plant extracts. The acetone extracts of *Allium sativum* and *Citrus limon* on clinical isolates of *Staphylococcus aureus* exhibited the indifferent effect. The combination of the equal concentration of the extracts exhibited synergistic antimicrobial activity on *Streptococcus pyogenes* while increasing the concentration of *Allium sativum*, the combination revealed indifferent effect (Jumare *et al*., 2015).

5.6. Antioxidant activity

Plants synthesize numerous compounds which are essential to life and are highly beneficial to human health. Secondary metabolites from plants that include large families of phenolic compounds, alkaloids, terpenes, steroids, carotenes and many other types of phytochemicals function as an antioxidant (Lin, 1994). In the present study, various phytochemicals namely alkaloid, flavonoid, saponin, steroid and tannin, as well as the general extracts (aqueous and methanol), extracted from *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don. were evaluated for antioxidant activity. Various extracts of the test plants exhibited antioxidant activity in terms of DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, nitric oxide radical scavenging activity, hydroxyl radical scavenging activity and ferric reducing ability. All the extracts exhibited antioxidant activity in a concentration-dependent manner.

In the case of *Cyphomandra betacea* (Cav.) Sendth., among the phytochemical extracts, the flavonoid extract exhibited significantly ($p<0.05$) higher antioxidant activity in terms of DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, nitric oxide scavenging activity, hydroxyl radical scavenging activity and ferric reducing ability as compared to the other extracts. It was interesting to note that the ferric reducing ability of the flavonoid extract was significantly higher than that of the standard ascorbic acid (Figure 4.51). Among the general extracts, the methanol extract exhibited significantly higher DPPH free radical scavenging activity than the aqueous extract. The methanol extract at the concentrations of 60 µg/mL, 70 µg/mL, 80 µg/mL, 90 µg/mL and 100 µg/mL also showed significantly ($p<0.05$) higher ferric reducing ability than the ascorbic acid (Figure 4.52). The results of the present study were similar with the findings of Olugbami *et al.*, (2015) which stated that the ethanol extract of the stem bark of *Terminalia glaucescens* exhibited significantly higher reducing power activity than the standard ascorbic acid and butylated hydroxytoluene (Olugbami *et al.*, 2015). In the present study, the total phenolic content of 8.70 ± 0.03 mg GAE/g of extract and flavonoid content of 10.39 ± 0.60 mg RE/g of extract were estimated in the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. Among the general extracts of the test plant, the total phenolic content of 17.43±0.01 mg GAE/g of extract and the flavonoid content of 5.28±0.24 mg RE/g of extract were estimated in the methanol extract of *Cyphomandra betacea* (Cav.) Sendth. However total phenolic content of 3.02±0.81 mg GAE/g of extract and total flavonoid content of 2.18±0.15 mg RE/g of extract were estimated in the aqueous extract (Table 4.8). Hence the higher antioxidant activity of the methanol extract could be attributed to the comparatively higher amount of flavonoid and

phenolic compound in the extract. The phenolic content of 7.63 ± 0.37 mg GAE/g edible portion and the flavonoid content of 6.44 ± 0.16 mg CE/g edible portion were reported in the ethanol extract of Tamarillo fruits from Malaysia. Phenolic content of 1.83 ± 0.50 mg GAE/g edible portion and flavonoid content of 2.22 ± 0.31 mg CE/g edible portion were estimated in the water extract (Noor Atiqah *et al*., 2014). It has been reported that the extract from the fruit of *Cyphomandra betacea* contained a high amount of phenolics, flavonoids, anthocyanin and carotenoid which possibly could have contributed to the antioxidant activity of the extract (Hassan and Bakar, 2013). The flavonoid present in plants acts as antioxidants by stabilizing the reactive oxygen species such as superoxide anion, hydroxyl radical or peroxy radicals and also act as quenchers of singlet oxygen (Harborne and Williams, 2000; Nijveldt *et al.*, 2001). The antioxidant property of the phenolic compound is attributed to its ability to scavenge free radicals by virtue of its hydrogen donating ability thereby exhibiting antioxidant activity (Patil *et al*., 2009).

Capsicum species are a rich source of vitamin C, vitamin E and carotene which are an important antioxidant component (Palevitch and Craker, 1995). In the present study among the phytochemical extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, the flavonoid extract exhibited significantly ($p<0.05$) higher DPPH free radical scavenging activity and hydrogen peroxide scavenging activity (Figure 4.45 and Figure 4.61). The total flavonoid content of 10.90 ± 0.47 mg RE/g of extract (Table 4.9) was estimated in the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish which could be related to the higher antioxidant activity of the flavonoid extract. The saponin extract of *Capsicum annuum* var. *cerasiforme* showed higher ferric reducing ability (Figure 4.53). Saponins isolated from plants have been reported to possess ferric reducing power, DPPH

free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities (Gulcin *et al*., 2004).

