Evaluation of some selected medicinal plants of Sikkim Himalaya for efficacy against rheumatoid arthritis

A Thesis Submitted

To

Sikkim University

In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

Ms. Aita Rani Subba (Limboo)

Department of Botany

School of Life Sciences

January 2022

Declaration

I hereby declare that the work which is being presented in this thesis entitled **"Evaluation of some selected medicinal plants of Sikkim Himalaya for efficacy against rheumatoid arthritis"** is for the fulfillment of the requirement for the award of Degree of **Doctor of Philosophy** submitted in the **Department of Botany, Sikkim University, Sikkim** is an authentic record of my work carried out under the supervision of **Dr. Santosh Kumar Rai**, Assistant Professor, Department of Botany, Sikkim University, Sikkim.

The work has not formed the basis for the award of any other degree or diploma, in this or any other Institution or University. In keeping with the ethical practice in reporting scientific information, due acknowledgments have been made wherever the findings of others have been cited.

Aita Rani Subba

Place: Gangtok
Date: 27/12/2021

Aita Rani Subba (Limboo)
Regd. No. 14/PhD/BOT/03
Ph. D Scholar
Department of Botany
School of Life Sciences
Sikkim University, Gangtok

6 माइल, सामदुर, तादोंग - 737102
गंगटोक, सिक्किम, भारत
फोन-03592-251212, 251415, 251656
टेलीफैक्स - 251067
वेबसाइट - www.cus.ac.in

6th Mile, Samdur, Tadong-737102
Gangtok, Sikkim, India
Ph. 03592-251212, 251415, 251656
Telefax : 251067
Website : www.cus.ac.in

सिक्किम विश्वविद्यालय
SIKKIM UNIVERSITY

(भारत के संसद के अधिनियम द्वारा वर्ष 2007 में स्थापित और नैक (एनएएसी) द्वारा वर्ष 2015 में प्रत्यायित केंद्रीय विश्वविद्यालय)
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CERTIFICATE

This is to certify that the Ph.D thesis entitled **"Evaluation of some selected medicinal plants of Sikkim Himalaya for efficacy against rheumatoid arthritis"** submitted to Sikkim University in partial fulfillment for the requirements of the degree of Doctor of Philosophy in Botany embodies the research work carried out by **Ms. Aita Rani Subba (Limboo)** at the Department of Botany, School of Life Sciences, Sikkim University. It is a record of a bonafide investigation carried out and completed by her under my supervision. She has followed the rules and regulations prescribed by the University. The results are original and have not been submitted anywhere else for any other degree or diploma.

Place: Gangtok
Date: 27/12/2021

Dr. Santosh Kumar Rai
Assistant Professor
Ph.D Supervisor
Department of Botany
School of Life Sciences
Sikkim University, Gangtok

6 माइल, सामदुर, तादोंग -737102
गंगटोक, सिक्किम, भारत
फोन-03592-251212, 251415, 251656
टेलीफैक्स -251067
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6th Mile, Samdur,Tadong -737102
Gangtok, Sikkim, India
Ph. 03592-251212, 251415, 251656
Telefax: 251067
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It is recommended that this Ph.D thesis be placed before the examiners for the evaluation.

Place: Gangtok
Date: 27/12/2021

Dr. Dhani Raj Chhetri
Professor/InCharge
Department of Botany
School of Life Sciences
Sikkim University, Gangtok

6 माइल, सामदुर, तादोंग - 737102
गंगटोक, सिक्किम, भारत
फोन-03592-251212, 251415, 251656
टेलीफैक्स - 251067
वेबसाइट - www.cus.ac.in

6th Mile, Samdur, Tadong-737102
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Ph. 03592-251212, 251415, 251656
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सिक्किम विश्वविद्यालय
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"Evaluation of some selected medicinal plants of Sikkim Himalaya for efficacy against rheumatoid arthritis"

Submitted by **Ms. Aita Rani Subba (Limboo)** under the supervision of **Dr. Santosh Kumar Rai**, Department of Botany, School of Life Sciences, Sikkim University

Aita Rani Subba.
Aita Rani Subba (Limboo)

Signature of the candidate

Dr. Santosh Kr. Rai
Assistant Professor
Department of Botany

27/12/2021
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Acknowledgements

This research work leading to a doctoral degree is an enduring and determined endeavor of my life. It would not have been accomplished without the selfless support and encouragement of many persons. I hereby take this opportunity to deliver my appreciation to all who have made this project fruition possible for me. Above all, I thank God for giving me the energy and astuteness necessary to understand and carry out this research project.

I am grateful to the Almighty for his fortune, grace, and benevolence upon my life and for his divine enablement. I am deeply indebted and wish to express my genuine gratitude to my supervisor, **Dr. Santosh Kumar Rai**, Department of Botany, School of Life Sciences, Sikkim University, for his invaluable guidance, encouragement, basic remarks, and constant motivation throughout this study.

I am deeply grateful to Professor, Dr. **Shanti S. Sharma**, Head, Department of Botany, Sikkim University for giving me the necessary facilities, help, and support in carrying out this study. I extend my appreciation to Dr. **Dhani Raj Chhetri**, Associate Professor, Department of Botany, Sikkim University, and Dr. **N. Satyanarayana**, Professor, Department of Botany, Sikkim University for their invaluable suggestions and support. I express my profound gratefulness to Dr. **Arun Chettri**, Assistant Professor, Dr. **N. Bijayalaxmi Devi**, Assistant Professor, and Dr. **Arun Kumar Rai**, Assistant Professor, Department of Botany, Sikkim University for their help and moral support during my research work.

It gives me great pleasure and I feel it's my solemn duty to express my endeavoring sincere thanks to traditional herbal healers of Sikkim Himalaya for sharing their

valuable knowledge and to BSI, Sikkim Himalayan Circle, Gangtok for identification and authentication of plant species. I'm also thankful to the Forest Management and Wildlife Department, the Government of Sikkim for the sample collection Permit, and Aakaar Biotechnologies Private Limited for GC-MS analysis.

I would also like to extend my special thanks to Dr. **Bishu Singh**, Assistant Professor, and Mr. **Jiwan Gurung**, Research Scholar, Department of Zoology, Sikkim University for helping me in the completion of the *in-vitro* study and for their help and suggestions at all stages of research.

I am extremely grateful to the non-teaching staff, Mr. **Deepak Chettri**, and Mr. **Roshan Rai**, Department of Botany, Sikkim University for their help and cooperation in the lab for the completion of my research work. I take this opportunity to sincerely thank my colleagues, Dr. **Pankaj Kr. Tripathy**, Dr. **Ranjit Kr. Pitalla**, Dr. **Dawa Lhendup Lepcha**, and **Bhupen Roka**, for their precious company and encouragement. Words are not enough to express my deepest gratitude to my juniors (**Patrush**, **Raksha**, **Abhijit**, **Hemant**, **Puja**, **Poonam**, **Binu**, **Bikram**, **Priyanka**, **Suman**) for their continuous monumental support in my life.

I am thankful to God for blessing me with good friends in my life. I am grateful to **Prasansha Subba** who not only is my best friend but also my perpetual encourager. My sincere thanks to **Sushila Subba**, **Passang Doma Lepcha**, **Seema Rai**, **Say Say Hang Subba**, **Sonam Doma Bhutia**, **Puja Sashankar**, **Poonam Chetry**, **Alice Rachael Subba**, **Kabita Subba** (Maiju), **N.B. Subba**, **Phu Tshering Bhutia**, and **Nima Tshering Lepcha** for providing me with moral and emotional support in my life.

I would also like to thank my eternal cheerleader **Aditya Pradhan**, and my best Bhai **Subhankar Gurung**, who has always helped me with open arms whenever I faced trouble spotting or had doubts regarding my research or writing.

I owe a great debt of gratitude to my beloved family without whom, I could not have made this possible. It is with due thankfulness that I remember all who have been influential in guiding or supporting me for the completion of this research investigation. My parents have been a guiding force behind the entire endeavor. I am grateful to my brothers, sisters, and my mother (**Suk Maya Subba**), who have provided me with moral and emotional support in my life.

I am also grateful to my other family members (Late Grandfather, Grandmother, my uncles, my aunties, cousins, and friends) who have supported me along the way. I am indebted to them for all the care, constant encouragement, and immense support. This work would not have been completed without their moral support. Lastly, I would like to thank all of my mentors, well-wishers, all kith and kin, near and dear ones, who helped and assisted me in their way.

I solemnly dedicate this doctoral thesis to my parents.

List of Abbreviations

%	Percentage
µg	Micro gram
5-LOX	5- Lipooxygenase
APCs	Antigen-presenting cells
BSA	Bovine serum albumin
BSTFA	N, O-Bis(trimethylsilyl) trifluoroacetamide
°C	Degree Celsius
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
COX2	Cyclooxygenase-2
DMARDs	Disease modifying anti-rheumatic drugs
DNA	Deoxyribonucleic acid
DPPH	2,2 – diphenyl- 1- picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
$Fecl_3$	Ferric chloride
FFNSC	Natural and Synthetic Compounds
FLS	Fibroblast like synoviocytes
GAE	Gallic acid equivalents

GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GM-CSF	Granulocyte-macrophage colony-stimulating factor
H_2SO_4	Sulphuric acid
HCL	Hydrochloric acid
HRBC	Human Red Blood Cell
IBD	Inflammatory bowel diseases
IC_{50}	Inhibition concentration by 50 percent
IKK	IκB kinase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
ITD	Ion trap detector
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP	Metacarpophalangeal
mg	Milligram
MHC	Major Histocompatibility complex
mM	Millimolar
MMP	Matrix metallopeptidase

MRI	Magnetic resonance imaging
MS	Mass spectrometry
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NIST	National Institute of Science and Technology
NKT cells	Natural killer T cells
nm	Nanometre
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
OD	Optical density
PARP	Poly (ADP-ribose) polymerase
PBS	Phosphate buffer saline
PGE_2	Prostaglandin E_2
pH	Potential of Hydrogen
PI3K-Akt	Phosphoinositide-3-kinase-Akt
RA	Rheumatoid arthritis
RA-FLS	Rheumatoid arthritis- Fibroblast like synoviocytes
RBC	Red blood cell

ROS	Reactive oxygen species
Rpm	Rotation per minute
RtE	Rutin equivalents
SD	Standard deviation
STAT-1	Signal transducer and activator of transcription 1
TFC	Total flavonoid content
TMCS	Trimethyl chlorosilane
TNF- α	Tumour necrosis factor- alpha
TPC	Total phenolic content
Viz.	Namely
WHO	World Health Organization

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Chapter 1

Introduction and Objectives

1.1. INTRODUCTION

Inflammation and the immune system are interrelated with each other (Kraneveld *et al.,* 2010). An immune reaction is a composite reaction due to injuries and infection and is denoted by the classical response of red a7ness, heat, swelling, pain, and loss of function (Carrillo *et al*., 2017). The immune system includes cells and soluble components that can intercede with the reaction for the elimination of immune stimulus and initiate the process of immunological memory. Inflammation and immunity disorders can occur due to improper inflammation or when normal inflammatory cells develop into chronic inflammation, either due to long-term improper response to stimuli (such as allergies) or due to offending agents such as chronic infection, transplantation, and autoimmunity (Royer & Armstrong, 2016). It is extensively known that acute inflammation is part of the body's defense mechanisms, but in few cases, acute inflammation fails to resolve and can lead to chronic inflammation that can cause a variety of inflammatory diseases including cardiovascular diseases, tumors, diabetes, arthritis, Alzheimer's disease, pulmonary diseases and autoimmune diseases (Hunter, 2012; Rea *et al*., 2018).

Rheumatoid arthritis (RA) is a systemic and chronic autoimmune inflammatory disease where the body's immune system targets and affects its own tissues. It is a painful inflammatory disease that can lead to disability due to pain, joint damage, bone erosion, and destruction of cartilage (Firestein, 2003; Yap *et al*., 2018). RA

affects 1% of the world's population and is more prevalent in women as compared to men (Costenbader & Karlson, 2006; Suzuki *et al*., 2008). In India, it affects over 20% of the population (Patwardhan *et al*., 2010; Wadekar *et al*., 2015). According to a cross-sectional report, 45% of RA patients in India have co-morbidities including hypertension, diabetes mellitus, and hypothyroidism (Gautam *et al*., 2020). Heart disease continues to be a serious issue in RA patients, with a 50% increase in the risk of cardiovascular (CV) mortality in rheumatoid arthritis patients compared to the general population (Yadav *et al*., 2019). As a result, early evaluation of associated co-existing conditions is critical for effective rheumatic disease treatment (Yadav *et al*., 2011). According to the Bone and Joint Decade (BJD) India Copcord 2001 survey, the ethnically unique northeast Indian population is susceptible to RA with a frequency rate of 0.2–0.4% (Das *et al*., 2019).

According to the WHO report, at least half of RA patients in developed countries are unable to work full-time due to impairment that develops within ten years of the disease's onset (Gautam *et al*., 2020). RA affects 0.92 % of adults in India. However, early diagnosis and intensive treatment may assist to avoid permanent disability (Amin *et al*., 2021). Every year, approximately 20-40 new cases per 100,000 people are recorded in India, with the female population being more susceptible to the disease. However, the RA condition in the female population is silent during pregnancy and only manifests after the birth of the infant (Gautam *et al*., 2020). Cigarette smoking, coffee consumption, and oral contraceptive pills have all been identified as risk factors for the development of RA (Oliver & Silman, 2006; Arthritis India, 2014).

RA usually develops in three stages: in the first stage, there is the synovial membrane swelling, pain, redness, warmth, stiffness, and joint swelling. In the second stage, there is rapid growth and division of pannus cells that cause synovium thickening; and in the third stage, the inflamed cells produce enzymes that can damage the bone and the cartilage, causing joint deformity, discomfort, and loss of joint movements (Tripathy *et al*., 2013). Although the exact etiology of RA is not clear, chronic inflammation plays a key role in the immunopathogenesis of RA along with genetic and environmental factors (Veselinovic *et al*., 2014; Rea *et al*., 2018). In RA, innate along with adaptive immune cells penetrate the synovium membrane causing pain and stiffness in the joint (van de Sande *et al*., 2012; van der Ven *et al*., 2017). In the progression of RA, immune cells and synovial fibroblasts produce proinflammatory cytokines including TNF-α, IL-1, and IL-6, IL-6, IL-7, IL-15, IL-1, IL-17, IL-6, IL-1, IL-18, GM-CSF, and TGF-β (Bottini & Firestein, 2013; Firestein & McInnes, 2017) and these complex proinflammatory cytokines have systemic effects in the body of RA patients. Besides immune cells, free radicals are also involved in the development of rheumatoid arthritis (Wruck *et al*., 2011; Veselinovic *et al*., 2014; Ponist *et al*., 2019), and highly reactive oxidative species (ROS) that have the potential to damage the joint tissues. RA patients have a higher threat of cardiovascular disease, including coronary artery disease, heart failure, cardiac arrest, myocardial infarction, etc. (Crowson *et al*., 2013; Urman *et al*., 2018).

Although RA is not a curable disease, therefore, several medications such as non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), and corticosteroids are prescribed to control and prevent the disease. The use of such medications results in harmful side effects including gastric ulceration and bleeding, belching, irritation of gastric mucosa, immunodeficiency, humoral

immunity disturbances, and damages in renal and hepatic systems. Because of such side effects of RA medications, there is an augment in the interest among the people for the search of novel alternative medicine preferably from plants and other natural sources without side effects or with minimum side effects.

People with chronic pain, such as those with RA, and those who are disappointed with existing treatment are more prone to consider alternative treatments. According to research, an estimated 60–90% of arthritis patients use complementary and alternative medicine (Wadekar *et al*., 2015). With the increased popularity of herbal medicines among people with RA, further research into their efficacy and safety is needed (Mur *et al*., 2002; Soeken *et al*., 2003). RA is to be treated using a multidisciplinary strategy aimed at reducing pain, reducing inflammation, and restoring joint function. The goal of intensive therapy is to control inflammation (Wadekar *et al*., 2015). Herbal remedies have recently gained popularity as a treatment for RA across the world (Asif *et al*., 2011).

Over the years people have been using medicinal plants for treating different kinds of diseases. The use of medicinal plants, along with other natural products for treating diseases exists in the form of the various traditional system of medicines such as Ayurveda, traditional Chinese medicine, Unani, traditional Korean medicine, and Kampo (Yuan *et al*., 2016). Medicinal plants can be any plant with therapeutic potential for treating diseases or precursors for the development of useful new drugs (Sofowora *et al*., 2013). Plant-based products are used in the development of modern drugs for treating various diseases. As per the data of research publications analyzed from 1981-2007 in search of new medicines, almost half of the modern medicines launched since 1994 have been developed from natural sources (Katiyar *et al*., 2012;

Krause & Tobin, 2013). As per the World Health Organization report, out of 252 essential drugs, 11% of them are of plant origin and the number of significant synthetic drugs is obtained through the use of natural precursors (Veeresham, 2012). Medicinal plant constituents such as morphine, quinine, vincristine, vinblastine, codeine, digoxin, atropine, etc. have a strong record of use in both traditional and modern medicines (Alamgir, 2017).

Even though plants have tremendous health benefits as a medication for the treatment of many diseases in human beings, several problems remain due to inadequate evidence to justify the use of medicinal plants. Therefore, the safety and efficacy evaluation of herbal remedies remains a challenge (WHO, 2005).

In fact, in the face of these challenges, therapeutic plants have a strong future to serve as preventive medicine against different diseases with more efficacy and fewer side effects than current therapies (Ekor, 2014). Various plant-derived compounds with high potential have been developed for the treatment of inflammatory diseases, arthritis, autoimmunity, and cancer (Dudics *et al*., 2018). Natural products are capable of controlling arthritic inflammation via multiple mechanisms, including inhibition of pro-inflammatory cytokines along with chemokines, initiation of anti-inflammatory agents and also the regulation of the Th17, and by the modulation of the immune system (Zhang *et al*., 2009; Nanjundaiah *et al*., 2012; Astry *et al*., 2015).

The inhabitants of the Sikkim Himalayan region have inherited rich traditional knowledge of plant uses for the treatment of common as well as chronic diseases. They often have information about the uses of plants as medicines for the treatment of different diseases and taking care of their good health. In Sikkim, a noticeable size of the population is found to be suffering from RA. The majority of the people living in

the village and far-flung areas usually prefer the traditional system of treatment using medicinal plants for the treatment and prevention of diseases like RA and other inflammatory conditions, and such practices exist since time immemorial. Plants are thought to be the safest and most effective treatment for RA patients. The present study focuses on the evaluation of medicinal plants which are being used by the indigenous people of the Sikkim Himalayan region for treating RA. Many medicinal plants are being utilized by the ethnic tribes of this region "to treat RA but there have not been detailed studies done so far to scientifically support these traditional healing practices as followed by the local healers". Therefore, this study is done to document and validate the ethnomedicinal plants which are being used by the local healers in Sikkim Himalaya for treating rheumatoid arthritis.

1.2. Objectives of the study

The main objectives of the study were:

1. To document plant-based traditional knowledge on rheumatoid arthritis (RA) treatment in Sikkim Himalayan region.
2. To use different solvent extraction and assess the phytochemical constituents.
3. To estimate anti-rheumatoid arthritis activity in different plant extracts through *in-vitro* method.
4. To identify possible phenolics involved in anti-rheumatoid arthritis activity.

Chapter 2

Review of Literature

2.1. Inflammation

Inflammation is the response of living tissues to harmful external factors like pathogens, damaged cells, toxic compounds, and injuries. Due to these, various inflammatory mediators (eicosanoids, biological oxidants, and cytokines) are produced which contribute to the initiation of inflammation resulting in redness, swelling, pain, heat, and loss of function (Highleyman, 2011; Adedapo & Ofuegbe, 2013). Inflammations are of two types: acute inflammation and chronic inflammation.

2.2. *Acute inflammation*

Acute inflammation lasts for a few days only and is the response of the body to adverse stimuli and is marked by increased leukocyte and plasma movement from the blood to the sites of the injury. Acute inflammation is recognized if the wound gets redness, heat, swelling, and pain (Jain & Bari, 2010).

2.3. *Chronic inflammation*

Chronic inflammation takes place over an extensive period, which increases the production of pro-inflammatory agents that contribute to tissue destruction (Adedapo & Ofuegbe, 2013). It is manifested by the infiltration of mononuclear cells, proliferation of fibroblasts, collagen fibers, along with the formation of connective tissues which lead to the formation of granuloma. Chronic inflammation led to tissues degeneration through the inflammatory mediators (reactive oxygen species, nitrogen

species, and protease) released from infiltrated inflammatory cells (Virgilio, 2004; Mittal *et al*., 2014; Abdulkhaleq *et al*., 2018). These oxidizing agents are potential mutagen and can cause permanent genomic alterations such as point mutations and deletion or rearrangement of tissue (Wiseman & Halliwell, 1996), in some cases, p53 mutations occur in rheumatoid arthritis and inflammatory bowel diseases (IBD), which are comparable to those in tumor diseases (Firestein *et al*., 1997; Cooks *et al*., 2014).

Although inflammation is a defense mechanism by the body, the process and the mediators involved can initiate chronic diseases counting rheumatoid arthritis, cardiovascular and bowel diseases, type-2 diabetes, neurodegenerative diseases, and cancer (Libby, 2007; Fürst & Zündorf, 2014).

2.4. *Rheumatoid arthritis* (RA)

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disease that causes joint deformity and permanent disability. It affects every third person of the world population and is three times more frequent in women as compared to men. RA is usually onset between the ages of 40-50, but people of any age can suffer from this disease. RA is typically manifested with inflammation, affected joints being swollen, red, warm, painful, and stiff especially in the morning time, and its diagnosis is based on the symptoms, physical examinations, MRI, radiography, and other related laboratory results. Patients with RA not only suffer from pain, stiffness, swelling, and joint deformity but also have a higher risk of cardiovascular diseases and lymphoma. RA targets synovial joints results in the massive collection of blood-borne cells (macrophages and T-cells) leading to progressive degeneration of cartilage

and bone erosion, which causes disability in RA patients (Leung, 2007; Naik & Wala, 2014; Guo *et al*., 2018).

2.5. *Pathogenesis of RA*

The exact causative agents of RA are still unclear, but previous studies indicate that the joint damage is due to chronic synovial membrane inflammation, i.e., synovitis (Wong & Lord, 2004; Sokolove & Lepus, 2013; Naik & Wala, 2014). The synovial membrane turns into hyperplastic, which comprises of synovial fibroblast, macrophages, natural killer cells, NKT cells, plasma cells, B-cells, CD4+ T-cells, CD8+ T-cells, and mast cells (McInnes & Schett, 2007; Brennan & McInnes, 2008; Rivellese *et al*., 2019). The autoreactive B-cells perform a crucial role in the production of autoantibodies and pro-inflammatory cytokines and T-cell activation, which eventually contributes to RA pathogenesis (Bugatti *et al*., 2014). These immune cells and their intermediaries are considered to be relevant in the pathogenesis of RA (McInnes & Schett, 2011; Min *et al*., 2020). Synovitis is related to extensive infiltrates of cellular inflammation and pathogenesis of RA is considered to be managed by antigen-specific responses due to the close association of antigen-presenting cells (APCs) that interact with T cells via MHC along with T cell receptors (Thomas *et al*., 2008), which will not only lead to T-cells activation (through the prevalence of co-stimulatory signals through the CD80/86 or CD28-B7 receptor) but will also lead to the formation of APCs. As a result of activation, T cells and APCs release pro-inflammatory cytokines, such as TNF-α, IL-6, IL-12, IL-23, and IL-1 (Burger *et al*., 1998; Commins *et al*., 2010; Brereton & Blander, 2011; Boissier, 2011; Turner *et al*., 2014; Tanaka *et al*., 2014). This cytokine environment promotes the naïve $CD4^+$ T cells to differentiate into alternative phenotypes such as T helper 1 and

Th 17, and IL-6 dependent T cell phenotypes that produce IL-17 (Bettelli *et al*., 2008; Zhou *et al*., 2009; Weaver & Hatton, 2009; Noack & Miossec, 2014). The IL-17 further initiates the creation of MMP1, MMP3, TNF-α, IL-6, IL-8, along with proinflammatory cytokines, which increases the infiltration of immune cells into synovium, causing tissue destruction (Mateen *et al*., 2016). Cytokines are involved in the complex pathogenesis of joint destruction in RA from initiation of chronic inflammatory synovitis to the promotion of articular destruction, through the activation of chondrocytes, osteoclasts, and synovial fibroblasts (Tak & Bresnihan, 2000; Choy & Panayi, 2001; Brzustewicz & Bryl, 2015; McInnes *et al*., 2016). The immune cells and soluble mediators that contribute to RA's pathogenesis are shown in **Figure 1**.