In the case of general extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, the methanol extract exhibited significantly ($p<0.05$) higher hydrogen peroxide, nitric oxide and hydroxyl radical scavenging activities than the aqueous extract (Figure 4.62, Figure 4.70 and Figure 4.78). In the quantitative phytochemical analysis, total phenolic content of 7.41±0.03 mg GAE/g and 4.03±0.07 mg GAE/g was estimated in the methanol and the aqueous extracts respectively (Table 4.9). Since the antioxidant activity of the plant extracts is closely related to the concentration of the phenolic compound in the extracts (Shan *et al*., 2007). The comparatively high amount of phenolic compound in the methanol extract may have contributed to its higher antioxidant activity than the aqueous extract. *Capsicum* is one of the richest sources of the most powerful antioxidant substances including carotenoids, vitamin C and vitamin E (Howard *et al*., 2000). Various extracts from fruits of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited potential antioxidant activity. Since the fruits of the plant are commonly consumed in Sikkim hence the consumption of this species of *Capsicum* would possibly provide protection against oxidative damage.

In the case of *Dicentra scandens* (D. Don) Walp., the phytochemical extracts exhibited varying antioxidant activity. The flavonoid extract exhibited significantly ($p<0.05$) higher DPPH free radical scavenging activity and nitric oxide scavenging activity than the other phytochemical extracts of the plant (Figure 4.47 and Figure 4.71). The saponin extract exhibited higher ferric reducing ability than the other phytochemical extracts of the test plant (Figure 4.55). The tannin extract exhibited higher hydrogen peroxide scavenging

activity than the other phytochemical extracts (Figure 4.63). The highest tannin content of 36.24±0.03 mg TAE/g of extract was estimated in the tannin extract of *Dicentra scandens* (D. Don) Walp. (Table 4.10). The antioxidant activity of the extract could be due to the presence of phenolic constituents including tannin in the extract.

Among the general extracts of *Dicentra scandens* (D. Don) Walp. the methanol extract exhibited significantly ($p<0.05$) higher ferric reducing ability, nitric oxide radical scavenging activity and hydroxyl radical scavenging activity than the aqueous extract (Figure 4.56, Figure 4.72 and Figure 4.80). Total phenolic content of 12.40 ± 0.03 mg GAE/g of extract and 5.05 ± 0.20 mg GAE/g of extract were estimated in the methanol and aqueous extracts respectively (Table 4.10). Hence comparatively higher antioxidant activity of the methanol extract could be related to the presence of relatively high phenolic content in the extract.

In the case of *Heracleum nepalense* D. Don, the saponin extract exhibited significantly ($p<0.05$) higher DPPH free radical scavenging activity than the other phytochemical extracts (Figure 4.49). Saponin has been reported to exhibit antioxidant activity. The DPPH radical scavenging activity of saponin extract from *Abutilon indicum* leaves has been reported by Lokesh *et al*., (2016). The flavonoid extract exhibited higher hydrogen peroxide scavenging activity (Figure 4.65). The tannin extract exhibited significantly ($p<0.05$) higher ferric reducing ability and nitric oxide scavenging activity than the other phytochemical extracts (Figure 4.57 and Figure 4.73). In the case of *Heracleum nepalense* D. Don, the total tannin content of 8.07±0.02 mg TAE/g of extract was estimated in the tannin extract (Table 4.11). Tannin has been reported to possess potent antioxidant property (Hagerman, 2002). Tannins act as primary antioxidants by donating

hydrogen atom or electrons and also function as secondary antioxidants. Tannins possess the capacity to chelate metal ions such as Fe (II). It affects the Fenton reaction steps and hence prevents the oxidation process (Amarowicz, 2007). Among the general extracts of *Heracleum nepalense* D. Don, the methanol extract exhibited significantly ($p<0.05$) higher hydrogen peroxide, nitric oxide and hydroxyl radical scavenging activities (Figure 4.66, Figure 4. 74 and Figure 4.82).