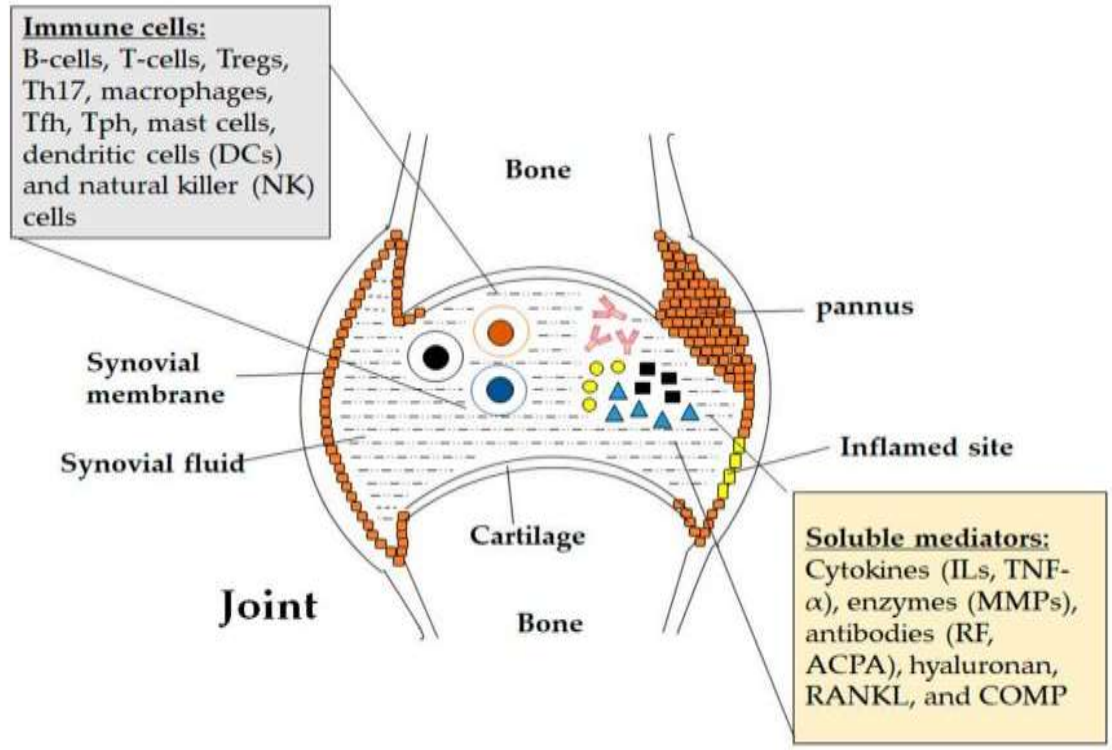

Figure 1: Autoimmune cells and soluble mediators in the pathogenesis of Rheumatoid arthritis (Yap *et al*., 2018).

2.6. *RA and cardiovascular risk*

There are several studies done to provide evidence that rheumatoid arthritis is related to an overall increase in cardiovascular morbidity along with mortality (Zegkos *et al*., 2016; Jagpal & Navarro-Millán, 2018; Rawla, 2019). Some of the previous data support that RA is related to an increase in the risk of untimely death due to cardiovascular disease (Charles-Schoeman, 2012; Lim *et al*., 2014). Nowadays, inflammation in RA is considered as a threat factor for the progression of atherosclerosis (Libby, 2008; Del Rincon *et al*., 2015) and both of these diseases share common disease mechanisms (Abou-Raya & Abou-Raya, 2006; Wu *et al*., 2013). The chronic inflammation and dysfunction of the immune system lead to the acceleration of atherogenesis and are involved in the entire phases of atherosclerosis (Matsuura *et al*., 2014).

2.7. *Oxidative stress as a basis of RA*

Free radicals are highly unstable compounds formed during the normal cellular metabolism of living organisms (Gupta *et al*., 2014). The development of free radicals or ROS (Reactive Oxygen Species) in living creatures is inevitable. Usually, there is a counterbalance between the generation of free radicals or ROS and internal antioxidant defense systems. However, if this equilibrium is distorted, it can lead to oxidative stress that is harmful to all major cellular components including proteins, DNA, and membrane lipids, and eventually causes cell death (Mahajan & Tandon, 2004). An antioxidant defense mechanism has been developed to deal with oxidative stress, including specific enzymes, specifically superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, along with many low molecular weight antioxidants, particularly ascorbate, alpha-tocopherol, glutathione,

thioredoxin, cysteine, vitamins, etc. But sometimes many pathological or environmental factors may influence this antioxidant protection mechanism, leading to the progression of different free radicals that affect the normal function of cells leading to human aging and the development of chronic diseases such as autoimmune disease, including rheumatoid arthritis, atherosclerosis, cancer, etc. (Fridovich, 1997; Wei *et al*., 2001; Rahman *et al*., 2012; Kumar *et al*., 2016). As a consequence of free radical generation at inflammation sites, there is the destruction of cartilage and joint along with degranulation of neutrophil, which increases the levels of isotopes and prostaglandins thus resulting in RA (Abbas & Monireh, 2008; Stamp *et al*., 2012.

2.8. *Treatment and Management of RA-inflammation*

Current pharmacotherapy for RA is used to reduce pain, joint inflammation, increase joint functions and prevent joint destruction along with deformity, and improve the overall quality of life. Medications including NSAIDs, corticosteroids, and analgesics are used to suppress the symptoms of RA along with DMARDs and biological response inhibitors are often used to inhibit or control the underlying immune process and to prevent long-term damage. Examples of some of the medicines are leflunomide, methotrexate, sulfasalazine, hydroxychloroquine, etanercept, infliximab, rituximab, abatacept, etc. Although these medications are effective in the treatment of rheumatoid arthritis, they are associated with various side effects, including gastrointestinal, cardiovascular, and other systems. Due to such undesirable side effects from current RA medication, natural plant products serve as both safer and more cost-effective for treatment with the least side effects. During the last few decades, interests are growing all over the world for the use of alternative and herbal therapies for treating RA (Callahan *et al*., 2009; Kikuchi *et al*., 2009; Marcus, 2009).

2.9. *Plant as sources of anti-RA agents*

From ancient times, people are using medicinal plants for treating their common ailments and this practice has been passed from one generation to the next, playing an imperative role in the evolution of human culture in the form of the common traditional system of medicines. According to the World Health Organization (WHO), the traditional medicine system is the knowledge, abilities, and practices based on the assumptions, beliefs, and experiences of various cultures to maintain good health and to diagnose, prevent and improve the overall physical and mental illness (WHO, 2013). For most traditional types of medicine, plants are considered the main source, and several conventional drugs are also developed by following ethnobotanical plants from the traditional medicine system. Nowadays uses of herbal plants are growing throughout the world for treating various diseases due to the existence of various phytochemicals (alkaloids, flavonoids, tannin, terpenoids, and phenolic compounds) with potential therapeutic activities (Njeru *et al*., 2013). Medicinal plants are considered as one of the major sources of anti-arthritic, antioxidants along with anti-inflammatory agents (Zubair *et al*., 2012; Atawodi *et al*., 2013) with fewer side effects.

Several medicinal plants along with their products have been used in the prevention of RA disease and studies are also done to scientifically validate the potency of plants for exhibiting anti-RA activity. Some of the reported medicinal plants with anti-RA activity are enumerated alphabetically below in **Table 1**, along with their families.

Table 1: List of plants reported for anti-RA activity

Sl.No	Botanical name (Family)	Part used	References
1.	*Abrus precatorius* L. (Fabaceae)	Fresh leaf	Choi *et al*., 1989; Georgewill & Georgewill, 2009
2.	*Acanthopanax trifoliatus* (L.)Merr. (Araliaceae)	Root, leaf	Chen *et al*., 2016
3.	*Acacia catechu* (L.f.) Willd. (Mimosaceae)	Fruit	Subramoniam *et al*., 2013
4.	*Acalypha indica* L. (Euphorbiaceae)	Inflorescence	George *et al*., 2016
5.	*Acanthopanax chiisanensis* Nakai (Araliaceae)	Leaf	Jung *et al*., 2005
6.	*Achyranthes aspera* L. (Amaranthaceae)	Shoot and root	Neogi *et al*., 1969; Manjunatha *et al*., 2012
7.	*Aconitum heterophyllum* Wall. (Ranunculaceae)	Root	Subramoniam *et al*., 2013
8.	*Aconitum vilmorinianum* Kom. (Ranunculaceae)	Root	Li *et al*., 2013a
9.	*Acorus calamus* L. (Arecaceae)	Rhizome	Subramoniam *et al*., 2013
10.	*Adhatoda beddomei* Clarke. (Acanthaceae)	Leaf	Subramoniam *et al*., 2013
11.	*Ailanthus triphysa* (Dennst.) Alston.	Stem bark	Subramoniam *et al*.,

	(Simaroubaceae)		2013
12.	*Ajuga bracteosa* Wall. ex Benth (Lamiaceae)	Whole plant	Kaithwas *et al*., 2012
13.	*Ajuga decumbens* Thunberg. (Lamiaceae)	Whole plant	Ono *et al*., 2008
14.	*Alangium salvifolium* (L.f.) Wangerin (Cornaceae)	Leaf	Prajapati *et al*., 2019
15.	*Alpinia galangal* L. (Zingiberaceae)	Rhizome	Huh *et al*., 2013
16.	*Alstonia scholaris* L. (Apocynaceae)	Latex	Thakurdesai *et al*., 2010
17.	*Ammania baccifera* L. (Lytraceae)	Whole plant	Tripathy *et al*., 2010
18.	*Anacardium occidentale* L. (Anacardiaceae)	Dried juice of the leaf	Huang, 1998
19.	*Anacyclus pyrethrum* DC. (Asteraceae)	Root	Usmani *et al*., 2016
20.	*Anacyclus valentinus* L. (Asteraceae)	Aerial part	Larbi *et al*., 2017
21.	*Andrographis paniculata* (Burm. f.) Wall. ex Nees (Acanthaceae)	Whole plant	Subramoniam *et al*., 2013
22.	*Annona montana* Macfad. (Annonaceae)	Leaf, fruit, seed, and stem bark	Chuang *et al*., 2008
23.	*Aphanamixis polystachya* Wall. (Meliaceae)	Bark	Hossain *et al*., 2009
24.	*Aquilaria agallocha* (Lour.) Roxb. (Thymeleaceae)	Wood and oil	Rahman *et al*., 2016

25.	*Aristolochia bracteolata* Lam. (Aristolochiaceae)	Whole plant	Chitme & Patel, 2009
26.	*Arctium lappa* L. (Asteraceae)	Seed	Farzaei *et al*., 2016
27.	*Argemone mexicana* L. (Papaveraceae)	Seed	Pandey & Tiwari, 2018
28.	*Argyreia speciosa* Sweet. (Convulvulaceae)	Root	Gokhale *et al*., 2002
29.	*Arnebia euchroma* Johnst. (Boraginaceae)	Root	Fan *et al*., 2012
30.	*Artocarpus tonkinensis* A. Cheval. (Moraceae)	Leaf	Ngoc *et al*., 2005
31.	*Artocarpus hypargyraea* Hance. (Moraceae)	Root	Chen *et al*., 2016
32.	*Asparagus racemosus* Willd. (Liliaceae)	Aerial part	Patwardhan *et al*., 2005
33.	*Asystasia dalzelliana* Sant. (Acanthaceae)	Leaf	Babushetty & Sultanpur, 2012
34.	*Azadirachta indica* A. Juss. (Meliaceae)	Leaf, seed, and stem bark	Biswas *et al*., 2002
35.	*Baccharis genistelloides* Linn. (Asteraceae)	Aerial part	Coelho *et al*., 2004
36.	*Bacopa monnieri* (L.) Pennell (Scrophulariaceae)	Whole plant	Vijayan *et al*., 2011
37.	*Bauhinia variegata* (L.) Benth. (Fabaceae)	Leaf bud, Leaf, Stem bark	SheshadriShekar *et al*., 2009

38.	*Berberis vulgaris* L. (Berberidaceae)	Stem, root bark, and fruit	Chopra *et al*., 2004
39.	*Barleria lupulina* Lindl. (Acanthaceae)	Leaf	Mazumder *et al*., 2012
40.	*Barringtonia racemosa* Linn. (Lecythidaceae)	Fruit	Kaur *et al*., 2012
41.	*Basella rubra* L. (Basellaceae)	Whole plant	Chen *et al*., 2016
42.	*Barleria prionitis* L. (Acanthaceae)	Leaf	Choudhary *et al*., 2014
43.	*Berberis orthobotrys* Bien ex Aitch. (Berberidaceae)	Stem bark	Alamgeer *et al*., 2017
44.	*Bergenia stracheyi* Linn. (Saxifragaceae)	Rhizome	Nazir *et al*., 2007
45.	*Boerhavia diffusa* L. (Nyctaginaceae)	Root	Dapurkar *et al*., 2013
46.	*Boswellia carterii* Birdw. (Burseraceae)	Resin	Fan *et al*., 2005
47.	*Boswellia serrata* Roxb. (Burseraceae)	Inflorescence	Sharma *et al*., 2010
48.	*Butea monosperma* Linn. (Fabaceae)	Whole plant	Yerragunta *et al*., 2011
49.	*Caesalpinia sappan* Linn. (Fabaceae)	Wood	Wu *et al*., 2011
50.	*Callicarpa loureiri* Hook. et Arn. (Lamiaceae)	Root, stem	Chen *et al*., 2016
51.	*Callicarpa macrophylla* Vahl. (Verbenaceae)	Flower	Gupta *et al*., 2013

52.	*Calluna vulgaris* (L.)Hull (Ericaceae)	Aerial part	Orhan *et al*., 2007
53.	*Calotropis gigantea* R.Br. (Asclepiadaceae)	Aerial part	Saratha & Subramanian, 2012
54.	*Calotropis procera* R.Br. (Asclepiadaceae)	Aerial part	Kumar & Roy, 2007
55.	*Caltha palustris* Linn. (Ranunculaceae)	Whole plant	Suszko & Obmińska-Mrukowicz, 2013
56.	*Cannabis sativa* Linn. (Cannabaceae)	Leaf	Malfait *et al*., 2000; Costa *et al*., 2004
57.	*Capparis decidua* (Forssk.) Edgew. (Capparaceae)	Fruit and Root	Marwat *et al*., 2011
58.	*Capparis spinosa* L. (Capparaceae)	Fruit	Feng *et al*., 2011
59.	*Camellia sinensis* (L.) Kuntze *(Theaceae)*	Leaf	Adcocks *et al*., 2002
60.	*Commiphora wightii* (Arn.) Bhandari (Burseraceae)	Stem	Mesrob *et al*., 1998
61.	*Cardiospermum halicacabum* L. (Sapindaceae)	Root	Venkatesh & Krishna kumara, 2006; Jeyadevi *et al*., 2013
62.	*Carthamus tinctorius* L. (Asteraceae)	Seed oil	Jun *et al*., 2011; Asgarpanah & Kazemivash, 2013
63.	*Cayaponia tayuya* Cogn. (Cucurbitaceae)	Root	Escandell *et al*., 2006
64.	*Cassia fistula* L. (Fabaceae)	Fruit	Sheikh *et al*., 2010

65.	*Cassia uniflora* Mill. (Fabaceae)	Stem	Chaudhari *et al*., 2012
66.	*Cedrus deodara* (Roxb.) G.Don (Pinaceae)	Wood	Kaur *et al*., 2012
67.	*Celastrus aculeatus* Merr. (Celastraceae)	Root	Venkatesha *et al*., 2011; Li *et al*., 2013b
68.	*Centella asiatica* (L.) Urban (Apiaceae)	Leaf	Chippada & Vangalapati, 2011
69.	*Centipeda minima* Lour. (Asteraceae)	Leaf	Sarkar *et al*., 2017
70.	*Chaenomeles speciosa* (Sweet) Nak. (Rosaceae)	Fruit	Li *et al*., 2009
71.	*Chelidonium majus* Linn. (Papaveraceae)	Aerial part	Lee *et al*., 2007a
72.	*Cinnamomum cassia* (L.) J.Presl. (Lauraceae)	Stem bark	Sharma *et al*., 2018
73.	*Cinnamomum camphora* (L.) J.Presl. (Lauraceae)	Oil	Lee *et al*., 2006
74.	*Cinnamomum parthenoxylon* (Jack) Nees. (Lauraceae)	Root, stem, leaf, and stem bark	Chen *et al*., 2016
75.	*Cinnamomum zeylanicum* Garcin ex Blume (Lauraceae)	Bark	Vetal *et al*., 2013
76.	*Cissampelos pareira* Linn. (Menispermaceae)	Root	Amresh *et al*., 2007
77.	*Citrus medica* L. (Rutaceae)	Fruit and peel of the fruit	Farzaei *et al*., 2016
78.	*Clematis chinensis* Osbeck.	Root	Hsieh *et al*., 2011

	(Ranunculaceae)		
79.	*Clematis ochroleuca* Aiton (Ranunculaceae)	Root	Farzaei *et al*., 2016
80.	*Clematis vitalba* L. (Ranunculaceae)	Aerial part	Yesilada & Küpeli 2007
81.	*Cleome gynandra* L. (Cleomaceae)	Leaf	Narendhirakannan *et al*., 2005; 2007
82.	*Cleome rutidosperma* DC. (Cleomaceae)	Aerial part	Chakraborty & Roy, 2010
83.	*Cocculus hirsutus* (L.) Diels (Menispermaceae)	Root	Bothara *et al*., 2011
84.	*Colchicum autumnale* L. (Colchicaceae)	Corm (bulb-like)	Farzaei *et al*., 2016
85.	*Coptidis rhizoma* Franch. (Ranunculaceae)	Root and rhizome	Wang *et al* 2011
86.	*Coriandrum sativum* Linn. (Apiaceae)	Seed	Nair *et al* 2012
87.	*Costos speciosus (*Koen) Smith. (Costaceae)	Aerial part	Srivastava *et al*., 2012
88.	*Crocus sativus* L.(Iridiaceae)	Flower	Zamani *et al*., 2015
89.	*Curculigo orchioides* Gaertn. (Liliaceae)	Tuber	Subramoniam *et al*., 2013
90.	*Curcuma longa* L. (Zingiberaceae)	Rhizome	Kohli *et al*., 2005
91.	*Curcuma zedoaria* Rosc. (Zingiberaceae)	Rhizome	Kaushik & Jalalpure, 2011

92.	*Commiphora caudata* (Wight & Arn.) Engl. (Burseraceae)	Leaf	Pashikanti *et al*., 2014
93.	*Cynodon dactylon* (L.) Pers. (Poaceae)	Whole plant	Bhangale & Acharya, 2014
94.	*Daphne genkwa* Siebold & Zucc. (Thymelaeaceae)	Flower bud	Jiang *et al*., 2014
95.	*Datura alba* L. (Solanaceae)	Seed	Pandey & Tiwari, 2018
96.	*Delonix elata* (L.) Gamble (Fabaceae)	Bark	Murugananthan *et al*., 2011
97.	*Desmodium gangeticum* (L.) DC. (Fabaceae)	Aerial part	Govindarajan *et al*., 2006
98.	*Diospyros melanoxylon* Roxb. (Ebenaceae)	Stem bark	Saluja *et al*., 2015
99.	*Dipsacus asperoides* Linn. (Dipsacaceae)	Root	Jung *et al*., 2012
100.	*Drynaria quercifolia* L. (Polypodiaceae)	Rhizome	Saravanan *et al*., 2013
101.	*Elaeocarpus munronii* (Wt.) Mast. (Elaeocarpaceae)	Leaf	Anusuya *et al*., 2018
102.	*Elaeocarpus sphaericus* (Gaertn.) K. Schum. (Elaeocarpaceae)	Whole plant	Ramasamy *et al*., 2012
103.	*Ephedra sinica* Staph. (Ephedraceae)	Aerial part	Yeom *et al*., 2006
104.	*Erythrina variegata* L. (Fabaceae)	Leaf, stem bark	Subramoniam *et al*., 2013

105.	*Eupatorium chinense* L. (Asteraceae)	Root, stem	Chen *et al*., 2016
106.	*Euphorbia antiquorum* L. (Euphorbiaceae)	Whole plant	Harpalani *et al*., 2011
107.	*Euphorbia thymifolia* L. (Euphorbiaceae)	Whole plant	Mamatha *et al*., 2014
108.	*Equisetum giganteum* L. (Equisetaceae)	Whole plant	Farinon *et al*., 2013
109.	*Ficus bengalensis* Linn. (Moraceae)	Stem bark	Manocha *et al*., 2011
110.	*Ficus microcarpa* L.f. (Moraceae)	Root, aerial part	Chen *et al*., 2016
111.	*Ficus simplicissima* Lour. (Moraceae)	Root stem	Chen *et al*., 2016
112.	*Fritillaria roylei* Hook. (Liliaceae)	Tuber	Subramoniam *et al*., 2013
113.	*Gendarussa vulgaris* Nees. (Acanthaceae)	Whole plant	Chen *et al*., 2016
114.	*Ginkgo biloba* Linn. (Ginkgoaceae)	Leaf	Han, 2005
115.	*Glycirrhiza glabra* L. (Fabaceae)	Rhizome	Mishra *et al*., 2011
116.	*Glycosmis pentaphylla* (Retz.) DC. (Rutaceae)	Stem bark	Ramesh Petchi & Vijaya, 2012
117.	*Gmelina arborea* Roxb. (Verbenaceae)	Root	Subramoniam *et al*., 2013
118.	*Hedera helix* Linn. (Araliaceae)	Leaf	Rai, 2013
119.	*Hemidesmus indicus* (L.) R.Br. (Apocynaceae)	Root	Abiraamasri & Lakshmi, 2016
120.	*Hibiscus plantifolius* L. (Malvaceae)	Leaf	Praveen *et al*., 2013

121.	*Hippocratea excelsa* H.B.K. (Hippocrataceae)	Bark	Perez *et al*., 1995
122.	*Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don. (Apocynaceae)	Seed, stem bark	Subramoniam *et al*., 2013
123.	*Holoptelea integrifolia* (Roxb.) Planch. (Ulmaceae)	Stem bark	Subramoniam *et al*., 2013
124.	*Hybanthus enneaspermus* (L.) F.Muell. (Violaceae)	Whole plant	Tripathy *et al*., 2009
125.	*Impatiens balsamina* L. (Balsaminaceae)	Flower	Chen *et al*., 2016
126.	*Jatropha curcas* L. (Euphorbiaceae)	Leaf	Baroroh *et al*., 2015
127.	*Jatropha isabellei* Mull. Arg. (Euphorbiaceae)	Underground part	Silva *et al*., 2013
128.	*Justicia gendarussa* Linn. (Acanthaceae)	Aerial part	Paval *et al*., 2009
129.	*Kaempferia galanga* L. (Zingiberaceae)	Rhizome	Subramoniam *et al*., 2013
130.	*Laportea bulbifera* Weddell. (Urticaceae)	Root	Luo *et al*., 2011
131.	*Lawsonia inermis* L. (Lythraceae)	Leaf	Kore *et al*., 2011
132.	*Lepidium sativum* L. (Brassicaceae)	Seed	Raval *et al*., 2013
133.	*Leucas aspera* Willd. (Lamiaceae)	Aerial part	Kripa *et al*., 2011
134.	*Lilium polyphyllum* D.Don ex Royle (Liliaceae)	Tuber	Subramoniam *et al*., 2013

135.	*Linum usitatissimum* L. (Linaceae)	Oil	Upadhyay, 2016
136.	*Litsea cubeba* (Lour.)Pers. (Lauraceae)	Root	Lin *et al*., 2013
137.	*Lonicera japonica* Thumb. (Caprifoliaceae)	Leaf	Thanabhorn *et al*., 2006
138.	*Luffa echinata* Roxb. (Cucurbitaceae)	Fruit	Chandel & Kushwaha, 2013
139.	*Lycoris radiata* (L Her.) Herb. (Amaryllidaceae)	Bulb	Chen *et al*., 2016
140.	*Machilus macrantha* Nees. (Lauraceae)	Bark	Tatiya & Saluja, 2011
141.	*Mallotus oppositifolium* Mull. (Euphorbiaceae)	Leaf	Nwaehujor *et al*., 2014
142.	*Merremia emarginata* Burm. (Convolvulaceae)	Whole plant	Purushoth Prabhu *et al*., 2012
143.	*Merremia tridentata* (L.) Hallier f. (Convolvulaceae)	Whole plant	Kamalutheen *et al*., 2009
144.	*Mirabilis jalapa* L. (Nyctaginaceae)	Root, leaf	Chen *et al*., 2016
145.	*Moringa oleifera* Lam. (Moringaceae)	Flower and leaf	Mahajan & Mehta, 2009
146.	*Nyctanthes arbortristis* L. (Oleaceae)	Leaf	Bhalerao *et al*., 2011
147.	*Nigella sativa* L. (Ranunculaceae)	Seed	Farzaei *et al*., 2016
148.	*Ocimum gratissimum* L. (Lamiaceae)	Leaf	Saluja *et al*., 2015
149.	*Oroxylum indicum* (L.) Kurz.	Root bark	Karnati *et al*., 2013