Among all the test plant extracts the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and the saponin extract of *Heracleum nepalense* D. Don exhibited the lowest IC_{50} value of 20 µg/mL in terms of DPPH free radical scavenging potential (Table 4.54 and Table 4.56). Similarly the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. followed by the methanol extract exhibited significantly higher ferric reducing ability than the other extracts as well as the standard ascorbic acid (Table 4.57, Figure 4.51 and Figure 4.52). In terms of the hydrogen peroxide scavenging potential, the lowest IC_{50} value of 10 µg/mL was exhibited by the flavonoid extract of *Heracleum nepalense* D. Don which was same as that of standard ascorbic acid (Table 4.64). The flavonoid extracts of *Cyphomandra betacea* (Cav.) Sendth. and *Dicentra scandens* (D.Don) Walp. exhibited the lowest IC_{50} value of 40 µg/mL in terms of nitric oxide radical scavenging potential (Table 4.65 and Table 4.67). Finally, the lowest IC_{50} value of 50 µg/mL was shown by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. thereby exhibiting the highest hydroxyl radical scavenging potential (Table 4.69). The phytochemical extracts, as well as the aqueous and the methanol extracts, exhibited a difference in scavenging potential of various free radicals and reduction of ferric ions which could be due to variation in the amount of phytoconstituents extracted in various

solvents (Pavithra and Vadivukkarasi, 2015). Among the phytochemical extracts of all the test plants studied, mostly the flavonoid extract exhibited the highest antioxidant activity. In some assay, the tannin, as well as saponin extracts, has shown the highest free radical scavenging activity and ferric reducing ability. Several studies suggested that phenolics such as flavonoid and tannin are well-known antioxidants since these secondary metabolites exhibited promising *in vitro* and *in vivo* antioxidant property (Kasote *et al.*, 2015). Similarly, saponin is reported to possess antioxidant activity (Gulcin *et al*., 2004). On the other hand various *in vitro* antioxidant assays reported that the radical scavenging potential of alkaloid is apparently moderate to nonexistent (Kasote *et al*., 2015).

6. CONCLUSION

- In the present study phytochemical extracts (alkaloid, flavonoid, saponin, steroid and tannin) of the four test plants namely *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don were evaluated for antimicrobial and antioxidant properties. General extracts (aqueous and methanol) of the test plants were also investigated for antimicrobial and antioxidant properties.

- The extracts exhibited a varying level of antimicrobial properties. Among the phytochemical extracts alkaloid, flavonoid, saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. exhibited antimicrobial activity against both Gram positive and Gram-negative test bacteria. The flavonoid extract exhibited significantly higher antimicrobial activity against most of the test microorganisms than the other phytochemical extracts. In the case of *Dicentra scandens* (D. Don) Walp. the alkaloid extract followed by the flavonoid extract inhibited the growth of most of the test microorganisms. The alkaloid extract exhibited significantly higher antimicrobial activity than the other phytochemical extracts. In *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don, the alkaloid extract exhibited higher antimicrobial activity than the other phytochemicals extracts.

- Among the general extracts of the test plants, methanol extract exhibited higher antimicrobial activity than the aqueous extract. The methanol extract of

Cyphomandra betacea (Cav.) Sendth. and *Dicentra scandens* (D. Don) Walp. inhibited the growth of all the test microorganisms.

- The lowest MIC value was exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. against *Staphylococcus aureus*. Based on the MIC index value, the test extracts exhibiting antimicrobial property were found to be bactericidal in nature.

- With respect to antimicrobial property, *Cyphomandra betacea* (Cav.) Sendth. followed by *Dicentra scandens* (D. Don) Walp. exhibited potential antimicrobial activity.

- The flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. were analyzed for time kill assay against *Escherichia coli* and *Staphylococcus aureus.* The extracts exhibited time-dependent killing of bacteria with more than two log reduction in the bacterial count.

- Bacterial cells treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. exhibited the leakage of cellular materials absorbing at 260 nm and 280 nm.

- The scanning electron microscope (SEM) study also revealed that the bacterial cells treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. showed deformed cell structure, breakage of cells and leakage of cellular contents.

- In the checkerboard assay, the combination of the flavonoid and the saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. exhibited synergistic activity. While in most of the combination of the phytochemical extracts of all the test plants against test microorganisms, the interaction was mostly additive.

- The phytochemical extracts also exhibited potential antioxidant activity in terms of free radical scavenging activity and ferric reducing ability. The flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. exhibited higher free radical scavenging activity than the other phytochemical extracts of the plant. The flavonoid extract exhibited significantly higher ferric reducing ability than the ascorbic acid.

- In the case of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, mostly the flavonoid extract exhibited higher free radical scavenging activity. The saponin extract exhibited higher ferric reducing ability than the other phytochemical extracts.