	(Bignoniaceae)		
150.	*Operculina turpethum* Linn. (Convolvulaceae)	Root	Sharma & Singh, 2013
151.	*Panax ginseng* C.A. Meyer. (Araliaceae)	Root	Kim *et al*., 2010
152.	*Paederia foetida* L. (Rubiaceae)	Whole plant	Subramoniam *et al*., 2013
153.	*Pholidota chinensis* Lindl. (Orchidaceae)	Bulb	Chen *et al*., 2016
154.	*Phyllanthus amarus* Schum. & Thonn. (Phyllanthaceae)	Aerial part	Mali *et al*., 2011
155.	*Phyllanthus emblica* L. (Phyllanthaceae)	Leaf, bark, and fruit	Rehman *et al*., 2007
156.	*Physalis angulata* Linn. (Solanaceae)	Leaf	Shravan *et al*., 2011
157.	*Pinus pinaster* Aiton (Pinaceae)	Stem bark	Tsubata *et al*., 2011
158.	*Piper betle* Linn. (Piperaceae)	Leaf	Pandey *et al*., 2010
159.	*Piper longum* Linn. (Piperaceae)	Seed	Choudhary *et al*., 2015
160.	*Piper nigrum* L. (Piperaceae)	Seed	Agrawal & Paridhavi, 2007
161.	*Pisonia grandis* R.Br. (Nyctaginaceae)	Leaf	Elumalai & Prakash, 2012
162.	*Pistia stratiotes* L. (Araceae)	Leaf	Kyei *et al*., 2012
163.	*Plantago ovata* Forssk.	Seed	Subramoniam *et al*

	(Plantaginaceae)		2013
164.	*Pleurotus sajorcaju* Singer. (Pleurotaceae)	Fruit	Pinal *et al*., 2012
165.	*Pongammia pinnata* (L.) Pierre (Fabaceae)	Leaf	Arote & Yeole, 2011
166.	*Premna serratifolia* L. (Verbenaceae)	Wood	Rajendran & Krishnakumar, 2010
167.	*Pseudocdrea kotschyi* Schweinf. (Meliaceae)	Leaf	Georgewill & Georgewill, 2008
168.	*Punica granatum* L. (Punicaceae)	Seed	Kothari *et al*., 2011
169.	*Randia dumetorum* (Retz.) Poir. (Rubiaceae)	Fruit	Patel *et al*., 2012
170.	*Ranunculus sceleratus* L. (Ranunculaceae)	Whole plant	Chen *et al*., 2016
171.	*Rheum palmatum* L. (Polygonaceae)	Root	Farzaei *et* al., 2016
172.	*Rhus verniciflua* Stokes. (Anacardiaceae)	Stem bark	Lee *et al*., 2009
173.	*Ricinus communis* L. (Euphorbiaceae)	Leaf	Prasad *et al*., 2011
174.	*Rosa centifolia* L. (Rosaceae)	Flower	Kumar *et al*., 2015a
175.	*Rosa multiflora* Thunb. (Rosaceae)	Hip	Guo *et al*., 2011
176.	*Rubia cordifolia* L. (Rubiaceae)	Root	Subramoniam *et al* 2013
177.	*Ruta graveolens* Linn. (Rutaceae)	Aerial part	Ratheesh *et al*., 2010
178.	*Salacia reticulata* Wight.	Leaf	Sekiguchi *et al*., 2012

	(Celastraceae)		
179.	*Salix alba* L. (Salicaceae)	Stem bark	Khodayari *et al*., 2013
180.	*Salix nigra* Linn. (Salicaceae)	Stem bark	Sharma *et al*., 2011
181.	*Santalum album* L. (Santalaceae)	Hardwood	Subramoniam *et al*., 2013
182.	*Saraca asoca* (Roxb.) Willd. (Fabaceae)	Stem bark	Mukhopadhyay & Nath, 2011
183.	*Saussaurea lappa* (Decne.) Sch.Bip. (Asteraceae)	Root	Chandur *et al*., 2011
184.	*Selaginella uncinata* (Desv.) Spring. (Selaginellaceae)	Whole plant	Chen *et al*., 2016
185.	*Semecarpus anacardium* Linn. (Anacardiaceae)	Nut	Ramprasath *et al*., 2006
186.	*Sesamum indicum* L. (Pedaliaceae)	Seed	Ruckmani *et al*., 2018
187.	*Sida rhombifolia* L. (Malvaceae)	Aerial part	Gupta *et al*., 2009
188.	*Sida cordifolia* L. (Malvaceae)	Aerial part	Polireddy, 2015
189.	*Smithia sensitiva* Aiton(Fabaceae)	Whole plant	Sreena *et al*., 2012
190.	*Smilax glabra* Roxb. (Liliaceae)	Root, stem	Chen *et al*., 2016
191.	*Sinomenium acutum* Rehd. (Menispermaceae)	Root	Liu *et al*., 1996
192.	*Sophora flavescens* Aiton (Fabaceae)	Root	Jin *et al*., 2010
193.	*Stephania longa* Lour. (Menispermaceae)	Whole plant	Chen *et al*., 2016
194.	*Strobilanthus callosus* Nees.	Root	Agarwal & Rangari,

	(Acanthaceae)		2003
195.	*Strychnus potatorum* Linn. (Loganiaceae)	Seed	Ekambaram *et al*., 2010
196.	*Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae)	Seed pulp	Subramoniam *et al*., 2013
197.	*Terminalia chebula* Retz. (Combretaceae)	Seed oil	Nair *et al*., 2010
198.	*Tinospora cordifolia* (Thunb.) Miers (Menispermaceae)	Leaf	Paval *et al*., 2011
199.	*Torilis japonica* Houtt. (Apiaceae)	Fruit	Endale *et al*., 2013
200.	*Toxicodendron pubescens* P. Mill. (Anacardiaceae)	Whole plant	Patil *et al*., 2011
201.	*Trewia polycarpa* Benth. (Euphorbiaceae)	Root	Chamundeeswari *et al*., 2003
202.	*Tribulus terrestris* L. (Zygophyllaceae)	Fruit	Mishra *et al*.,2013
203.	*Tridax procumbens* Linn. (Asteraceae)	Leaf	Jain *et al*., 2012
204.	*Trigonella foenum-graecum* Linn. (Fabaceae)	Seed	Sindhu *et al*., 2012
205.	*Tripterygium wilfordii* Hook.f. (Celastraceae)	Root	Kaur *et al*., 2012
206.	*Urginea indica* (Roxb.) Kunth (Liliaceae)	Bulb	Rahman *et al*., 2011
207.	*Urtica dioica* L. (Urticaceae)	Root	Tabad & Jalilian, 2015

208.	*Urtica pilulifera* L. (Urticaceae)	Leaf	Abudoleh *et al*., 2011
209.	*Valeriana wallichii* DC. (Valerianaceae)	Root	Subramoniam *et al*., 2013
210.	*Vernonia anthelmintica* (L.) Willd. (Asteraceae)	Seed and root	Otari *et al*., 2010
211.	*Vernonia cinerea* Less. (Asteraceae)	Flower	Latha *et al*., 1998
212.	*Vitex negundo* L. (Verbenaceae)	Leaf	Pavithra *et al*., 2015
213.	*Wedelia calendulacea* (L.) Less. (Asteraceae)	Leaf	Panchal *et al*., 2012
214.	*Withania somnifera* (L.) Dunal (Solanaceae)	Root	Mirjalili *et al*., 2009
215.	*Xanthium strumarium* Linn. (Asteraceae)	Leaf	Patil *et al*., 2012a
216.	*Yucca schidigera* Roezl. (Liliaceae)	Bark	Cheeke *et al*., 2006
217.	*Zingiber officinale* Roscoe (Zingiberaceae)	Rhizome oil	Ahmed *et al*., 2011
218.	*Ziziphus mauritiana* Lam. (Rhamnaceae)	Seed	Subramoniam *et al*., 2013

2.10. *Phytoconstituents with anti-RA activity*

Rheumatoid arthritis can also be regulated by phytoconstituents extracted from medicinal plants that are capable of modifying and preventing the activation of pro-inflammatory mediators. The various classes of phytochemicals that have anti-inflammatory properties, including flavonoids, terpenes, catechins, quinones,

anthocyanins, alkaloids, polyphenols, anthoxanthins, etc., are capable of inhibiting the activation of various proinflammatory agents that are involved in RA outcomes. Phytoconstituents that have been reported to inhibit proinflammatory mediator activation are listed below in **Table 2**.

Table 2: List of Phytochemicals containing anti-inflammatory and anti-RA property

Phytochemical	**Natural sources**	**Inhibitory activity (mechanism of action)**	**References**
Andrographolide	*Andrographis paniculata* (Burm.f.) Nees	Inhibit NF-κB binding to its consensus sequence.	Burgos *et al*., 2009
Apigenin	Fruits and vegetables	Inhibits NO-production, COX2 expression, and PGE_2 production Cytokines TNF-α, IL-1β production, IL-6, IL-12 production	Lee *et al*., 2007b; Jeong *et al*., 2009
Boswellic acid	*Boswellia serrata* Triana & Planch	Inhibit the activation of NF-kB, COX-2, 5-LOX, MMP-9.	Kimmatkar *et al*., 2003; Takada *et al*., 2006
Berberine	*Berberis vulgaris* L.	Inhibit the proinflammatory factors like NF-kB, COX-2, TNF-a, IL-1b, IL-6	Ivanovska *et al*., 1999
Capillarisin	*Artemisia capillaries* Thunb.	Inhibits the activation of iNOS, COX-2, and proinflammatory cytokines.	Han *et al*., 2013
Capsaicinoids	Sweet pepper	Inhibit NFkB activation in response to different agents including TNF-α and prevent IkB kinase activation and IkB-α degradation in a dose-dependent manner.	Sancho *et al*., 2002
Celastrol	Plants from the	Prevent the release of	Li *et al*., 2013b

	Celastraceae family	proinflammatory cytokines from macrophages and monocytes in LPS-induced RA-FLS invasion and suppressed nuclear translocation of NF-κB through inhibition of LPS-induced phosphorylation of IκBα and its degradation.	
Cucurbitacin R	*Cayaponia tayuya* (Vell.) Cogn.	Inhibit the activation of NF-kB, COX-2, TNF-α.	Escandell *et al*., 2007
Curcumin	Turmeric	Inhibit NF-kB activation in a concentration-dependent manner in endothelial cells.	Kumar *et al*., 1998
Cryptotanshinone	*Salvia miltiorrhiza* Bunge	Prevent IL-1β and IL-17α secretions.	Wang *et al*., 2015
Daidzein	Soybeans and other legumes	Inhibit LPS-induced STAT-1 and NF-$_{\kappa}$B activations.	Hämäläinen *et al*., 2007; Choe *et al.,* 2012
Epicatechin	*Camellia sinensis* (L.) Kuntze	NF-$_{k}$B activity, NO-production PGE_2 production Cytokines TNF-α, IL-6 production	Morrison *et al*., 2014; Wang & Cao, 2014.
Epigallocatechin-3-galate	*Camellia sinensis* (L.) Kuntze	Suppress IL-1β-induced chemokine production and activation of MMP-2 in RA-FLS and obstruct the processes responsible for cartilage degradation.	Ahmed, 2010
Eugenol	*Syzygium aromaticum* (L.) Merr.& L.M.Perry	Inhibits the activation of NF-κB, 5-LOX, TNF-α, IL-1β.	Sharma *et al*., 1994
Ferulic acid	*Angelica Sinensis* (Oliv.) Diels	Suppress the activation of IL-1β, TNF-α, MMP-1, and MMP-13	Chen *et al*., 2010
Glabridin	*Glycyrrhiza glabra* L.	Inhibits LPS-stimulated nitric oxide, IL-1β, IL-6, and PGE2 production.	Chandrasekaran *et al*., 2011; Thiyagarajan *et*

			al., 2011
Guggulsterone	*Commiphora mukul* (Arn.) Bhandari	Inhibits the IL-1β-mediated inflammatory responses by inhibiting NF-κB in FLS.	Lee *et al*., 2008
Genistein	*Gycine max* (L.) Merr.	Inhibit TNF-α and decrease the release of proinflammatory cytokines.	Hämäläinen *et al*., 2007; Li *et al*., 2014
Herpagoside	*Harpagophytum procumbens* (Burch.) DC. ex Meisn.	Suppress the pro-inflammatory factors like iNOS, COX-2, NF-κB.	Huang *et al*., 2006
Hesperidin	Citrus fruits	Inhibits the activation of NF-Kb, iNOS expression, and NO production.	Hämäläinen *et al*., 2007
Indole-3-carbinol	Vegetables from family Brassicaceae	Inhibits the activation of NF-κB and NF-κB-regulated gene expression.	Takada *et al*., 2005
Isoliquiritigenin	*Glycyrrhiza glabra* L.	Inhibits production of LPS-stimulated nitric oxide, IL-1β, IL-6, and PGE_2.	Chandrasekaran *et al*., 2011; Thiyagarajan *et al*., 2011
Isorhamnetin	*Olea europaea* L.	Inhibits the activation of NF-κB, iNOS expression, and NO production.	Hämäläinen *et al*., 2007
Kaempferol	Beans and vegetables	Inhibits the LPS-induced STAT-1 and NF-$_k$B activations, iNOS expression and PGE_2 production, COX2 expression, PGE_2 and Cytokine TNF-α, IL-1β production.	Hämäläinen *et al*., 2007; Kong *et al*., 2013; Liu *et al*., 2014
Kynurenic acid	*Equisetum arvense* L.	Inhibits the proliferation of synoviocytes.	Parada-Turska *et al*., 2006
Luteolin	*Thymus vulgaris* L.	Suppress the production of TNF-α and inhibit the activation of NF-κB.	Kumazawa *et al*., 2006