- Among the phytochemical extracts of *Dicentra scandens* (D. Don) Walp. varying antioxidant activity was exhibited by the phytochemical extracts. The flavonoid extract exhibited higher DPPH radical and nitric oxide radical scavenging activity. The saponin extract showed higher ferric reducing ability.

- In *Heracleum nepalense* D. Don mostly the tannin extract exhibited higher free radical scavenging activity and ferric reducing ability. The flavonoid extract exhibited significantly higher hydrogen peroxide scavenging activity than the other phytochemical extracts. In terms of hydrogen peroxide scavenging activity,

the concentration of the flavonoid extract at which the lowest IC_{50} value achieved was 10 µg/mL, which was the same as that of standard ascorbic acid.

- Among the general extracts of all the four test plants, the methanol extract exhibited higher free radical scavenging activity and ferric reducing ability in most of the antioxidant assays as compared to the aqueous extract.

- Characterization of the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish by GC-MS analysis revealed the presence of capsaicin and dihydrocapsaicin as two major alkaloids in the extract. The GC-MS analysis of the methanol and the alkaloid extracts of *Dicentra scandens* (D. Don) Walp. detected protopine and corydine as major alkaloid compound in the extracts.

- From the present study, it was observed that the phytochemicals namely flavonoid, alkaloid, saponin are the important components that possibly contribute to the antimicrobial property of the test plants. The phenolic compounds, most importantly flavonoid and tannin are the crucial antioxidant components. Among the general extracts, the methanol extract exhibited potential antimicrobial and antioxidant activities. The efficacy of the solvent to extract these phytochemical may have resulted in the higher bioactivity. Hence, it can be inferred from the results that the synergistic interaction among these phytochemicals in the extracts plays a crucial role in therapeutic efficacy.

- Antimicrobial resistance is a global problem. Phytochemicals are the rich source of antimicrobial agents and can serve as an alternative source. Similarly, intake of

natural dietary antioxidants from plants helps in combating oxidative stress thereby preventing many disease conditions including cancer.

- The phytochemical extracts of the test plants exhibited potential antimicrobial and antioxidant activities. The results of the present study indicated that the fruits of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Heracleum nepalense* D. Don and the roots of *Dicentra scandens* (D. Don) Walp. are the rich source of many bioactive compounds. The antimicrobial and antioxidant properties could be attributed to these bioactive compounds. Hence, these extracts can be explored further for therapeutic purposes with respect to antimicrobial action and in combating oxidative stress.

7. SUMMARY

Plants have been used since antiquity to prevent and treat various diseases. Most of the medicinal properties of the plants have been attributed to the presence of the phytochemicals in the plants. In addition, the major source of nutritional requirements in our diet is fulfilled by plant sources. Due to the emergence of antibiotic-resistant strains of microorganisms to commonly used antibiotics, phytochemicals serve as an alternative source of antimicrobial agent. In the past decades, antioxidants from natural sources including plants have attracted considerable interest among nutritionists and consumers due to their safety and potential therapeutic properties.

Sikkim, a North Eastern state of India has been identified as one of the biodiversity hotspots in the Eastern Himalayas. The region harbors a number of species of plants and also has a rich culture of traditional medicine. There are numerous plants which still remain unexplored for its therapeutic properties. Hence in the present study four plants were selected from Sikkim namely *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don. *Cyphomandra betacea* (Cav.) Sendth and *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are used in culinary preparations and also possess medicinal value. *Dicentra scandens* D. Don Walp. and *Heracleum nepalense* D. Don are used in the traditional medicine.

The therapeutic properties including antimicrobial and antioxidant properties of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don from

this region has not been examined in detail. The medicinal properties of the plants are attributed to the phytochemicals and their synergistic interactions. Phytochemicals such as alkaloid, flavonoid, saponin, steroid and tannin are reported to possess various biological properties including antimicrobial and antioxidant properties. Hence, the present study was intended to extract these phytochemicals from the fruits of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Heracleum nepalense* D. Don and the roots of *Dicentra scandens* (D. Don) Walp. and to evaluate the antimicrobial and antioxidant properties of various phytochemicals extracted from these test plants.

For the extraction of the phytochemicals, respective solvents and specific extraction procedures were used. Further, the general extracts namely aqueous and methanol extracts were also prepared and were studied for antimicrobial and antioxidant properties.

The percentage yield of various extracts of the test plants was estimated. Among all the extracts the highest yield of 29.09±1.40 % was obtained from tannin extract of *Dicentra scandens* (D. Don) Walp. While the steroid extract has the least percentage yield among all the extracts of the test plants.

The phytochemical analysis of the extracts revealed the presence of various phytochemicals namely alkaloid, flavonoid, saponin, steroid, tannin, anthocyanin, glycoside, carbohydrate, protein and fat. In the specific extracts (phytochemical extracts) mostly the respective phytochemical along with few other phytoconstituents were detected. However, in the general extracts (aqueous and methanol) most of the phytochemicals were detected. Quantitative phytochemical

analysis was performed to determine the total phenolic content, total flavonoid content, total tannin content in terms of milligrams of gallic acid equivalent (GAE) per gram of extract, rutin equivalent (RE) per gram of extract and tannic acid equivalent (TAE) per gram of extract respectively. Among the test plants extracts, the highest phenolic content of 17.43±0.01 mg GAE/g of extract was estimated in the methanol extract of *Cyphomandra betacea* (Cav.) Sendth. Highest flavonoid content of 10.90±0.47 mg RE/g and 10.39±0.60 mg RE/g of extracts were estimated respectively in the flavonoid extracts of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish and *Cyphomandra betacea* (Cav.) Sendth. Similarly, the highest tannin content of 36.24±0.38 mg TAE/g of extract was estimated in the tannin extract of *Dicentra scandens* (D. Don) Walp.

The phytochemical extracts namely alkaloid, flavonoid, saponin, steroid, tannin as well as aqueous and methanol extracts of fruits of *Cyphomandra betacea* (Cav.) Sendth., *Capsicum annuum* var. *cerasiforme* (Mill.) Irish, *Heracleum nepalense* D. Don and roots of *Dicentra scandens* (D. Don) Walp. were evaluated for antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella enterica* ser. *typhi, Shigella flexneri* and *Vibrio cholerae* O139 using agar well diffusion method. The extracts exhibited varying levels of antimicrobial activity in terms of the diameter of the zone of inhibition.

In the case of *Cyphomandra betacea* (Cav.) Sendth., at the concentration of 400 mg/mL, the phytochemical extracts namely alkaloid, flavonoid, saponin inhibited the growth of all the test microorganisms. Tannin extract inhibited the growth of *Shigella flexneri* and *Vibrio cholerae* O139. Methanol extract exhibited

antimicrobial activity against all the test microorganisms. The aqueous extract inhibited the growth of *Vibrio cholerae* O139 and *Bacillus cereus*.

Similarly at the concentration of 400 mg/mL, the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited antimicrobial activity against all the nine test microorganisms. The zone of inhibition formed by alkaloid extract was significantly ($p<0.05$) larger than the other extracts. The zone of inhibition was not observed with the flavonoid and tannin extracts. Among the general extracts, the methanol extract inhibited the growth of some of the test microorganisms. Zone of inhibition was not observed with the aqueous extract.

In case of *Dicentra scandens* (D. Don) Walp., the alkaloid extract inhibited the growth of all the test microorganisms. At the concentration of 400 mg/mL, the alkaloid extract exhibited significantly ($p<0.05$) higher antimicrobial activity than the other phytochemical extracts. Phytochemical extract namely flavonoid, saponin and tannin also exhibited varying antimicrobial activity against test microorganisms. Among the general extracts, the methanol extract inhibited the growth of all the test microorganisms. The aqueous extract inhibited the growth of *Proteus vulgaris* and *Staphylococcus aureus.* In Sikkim, the root of *Dicentra scandens* (D. Don) Walp. is used by traditional healers to treat various enteric infections including gastritis. In the present study, it was found that phytochemical extracts and the methanol extract of *Dicentra scandens* (D. Don) Walp. exhibited antimicrobial activity against *Escherichia coli* and *Shigella flexneri* which are related to enteric infections.

At the concentration of 400 mg/mL, the alkaloid extract of *Heracleum nepalense* D. Don exhibited antimicrobial activity against most of the test microorganism.

The extract exhibited significantly ($p<0.05$) higher antimicrobial activity than the other phytochemical extracts. Flavonoid, saponin and tannin extracts exhibited antimicrobial activity mostly against Gram-positive test bacteria. The methanol extract of *Heracleum nepalense* D. Don exhibited antimicrobial activity, whereas the zone of inhibition was not observed with the aqueous extract.

Among all the phytochemical extracts of the test plants, the steroid extract did not exhibit the zone of inhibition against any of the test microorganisms under study.

The minimum inhibitory concentration (MIC) assay was performed by broth macrodilution method. The minimum bactericidal concentration (MBC) was also determined. The MIC values for various extracts ranged from 3.125 mg/mL to 200 mg/mL against the different test microorganisms. The MBC values ranged from 6.25 mg/mL to 200 mg/mL. Based on MIC index values, the extracts exhibiting antimicrobial activity from all the test plants were bactericidal in nature.

The flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. inhibited growth of all the test microorganisms. The extract exhibited significantly higher antimicrobial activity than the alkaloid extract against most of the test microorganisms under study. As compared to the other extracts the flavonoid extract exhibited the largest zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*. Among all the test extracts of the plants under study, the lowest MIC value of 3.125 mg/mL was exhibited by the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. against *Staphylococcus aureus.* Similarly the alkaloid extract of *Dicentra scandens* (D. Don) Walp. also exhibited significantly higher antimicrobial activity than the other phytochemical extracts of the plant. The extract showed the largest zone of inhibition against *Escherichia*

coli and *Staphylococcus aureus.* Hence, the two potential extracts namely the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. were analyzed further for time kill assay and membrane damaging properties against *Escherichia coli* and *Staphylococcus aureus*. The extracts exhibited potent time and dose-dependent bacterial killing property exhibiting more than two log reduction in bacterial count. The extracts exhibited membrane damaging property resulting in the leakage of cellular material absorbing at 260 nm and 280 nm which are in the range of nucleic acid and protein. With respect to cellular leakage, the leakage of cellular material absorbing at 280 nm was higher than the leakage of material absorbing at 260 nm. The protein content of the supernatant of *Escherichia coli* and *Staphylococcus aureus* suspension treated with the extracts was significantly ($p<0.05$) higher than that of untreated bacteria.

To further validate the possibility of membrane damaging property of the extracts, scanning electron microscope (SEM) study was performed. The SEM analysis revealed that the test bacteria treated with the flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. exhibited cell wall breakage, swelling of cells and leakage of cellular contents. The untreated bacterial cell revealed normal cell morphology.

To evaluate the possible synergistic antimicrobial property of the extracted phytochemicals checkerboard assay was performed. Based on the fractional inhibitory concentration (FIC) index value, the combination of flavonoid and saponin extracts of *Cyphomandra betacea* (Cav.) Sendth. exhibited synergistic activity against *Staphylococcus aureus* ($\Sigma FIC \leq 0.5$). The extracts in combination exhibited synergism at two concentrations (flavonoid extract 3.125 mg/mL and

saponin extract 6.25 mg/mL) with FIC index of 0.375 and (flavonoid extract 3.125 mg/mL and saponin extract 12.5 mg/mL) with FIC index of 0.5. Most of the combinations of the phytochemicals from all the four test plants exhibited additive interaction ($0.5 < \Sigma FIC \leq 2$) against the test microorganisms. The phytochemicals in combination may possibly have interacted thereby increasing the efficacy of each component in imparting additive and synergistic activity.

In the present study, various extracts of the test plants were evaluated for antioxidant activity. The extracts exhibited antioxidant activity in terms of DPPH free radical scavenging activity, hydrogen peroxide radical scavenging activity, nitric oxide radical scavenging activity, hydroxyl radical scavenging activity and ferric reducing antioxidant power. All the extracts exhibited antioxidant activity in a concentration-dependent manner. Mostly the flavonoid extract of all the test plants exhibited higher antioxidant activity than the other phytochemical extracts.

Among all the phytochemical extracts of *Cyphomandra betacea* (Cav.) Sendth. the flavonoid extract exhibited significantly higher free radical scavenging activity and ferric reducing ability than the other phytochemical extracts. The flavonoid extract showed significantly ($p<0.05$) higher ferric reducing antioxidant power than the ascorbic acid. In the quantitative phytochemical analysis, the total flavonoid content of 10.39±0.60 mg RE/g of extract was estimated in the extract which could possibly relate to the higher antioxidant activity. Among the general extracts, mostly the methanol extract exhibited higher antioxidant activity as compared to the aqueous extract.

Similarly the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish exhibited significantly ($p<0.05$) higher DPPH radical and hydrogen peroxide

scavenging activities. Highest flavonoid content of 10.90±0.47 mg RE/g of extract was estimated in the flavonoid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish which could be attributed to the higher antioxidant activity of the flavonoid extract. The saponin extract of the plant exhibited higher hydroxyl radical, nitric oxide radical scavenging activities and ferric reducing ability. Among the general extracts, the methanol extract exhibited higher antioxidant activity than the aqueous extract.

Flavonoids are polyphenolic compounds produced by plants and are the rich source of natural antioxidant. These components are essentially supplied to the human body through diet. Fruits of the test plants under study namely *Cyphomandra betacea* (Cav.) Sendth. and *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are taken in the diet. Since various extracts of the plants exhibited potential antioxidant activity hence the consumption of these plant species can be beneficial in combating oxidative stress.