Madecassoside	*Centella asiatica* (L.) Urban	Reduce the production of nitric oxide, PGE2, TNF-α, IL-1β, and IL-6.	Won *et al*., 2010
Morin	*Chlorophora tinctoria* (L.) Gaudich.ex Benth.	Inhibits the production of NF-kB, 5-LOX, MMP-9, TNF-α, IL-1β, IL-6.	Rotelli *et al*., 2003
Naringenin	Fruits and vegetables	Inhibits the LPS-induced NF-$_k$B activation iNOS expression, NO-production, COX-2 expression cytokines, TNF-α, IL-1β, IL-6 production.	Bodet *et al*., 2008, Jayaraman *et al*., 2012
Oleuropein	*Olea europaea* L.	Suppress the activation of the nuclear enzyme poly (ADP-ribosyl) polymerase (PARP) involved in inflammation of the joint.	Genovese *et al*., 2005
Pelargonidin	Berries	Suppress the activation of NF-$_k$B, iNOS expression, and NO-production.	Hämäläinen *et al*., 2007
Quercetin	*Allium cepa* L.	Inhibits the activation of NF-kB, AP-1 and suppresses the production of TNF-α, IL-1β, NO, MCP-1.	Guardia *et al*., 2001; Mamani-Matsuda *et al*., 2006; Ji *et al*., 2013
Resveratrol	*Vitis vinifera* L.	Inhibit TNF-α-induced production of IL-1β and matrix metalloproteinase (MMP-3) by inhibition of PI3K-Akt signaling pathway in Rheumatoid arthritis fibroblast-like synoviocytes (FLS).	Tian *et al*., 2013
Rosmarinic acid	*Rosmarinus officinalis* L.	Inhibit NF-κB, COX-2, and TNF-α.	Hur *et al*., 2007
Rutin	*Fagopyrum esculentum*	Inhibits the activation of NF-κB, iNOS expression, and NO	Guardia *et al*., 2001

	Moench. and other fruits	production.	
Salicin	Bark of *Salix alba* L.	Suppress the production of pro-inflammatory factors.	Khayyal *et al*., 2005
Sesamin	*Sesamum indicum* L.	Prevent the degeneration of cytokine-induced cartilage by slowing down the degradation of constitutive glycosaminoglycans and collagen.	Khansai *et al*., 2016
Sesamol	*Sesamum indicum* L.	Decrease the level of production of pro-inflammatory cytokines and inhibits the activity of tissue destructive enzymes.	Hemshekhar *et al*., 2013
Sesquiterpene lactones	*Saposhnikovia divaricata* (Turcz.) Schischk.	Inhibit NF-kB pathway by targeting IKK and preventing the degradation of IkB-α and IkB-β, without interfering with ROS formation, in vitro experiments.	Hehner *et al*., 1998; Siedle *et al*., 2004
Sibyllenone	*Ocotea bullata* (Burch.) E. Meyer in Drege	Inhibit the activation of LOX-5	Zschocke *et al*., 2000
Silymarin	*Silybum marianum* (L.) Gaertn.	Inhibition of 5-lipooxygenase	Gupta *et al*., 2000
Statins	*Aspergillus terreus* Thom	Inhibits the pro-inflammatory factors including NF-κB, COX-2, MMP-9	Ahn *et al*., 2007
Tocotrienol	Nuts and vegetable oil	Inhibits TNF-α and the activation pathway of NF-κB.	Radhakrishnan *et al*., 2014
Triptolide	*Tripterygium wilfordii* Hook.f.	Inhibits the activation of NF-κB	Lin *et al*., 2007
Thymoquinone	*Nigella sativa* L.	Prevent LPS-induced p38 MAPK and NFκB-p65 phosphorylation.	Vaillancourt *et al*., 2011
Type-A	*Cinnamomum*	Anti-arthritic effects in an	Vetal *et al*.,

Procyanidine	*zeylanicum* Garcin ex Blume	experimental model.	2013
Ursolic acid	*Ocimum sanctum* Linn.	Inhibits the proinflammatory cytokine Th1.	Ahmad *et al*., 2006
Withanolides	*Withania somnifera* (L.) Dunal	Inhibits the activation of NF-κB	Heyninck *et al*., 2014

Most plants have been confirmed to have anti-RA activities **(Table 1)** i.e. *Alstonia scholaris* L., *Abrus precatorius* L., *Acanthopanax trifoliatus* (L.)Merr., *Acacia catechu* (L.f.) Willd., *Acalypha indica* L., *Achyranthes aspera* L., *Aconitum heterophyllum* Wall., *Acorus calamus* L., *Adhatoda beddomei* Clarke., *Ailanthus triphysa* (Dennst.) Alston., *Ajuga bracteosa* Wall., *Alangium salvifolium* (L.f.) Wangerin, *Ammania baccifera* L., *Anacardium occidentale* L., *Aquilaria agallocha* (Lour.) Roxb., *Aristolochia bracteolata* Lam., *Argemone Mexicana* L., *Argyreia speciosa* Sweet., *Asparagus racemosus* Willd., *Azadirachta indica* A. Juss, *Bacopa monnieri* Penell., *Bauhnia variegata* (L.) Benth., *Berberis vulgaris* L., *Boerhaavia diffusa* L., *Boswella serrata* Roxb., *Caesalpinia sappan* Linn., *Cannabis sativa* Linn., *Cedrus deodara* (Roxb.) G.Don, *Celastrus aculeatus* Merr., *Centella asiatica* (L.) Urban, *Centipeda minima* Lour., *Cleome gynandra* L., *Cleome rutidosperma* DC., *Costos speciosus (*Koen) Smith., *Crocus sativus* L., *Cynodon dactylon* (L.) Pers., *Kaempferia galanga* L., *Cinnamomum zeylanicum* Garcin ex Blume, *Coriandrum sativum* Linn., *Curcuma longa* L., *Luffa echinata* Roxb., *Machilus macrantha* Nees., *Mallotus oppositifolium* Mull., *Merremia emarginata* Burm., *Mirabilis jalapa* L., *Moringa oleifera* Lam., *Nigella sativa* L., *Nyctanthes arbortristis* L., *Ocimum gratissimum* L., *Oroxylum indicum* (L.) Kurz., *Operculina turpethum* Linn., *Paederia foetida* L., *Plantago ovata* Forssk., *Phyllanthus amarus* Schum. & Thonn.,

Phylanthus emblica L., *Physalis angulata* Linn., *Piper nigrum* L., *Piper longum* Linn., *Pisonia grandis* R.Br., *Pistia stratiotes* L., *Pongammia pinnata* (L.) Pierre, *Premna serratifolia* L., *Punica granatum* L., *Randia dumetorum* (Retz.) Poir., *Rheum palmatum* L., *Ricinus communis* L., *Rosa centifolia* L., *Rubia cordifolia* L., *Ruta graveolens* Linn., *Salix alba* L., *Salix nigra* Linn., *Santalum album* L., *Saraca asoca* Roxb., *Saussaurea lappa* (Decne.) Sch. Bip., *Semecarpus anacardium* Linn., *Sesamum indicum* L., *Sida rhombifolia* L., *Sida cordifolia* L., *Smilax glabra* Roxb., *Smithia sensitiva* Smith., *Stephania longa* Lour., *Strobilanthus callosus* Nees., *Strychnus potatorum* Linn., *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* Retz., *Tinospora cordifolia* (Thunb.) Miers, *Toxicodendron pubescens* P. Mill., *Trewia polycarpa* Benth., *Tribulus terrestris* L., *Tridax procumbens* Linn., *Trigonella foenum-graecum* Linn., *Tripterygium wilfordii* Hook.f., *Urginea indica* (Roxb.) Kunth, *Urtica dioica* L., *Urtica pilulifera* L., *Valeriana wallichii* DC., *Vernonia anthelmintica* (L.) Willd., *Vernonia cinerea* Less., *Vitex negundo* L., *Wadelia calendulacea* (L.) Pruski, *Withania somnifera* (L.) Dunal, *Xanthium strumarium* Linn., *Yucca schidigera* Roezl., *Zingiber officinale* Roscoe, and *Ziziphus mauritiana* Lam. are found in most parts of India and are used by local inhabitants in the form of various traditional medicines to treat arthritis.

2.11. *Plants used for the treatment of RA in the Sikkim Himalayan region*

Sikkim Himalaya is home to not only a diverse range of medicinal plants but also to many distinct ethnomedicinal systems that have evolved over time. The Lepcha herbal, Nepali *jaributi,* and Tibetian systems are the most well-known among these. Each of these systems depends on local plant resources, and the enumeration of ethnomedicinal plants in this area includes all of the traditional practices. Biswas

(1956) has described 147 medicinal plants found in the Darjeeling and Sikkim Himalayas and 18 plant species out of them are reported for the treatment of RA. Srivastava & Kapaki (1990) have recorded over 400 plant species with the therapeutic potential of which many of them are used in the traditional system of medicines in the region. Further Rai & Sharma (1994) reported the potential of 40 medicinal plants. Rai *et al.*, (1998) have enumerated 47 plant species having ethnomedicinal values. Bejoy (2002) has enumerated 423 medicinal plants from Sikkim and reported 50 plant species for treating RA. Singh *et al*., (2002) reported the 64 plants species used as folk medicines for the treatment of numerous ailments of which 4 species are reported for the treatment of RA. Maity *et al*., (2004) have enumerated 15 medicinal plants of which 2 species are used for treating RA. Chhetri (2005) has reported 110 ethnomedicinal plants species of which 4 species are reported for treating RA. Sharma & Sharma (2010) have enlisted 490 medicinal plants from Sikkim and they reported about 50 plant species for the treatment of RA. Panda & Misra (2010) have reported 31 species of medicinal plants which are used in the traditional healing system of Sikkim, 5 out of them are reported for the treatment of RA. Lepcha *et al*., (2011) have enlisted 25 important medicinal plants from the landslide-prone regions of Sikkim of which 3 species are reported for treating RA. Panda (2012) has enlisted 23 medicinal plants used by the people of Sikkim for the treatment of various ailments and *Rhododendron arboreum* is reported for treating RA. Mandal *et al*., (2013) have recorded 30 medicinal plants used by the ethnic inhabitants of Sikkim to cure human as well as livestock diseases of which *Oroxylum indicum* is reported for treating RA. Badola & Pradhan (2013) have recorded the use of 124 species of ethnomedicinal plants which are used by the Limboo community to cure 77 ailments and *Juglans regia* is recorded for treating RA. Tamang *et al*., (2017) have enumerated 54 species

of medicinal plants which are used by the ethnic inhabitants for treating numerous ailments, and 4 plant species out of them are reported for RA. Some of the reported medicinal plants from Sikkim Himalaya with anti-RA activity are enumerated alphabetically below in **Table 3**, along with their families and part used.