In the case of *Dicentra scandens* (D. Don) Walp. the flavonoid extract exhibited significantly ($p<0.05$) higher DPPH radical scavenging and nitric oxide radical scavenging activity as compared to other extracts. The tannin extract exhibited higher hydrogen peroxide scavenging activity. The highest tannin content of 36.24±0.38 mg TAE/g of extract was estimated in the tannin extract of *Dicentra scandens* (D. Don) Walp. Hence the activity of the extract could be attributed to the presence of a high amount of tannin in the extract. The saponin extract exhibited higher ferric reducing antioxidant activity. Among the general extracts, mostly the methanol extract exhibited higher antioxidant activity than the aqueous extract.

In the case of *Heracleum nepalense* D. Don, the saponin extract exhibited significantly ($p<0.05$) higher DPPH radical scavenging activity than the other phytochemical extracts. Free radical scavenging potential was found to vary among the extracts. The flavonoid extract exhibited significantly ($p<0.05$) higher hydrogen peroxide scavenging activity. In terms of hydrogen peroxide scavenging activity, the IC_{50} value of 10 µg/mL exhibited by the flavonoid extract was the same as that of standard ascorbic acid.

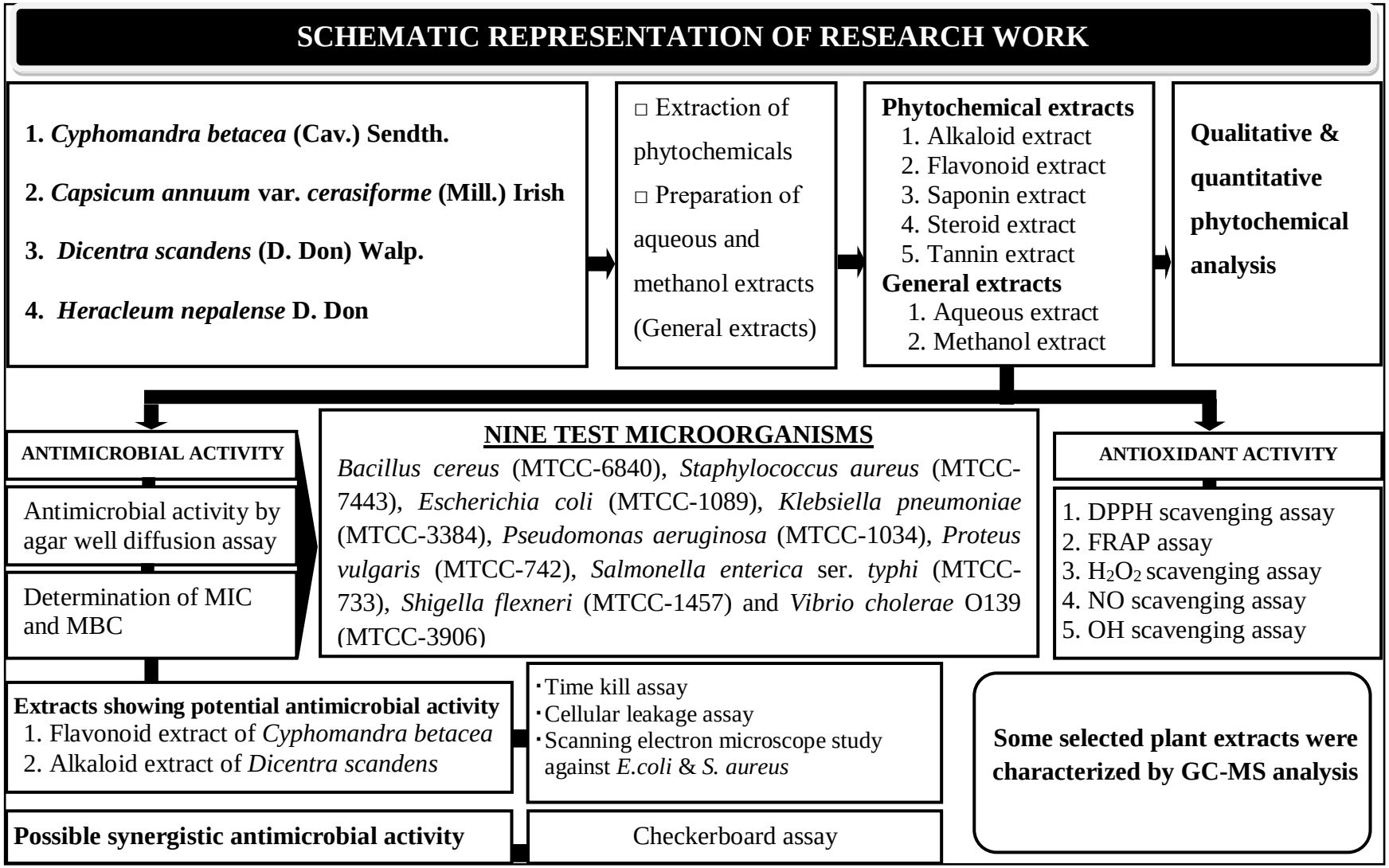
Capsicum annuum var. *cerasiforme* (Mill.) is a valuable cash crop of Sikkim. It is also useful in gastritis. *Dicentra scandens* (D. Don) Walp. is a rare species of medicinal plant in this region and is used to cure enteric diseases by the traditional healers. The alkaloid extract of both the test plants exhibited potential antimicrobial activity against the test microorganisms. Hence the extracts from these plant species were subjected to GC-MS analysis to know the bioactive constituents in the extracts. The analysis of the extracts revealed the presence of various bioactive components in the extracts which could be responsible for the antimicrobial activity.