Table 3: List of plants reported from Sikkim Himalaya for the treatment of RA

S.no	**Scientific name**	**Local Name**	**Family**	**Part used**
1	*Achyranthes aspera* L.	*Apamarg*	Amaranthaceae	Seed and leaf
2	*Aconitum bisma* (Buch.-Ham.) Rap.	*Bikhma*	Ranunculaceae	Root
3	*Aconitum ferox* Wall. ex Ser.	*Bikh*	Ranunculaceae	Tuberous root
4	*Aconitum heterophyllum* Wall. ex Royle	*Bikhjhar*	Ranunculaceae	Root
5	*Acorus calamus* L.	*Bojho*	Acoraceae	Rhizome
6	*Aesculus indica* (Wall. ex Camb.) Hook.	*Pangra*	Sapindaceae	Seed oil
7	*Ageratum conyzoides* L.	*Elamey*	Asteraceae	Whole plant
8	*Aloe barbadensis* Mill.	*Ghew kumari*	Liliaceae	Leaf
9	*Alpinia galanga* (L.) Willd.	*Kulingan*	Zingiberaceae	Rhizome
10	*Alstonia scholaris* Lin.	*Chatiwan*	Apocynaceae	Leaf
11	*Ammannia baccifera* L.	*Amber*	Lythraceae	Leaf
12	*Aphanamixis polystachya* (Wall.) R.N. Parker	*Bandri phal*	Meliaceae	Seed oil
13	*Argemone Mexicana* L.	*Sungure kara*	Papaveraceae	Leaf
14	*Bidens pilosa* L.	*Kuro*	Asteraceae	Young shoot
15	*Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl.	*Dhokrey phul*	Solanaceae	Leaf
16	*Callicarpa arborea* Roxb.	*Guenlo*	Verbenaceae	Bark

17	*Callicarpa macrophylla* Vahl.	*Patharman*	Verbenaceae	Bark
18	*Canarium bengalense* Roxb.	*Gokuldhup*	Burseraceae	Leaf and bark
19	*Cassia fistula* L.	*Rajbriksha*	Fabaceae	Leaf
20	*Cedrus libani* Barrl.	*Deodar*	Pinaceae	Wood
21	*Celastrus paniculata* Willd.	*Malkaguni*	Celastraceae	Seed
22	*Cinnamomum tamala* Fr.	*Chotasinkoli*	Lauraceae	Leaf
23	*Datura fastuosa* Lin.			
24	*Dioscorea deltoidea* Wall. ex Griseb.	*Bhyagur*	Dioscoreaceae	Tuber
25	*Diploknema butyracea* (Roxb.) H.J. Lam	*Chewri*	Butyracea	Seed
26	*Ephedra sikkimensis* var. *sikkimensis* Stapf	*Somlata*	Ephedraceae	Whole plant
27	F*raxinus floribunda* Wall.	*Lankhuri*	Oleaceae	Bark
28	*Gaultheria fragrantissima* Wall.	*Dhasingare*	Ericaceae	Fruit oil
29	*Gloriosa superb* L.	*Langaraytarul*	Liliaceae	Seed
30	*Gynocardia odorata* R. Br.	*Gantey*	Flacourtiaceae	Seed oil
31	*Juglans regia* L.	*Okhar*	Juglandaceae	Fruit
32	*Kaempferia galanga* L.	*Kharabe*	Zingiberaceae	Leaf
33	*Lycopodium clavatum* L.	*Nagbeli*	Lycopodiaceae	Whole plant
34	*Mentha viridis* Lin.	*Pudina*	Lamiaceae	Leaf
35	*Michelia champaca* L.	*Aulechamp*	Magnoliaceae	Flower
36	*Mirabilis jalapa* L.	*Lankasaani*	Nyctaginaceae	Leaf
37	*Oroxylum indicum* (L.) Benth. ex Kurz	*Totola*	Bignoniaceae	Bark
38	*Paederia foetida* L.	*Padelahara*	Rubiaceae	Whole plant
39	*Piper longum* Lin.	*Pipala*	Piperaceae	Fruit
40	*Pittosporum napaulense* (DC.) Rehder & Wilson	*Phurke*	Pittosporaceae	Bark
41	*Plantago major* L.	*Ishabgol*	Plantaginaceae	Whole plant

42	*Plumeria acutifolia* Poir.	*Chuwa*	Apocynaceae	Bark
43	*Plumbago indica* L.	*Chitu*	Plumbaginaceae	Root
44	*Polygonum viviparum* L.	*Ratnaulo*	Polygonaceae	Root
45	*Potentilla fruticosa* L.	*Chiniaphal*	Rosaceae	Leaf
46	*Randia dumetorum* Lam.	*Amuki, Maidal*	Rubiaceae	Bark
47	*Rheum nobile* Hook.f. & Thom.	*Kenju*	Polygonaceae	Rhizome
48	*Rhodiola himalensis* (D. Don) S.H. Fu	*Lakpaguru*	Crassulaceae	Root
49	*Rhododendron arboreum* Sm.	*Laligurans*	Ericaceae	Leaf
50	*Rhododendron campanulatum* D. Don	*Nilochimal*	Ericaceae	Leaf
51	*Ricinus communis* L.	*Arandi*	Euphorbiaceae	Leaf
52	*Rubia cordifolia* L.	*Majitho*	Rubiaceae	Root
53	*Saccolabium papillosum* Lindl.	*Nakuli*	Orchidaceae	Root
54	*Saussurea tridactyla* Sch. Bip. ex Hook.f.	*Kapasephul*	Asteraceae	Whole plant
55	*Scindapsus officinalis* (Roxb.) Schott.	*Thoolo pipalaa*	Araceae	Fruit
56	*Semecarpus anacardium* L.f.	*Bhalayo*	Anacardiaceae	Fruit juice
57	*Solanum dulcamara* L.	*Dogwood*	Solanaceae	Fruit
58	*Spondias magnifera* Willd.	*Amaru*	Anacardiaceae	Fruit
59	*Stephania glabra* Roxb.	*Tamarkey*	Menispermaceae	Tuber
60	*Taraxacum officinale* Weber	*Tukiphool*	Asteraceae	Leaf
61	*Tinospora cordifolia* (Willd.) Hook.f. & Thom.	*Gurjo*	Menispermaceae	Stem
62	*Toddalia asiatica* (L.) Lam.	*Mainkanra*	Rutaceae	Fruit and root
63	*Trewia nudiflora* L.	*Ramritha*	Euphorbiaceae	Root
64	*Urtica dioica* L.	*Sisnu*	Urticaceae	Root
65	*Vitex negundo* L.	*Sewali*	Verbenaceae	Leaf
66	*Xanthium strumarium* L.	*Bhedekuro*	Asteraceae	Seed oil

69	*Zanthoxylum budrunga* Wall.	*Timursil*	Rutaceae	Fruit
67	*Zingiber officinale* Rosc.	*Aduwa*	Zingiberaceae	Rhizome
68	*Zingiber zerumbet* (L.) Roscoe ex Sm.	*Phacheng*	Zingiberaceae	Rhizome

Chapter 3

Study Area

3.1. *Description of the Study Area*

Sikkim is one of the mountainous states of India lies between 27˚04'46" to 28˚07'48" N latitudes and 88˚00'58" to 88˚55'25" E longitude covering an area of 7096 km^2 with wide altitudinal variation from 300m to 8586m asl. Sikkim is known for its scenic beauty with rich biological diversity indicated by varied eco-climatic conditions where the mount Khangchendzonga and the adjacent Singalila mountain range play a vital role to determine the climatic condition in the western region bordering Darjeeling and Nepal while Chola range in the eastern region bordering Bhutan and southwestern part of Tibet Autonomous Region (TAR) of China. These two ranges not only have a significant effect on the atmospheric condition and regional climate of Sikkim and its surrounding areas. In the north, the state forms an international boundary with China, while in the south it is bordered with West Bengal. Sikkim constitutes about 0.22 % of the total geographical area in the country but contributes about 26.95% of floristic elements in the country (Gogoi *et al*., 2021).

Historically, the name Sikkim is derived from the Limbu word *Sukhim* which means "new Home". The Tibetan people called it *Drejong* which means "valley of rice" while the Bhutias called it "*Beyul Demazong*" which means "the hidden valley of rice" and the Lepcha people called it *Mayel Lyang*, which "Land of hidden paradise".

3.2. *A brief history of Sikkim*

The earliest history of Sikkim dates back to the 13th century and the origin of the Namgyal dynasty begins from the Mik Yak House of Tibet. The rulers of this dynasty were the descendants of Tri Srong Den Tsen who was the most illustrious of all the

rulers of Tibet. The Min Yak chief was prophesied to go south from Tibet where his descendants would rule. Impelled by the divine command; he started forth from his kingdom with his sons and passed through Sakya to pay homage to the hierarchs there. At Sakya, the eldest son of the emigrant chief helped in the construction of a monastery after he obtained the name Gyad-hBum-bSar (Kye-Bumsa). He married the daughter of the Sakya hierarch and dwelt in the nearby Chumbi valley which later became the nucleus of the kingdom of Sikkim. Kye Bumsa headed further south to meet the Lepcha head, Thekong-Tek to receive the blessings. This meeting was commemorated with the signing of a treaty of brotherhood between the two chieftains at a place called Kabi (Karbi) 21 km north from Gangtok where nine stones were erected to mark the place. After the death of Kye Bumsa, his son Mipon Rab was succeeded to the chieftainship. He also married Gurumo, a lady from Sakya hierarch's family, and had four sons together namely Sangpodar, Tsechudar, Nima Gyalpo, and Guru Tashi. Among four brothers, Guru Tashi's family became pre-eminent and inherited the princely dignity and name. The modern history of Sikkim begins in 1642 with the consecration of the first prescribed *Chogyal* (king) Phuntsog Namgyal, the great-great-grandson of Guru Tashi. He was consecrated at Yuksom by the three venerated monks. Phuntsog Namgyal is known for the foundation of the Namgyal dynasty in Sikkim whose successors continued to rule till the merger with India in the year 1975 (Kharel & Bhutia, 2013).

In 1670 Phuntsog Namgyal was succeeded by his son, Tensung Namgyal, who shifted the capital from Yuksom to Rabdentse near Geyzing, the present headquarter of West district of Sikkim. Owing to a family dispute over the succession of kingship to his brother, Pende Ongmo, the daughter of Tensung Namgyal with the help of Bhutanese force attacked Sikkim in 1700 and held the country for about five to eight years. Ten

years later, the Bhutanese forces were sent back to Bhutan on the request of the then Dalai Lama, The kingdom remained in unrest during 1717-1733 due to frequent attacks from Nepal in the West and Bhutan from the East that led to the reduction of boundary and destruction of the capital at Rabdentse by the Nepalese force. In 1814, Tsudphud Namgyal shifted the capital from Rabdentse to Tumlong in North Sikkim.

To the southwest of Sikkim, a war between the forces of East India Company and Gorkha was fought in the year 1814 and the war ended with the treaty of Sugauli on 2nd December 1815 which was rectified on 4th March 1816. By this year Sikkim had lost the territories west to river Teesta and east to river Mechi. This treaty led the Gorkha forces to evacuate the territories East to river Mechi including present-day Darjeeling and its adjoining areas bordering Nepal and restored to Sikkim. A year later, the treaty of Titalia was signed between the East India Company and the King of Sikkim on 10th February 1817, and the territories evacuated and restored from Nepal were ceded to the East India Company. This treaty helped Sikkim to restore peace but made them dependent on the East India Company followed by the treaty of Tumloong signed between the British Government and the King of Sikkim on 28th March 1861 lost all freedom of action and became a protectorate of the British Government of India. Thutob Namgyal shifted the capital of Sikkim to Gangtok in 1894 and since then it remained the capital of Sikkim. On 5th December 1950, a treaty was signed between India and Sikkim which gave Sikkim the status of protectorate state, and on 16th May 1975, Sikkim became the 22nd state of the Union of India and the monarchy was abolished (Moktan, 2004).

3.3. *Description of the studies people*

Lepcha people are believed to be the earliest settlers of Sikkim but over the centuries many people from its neighboring countries settled here and constituted the homogenous blend of distinct Sikkimese identity. The predominant communities are the Lepchas, Bhutias, and Nepalese. In urban and city areas people from many states of India have also settled and they are engaged in business, government services and construction work.