The GC-MS analysis of the alkaloid extract of *Capsicum annuum* var. *cerasiforme* (Mill.) Irish revealed the presence of Capsaicinoids including capsaicin and dihydrocapsaicin as two major alkaloids in the extracts. The analysis of the methanol and the alkaloid extracts of *Dicentra scandens* (D. Don) Walp. revealed the presence of alkaloids such as protopine and corydine as the predominant components in the extracts.

The plants investigated in this study namely *Cyphomandra betacea* (Cav.) Sendth. and *Capsicum annuum* var. *cerasiforme* (Mill.) Irish are part of the diet and also

possess medicinal properties. Similarly, *Dicentra scandens* (D. Don) Walp. and *Heracleum nepalense* D. Don are used in folklore medicine. The results of the present study revealed that the various phytochemical extracts, as well as the general extracts, exhibited significant ($p<0.05$) antimicrobial and antioxidant properties. The flavonoid extract of *Cyphomandra betacea* (Cav.) Sendth. and the alkaloid extract of *Dicentra scandens* (D. Don) Walp. revealed significant membrane damaging property resulting in the leakage of cellular materials. Hence this study provides baseline data for the exploration of the phytochemicals as therapeutic agents to develop new antimicrobial agents. The phytochemical extracts can be further purified and investigated to develop the natural antioxidants.

Further studies are required for the purification of the phytochemical extracts using different techniques and their possible modes of antibacterial and antioxidant activities can be determined.


SCHEMATIC REPRESENTATION OF RESEARCH WORK
1. Cyphomandra betacea (Cav.) Sendth.
2. Capsicum annuum var. cerasiforme (Mill.) Irish
3. Dicentra scandens (D. Don) Walp.
4. Heracleum nepalense D. Don
□ Extraction of phytochemicals
□ Preparation of aqueous and methanol extracts (General extracts)
Phytochemical extracts
1. Alkaloid extract
2. Flavonoid extract
3. Saponin extract
4. Steroid extract
5. Tannin extract
General extracts
1. Aqueous extract
2. Methanol extract
Qualitative & quantitative phytochemical analysis
ANTIMICROBIAL ACTIVITY
Antimicrobial activity by agar well diffusion assay
Determination of MIC and MBC
NINE TEST MICROORGANISMS
Bacillus cereus (MTCC-6840), Staphylococcus aureus (MTCC-7443), Escherichia coli (MTCC-1089), Klebsiella pneumoniae (MTCC-3384), Pseudomonas aeruginosa (MTCC-1034), Proteus vulgaris (MTCC-742), Salmonella enterica ser. typhi (MTCC-733), Shigella flexneri (MTCC-1457) and Vibrio cholerae O139 (MTCC-3906)
ANTIOXIDANT ACTIVITY
1. DPPH scavenging assay
2. FRAP assay
3. H2O2 scavenging assay
4. NO scavenging assay
5. OH scavenging assay
Extracts showing potential antimicrobial activity
1. Flavonoid extract of Cyphomandra betacea
2. Alkaloid extract of Dicentra scandens
·Time kill assay
·Cellular leakage assay
·Scanning electron microscope study against E.coli & S. aureus
Some selected plant extracts were characterized by GC-MS analysis
Possible synergistic antimicrobial activity
Checkerboard assay

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