3.3.1. *Lepchas*

Lepcha people believe that their home has always been the legendary kingdom of "*Mayel*" in the vicinity of Mount Khangchendzonga and have been settled there several years before the Bhutias and Nepali communities settled in Sikkim. Lepchas are traditionally believers of nature worship and now many of them follow other religions. They worship the spirits of mountains, rivers, and forests as their deity as believed that their deity enables them to co-exist with nature harmoniously. Spirit possession is common among the spiritual priests and they are called *Boongthing* for males and *Mun* for the female priest. Dzongu, an area in the North district is designated as a reserved area for the Lepcha community. However, the population of Lepcha is spread in all parts of Sikkim, districts of Darjeeling, Kalimpong of West Bengal, and the Eastern part of Nepal.

a) *Culture and Tradition*

Lepchas have diverse cultures and traditions of which *Eng-Rum-Faat* is performed for the newborn baby. The marriage ceremony is called *Bree-Rum-Faat* during which *Boongthing* or *Mun* and the religious priest called Lamas to solemnize the ceremony. The priest calls the local spirits and local deity uttering the name of God, places,

mountains, and rivers while the blessing to the wedding couples. The priest gives a sermon to the couples and wishes them to remain bonded forever.

The death ceremony is called *Aamek Sunglyon* and they use two different methods to cremate the dead bodies. The dead bodies of children are usually left in the caves or bury whereas the dead body of adults is cremated performing rites for 49 days by the Lamas. After performing the last rites, many prayer flags are hoisted by the family members and their relatives for the heavenly peace of the deceased.

Lepchas celebrate many festivals like *Tendong-Lho-Rum-Faat, Chu-Rum-Faat, Dho-Rum-Faat, Sugi-Rum-Faat* and *Gaeboo Achyok Aagek Singnim.*

b) *Language and script*

Lepchas have their own language called *Rongring*, and although the language is not very well developed it is very rich in vocabulary related to nature which include many plant and animal names found in Sikkim and its adjoining areas. Lepchas have their script called *Rongchyaming* or Lepcha script which is believed to be of Tibeto-Burman origin.

3.3.2. *Bhutias*

Bhutia people are of Tibetan origin whose ancestors must have emigrated to Sikkim from Tibet probably in the fifteenth century. In earlier days they were mostly found settled in the snow-bound areas of Sikkim, and now they are settled in almost all parts of Sikkim and its adjoining areas of Darjeeling, Kalimpong districts of West Bengal. Bhutias follow Buddhism and organize religious activities regularly in their houses and the monastery. They perform prayers on every religious occasion and raise prayer flags around their houses and monasteries to be protected from evils. A traditional Bhutia house is called *Khim* which is usually rectangular in shape. Elderly people in the Bhutia community turn the prayer wheel called *Khorlo* while praying.

a) *Culture and tradition*

Bhutias follow Mahayana Buddhism and perform all types of ceremonies and festivals following the Buddhist religion. The marriage ceremony is performed by the Lamas in both the houses of bride and groom on which gold ornaments and other valuable items are given to the bride. Both the birth and death ceremonies are performed by the religious priest. The dead body of the adult is cremated in a specific location where the dead body of the infant is buried after consulting the constellation of stars. After performing the last rites of the dead member, many prayer flags are raised for the heavenly peace of the deceased. Bhutias celebrate many festivals in a year. The most important festivals are *Bumchu, Drukpa Teshi, Kagyed Dance, Kalchakra Puja, Lhabab Dhuechen, Losar, Losoong, Saga Dawa, Pang Lhabsol,* and *Namsoong.*

3.3.3. *Nepali*

Nepali community is composed of about twenty different heterogeneous groups they speak the Nepali language as the *lingua franca* which include Bahun, Bhujel, Chhetri, Damai, Gurung, Kami, Majhi, Mangar (Thapa), Newar (Pradhan), Rai, Sarki, Sherpa, Subba (Limboo), Sunuwar (Mukhia), Tamang, Thakuri, Thami, and Yolmo. Each of them has their belief system, custom, culture, tradition and they have their dialect and celebrate their festivals. They follow the *Devnagari* script in general however, some of them claimed to have their own scripts.

a) *Culture and Tradition*

Among the Nepali-speaking communities Bahun, Bhujel, Chhetri, Damai, Kami, Majhi, Newar, Thakuri, and Sarki follow the Hindu religion whereas Sherpa, Tamang, and Yolmo follow the Buddhist religion. Gurung communities partially follow the Hindu religion and partially the Buddhist religion whereas the Mangar community

partially follows the Hindu religion. The remaining communities have their own belief system of animism. Nature worship is and animal sacrifice is common while performing religious festivals among this group. However, the Nepali community has maintained more or less uniform tradition right from birth to death. They perform a name-giving ceremony called *Nauran* when a new baby is born in the family. When a baby attains the age of six months, a ceremony is organized called *Pasni* or *bhat khuwai*. When a male child reaches five to six years of age a ceremony is observed called *Chewar* where tonsure of the child is performed by the maternal uncle.

Followers of Hindu and Buddhist communities cremate the dead body of the adult and the dead body of the infant is buried. The last rites are performed by the community priest. Such rituals of the remaining communities are performed by their community priest and the dead bodies are usually buried. In Rai and Subba (Limboo) communities the death rites are performed by their community priest called *Mangpa* by Rai and *Phedangma* by Subba community. These community priests are believed to have divine gifts who can communicate with the spirit of the deceased person.

Nepali communities have many festivals where some of them are celebrated whereas others are simply observed. They follow the English/Gregorian calendar as well as the *Vikram Sambat* calendar which is popular in the agrarian society. The first day of *Vikram Sambat* is celebrated as a new year and a mark of cultural unity. The most common festivals celebrated by the Nepali community include *Asar pandrah, Barahimzong* (Mangar community), *Chaite Dasai, Sahune Sankranti, Bhadaure Purne* (Gurupuja), *Dashai* (Dashera), *Tihar* (Diwali/Dipawali), *Maghe Sankrati, Sansari Puja, Sakewa* (Kirat Rai community), *Geel, Sunwar Sagoon* (Sunwar/Mukhia community), *Sonam Losar* (Tamang community), *Chasok Tangnam* (Subba/Limboo community) and *Buddha Jayanti* (birth anniversary of Lord Buddha).

A tradition of blood brotherhood has a very significant place among the indigenous communities of Sikkim. The Min Yak chieftain Kye Bumsa and the Lepcha head Thekong-Tek had the first blood brotherhood that makes the bonding of two families stronger. A similar tradition is also found among the heterogeneous group of Nepali communities called *"Mit"*. In this community, even the female member of the family also takes part in *"Mit"* relationships.

3.4. *Mountain system*

A major portion of Sikkim is composed of Precambrian rock and is much younger in age. The mountains rise in elevation northward. The Northern, Eastern, and Western regions of the state are constituted with hard massive gneisses rock capable of resisting denudation. The Central and Southern areas are constituted with comparatively soft, thin, slaty, and half-schist ore rocks which denude easily. The trend of the mountain system is in an East-West direction. However, chief ridges run more or less in a North-South direction. The valleys are rather open towards the top but usually attain step gorge-like structures as the bed of the rivers is approached. Sikkim encompasses the parts of Lesser Himalaya, Higher Himalaya, and the Trans Himalaya and bears the magnificent mountain peaks of the Himalaya. There are 58 snow-covered peaks located in three districts of Sikkim. Some important mountain peaks are Mt.Khangchendzonga (8596m), Mt Kabru (7361.36m), Mt.Talung (7356.8m), Mt.Siniolchu (6870.4m), Mt.Simvo (6832.7m), Mt.Pandim (6718.4m), Mt.Rathong (6718.4m), Mt.Paunhri (6688m), Mt.Kokthang (6129.2m),Mt. Lama Wangden (5887.26m) and Mt. Masunyange (5867.2m) (Kharel & Bhutia, 2013). Mt.Khangchendzonga is the most spectacular mountain peak located in the West district bordering Nepal.

3.5. *River system*

Sikkim is endowed with more than one hundred perennial rivers and is rich in water resources. River Teesta and Rangit constitute the major river system with a number of tributaries. Rangit River is the major river in the western region of Sikkim originating from Rathong glacier, the base of Mt. Khangchendzonga, and flows southwards. Flowing from the north, it joins with river Ramam at Nayabazar and becomes the great Rangit river which further flows southeast direction extending about 51 km and finally meets with river Teesta at Triveni bordering with the district of Darjeeling. Some of the major tributaries of river Rangit are Prek Chu, Rathong Chu, Kalej Khola, Reshi Khola, Rambhang Khola, Rimbi Khola, and little Rangit. Rathong Chu is considered a holy river in Sikkim and has religious significance. Bum Chu festival is celebrated every year offering the holy water of Rathong Chu in the Tashiding monastery of the West district of Sikkim.

River Teesta originates from a glacial lake, Cho Lhamu in the northeastern corner of Sikkim and is joined by several tributaries on way to down that spread the North and South district of the state. Some of the major tributaries of the river Teesta are Lachung Chu, Rakchum Chu, Rani Khola, Rangpo Chu, Rongli Chu, Rang Chu, and Rora Chu.

3.6. *Lakes and glaciers*

Sikkim is endowed with some of the picturesque lakes serving as an important water source in the state. There are several lakes of various sizes and most of them are in the high altitudinal regions. These lakes are originated as depressions scooped by the glaciers in valleys through which they flowed and subsequently filled by glacial melt. Others have been formed by the damming of glacial water by terminal moraines (Kharel & Bhutia, 2013).

Lakes are locally called Cho (Bhutia) and Pokhri (Nepali). Some of the prominent and beautiful lakes are Gurudongmar lake, Thang Chhojo, Lam Pokhari, Kali Pokhri, Kanahailal Pokhri, Deoningale Pokhri, Torepool Pokhri, Dudh Pokhri, Rakta Pokhri, Kalmoi Pokhri, Paley Pokhri, Hans Pokhri, Dhungey Pokhri, Thuley Pokhri, Laxmi Pokhri, Nir Pokhri, Mayur Pokhri, Ram Laxuman Pokhri, and Jyamte Pokhri, Tsomgo lake (Chhangu), Syeberuka Cho and Serabthang Cho. These lakes are located in subtropical to temperate regions. Gurudongmar lake is the largest lake and is located at the highest point among the lakes in North Sikkim. Khechopelri lake is known as a wish fulfilling lake located in West Sikkim and is considered as the most sacred lake in Sikkim.

Glaciers are restricted to the western and northern parts of the state. Waters from the glaciers drain into the Teesta River and are hence generally known as the Teesta basin glaciers. There are four major glacierized basins in Sikkim namely East Rathong Basin, Talung Basin, Changme Khangpu Basin, and Zemu Basin covering an area of 7172.21 sq. km.

3.7. *Vegetation and forest types*

Vegetation and forest types of Sikkim differ from its neighboring regions of the Eastern Himalaya when considered from the viewpoint of its rich biodiversity and distribution within the limit of this region. It is because of the great diversities in physiographic, climatic, and edaphic conditions often aided by biotic factors. Considering the uniqueness and diversity of the flora, many attempts were made from time to time to classify the vegetation by various botanists and ecologists Sir J.D Hooker (1872-1897) classified the vegetation of Sikkim into six different types namely Tropical Semi-Evergreen Forest (300-900m), Sub Tropical Mixed Broad Leaves Hill Forest (900-1800m), Himalayan Wet Temperate Forest (1800-2700m),

Sub Alpine Forest (2700-3700m), Moist Alpine Forest (3700-4000m) and Dry Alpine Forest (Above 4000m). Almost after one hundred years Champion & Seth (1968) and Sinha & Chauhan (1996) classified the vegetation of the Sikkim Himalayan region with few modifications however, the composition of vegetation is unchanged (Chetia & Rai, 2021). According to the Forest Survey of India report (FSI, 2017), the total forests covered in Sikkim are 47.13% that harbors large varieties of flora and fauna that delight nature lovers, biologists, conservationists, and environmentalists. At present, Sikkim has eight protected areas that cover an area of 3019.1 km with altitudinal gradients ranging from 310–8598m of which Kanchandzonga Biosphere Reserve is the largest protected area covers 2620 km^2. It shares its border with Nepal in the West, Tibet Autonomous Region of China in the North, and Darjeeling district in the South and it is inscribed to the UNESCO World Heritage Site list in 2016. Due to its immense altitudinal gradient and physiographic contrast, Sikkim shows an immense existence of eco-climatic zones from tropical to alpine. There are a large number of flowering plant species, ferns, gymnosperms, bamboos, and canes that attracted many botanists from Asia and Europe during the nineteenth century.

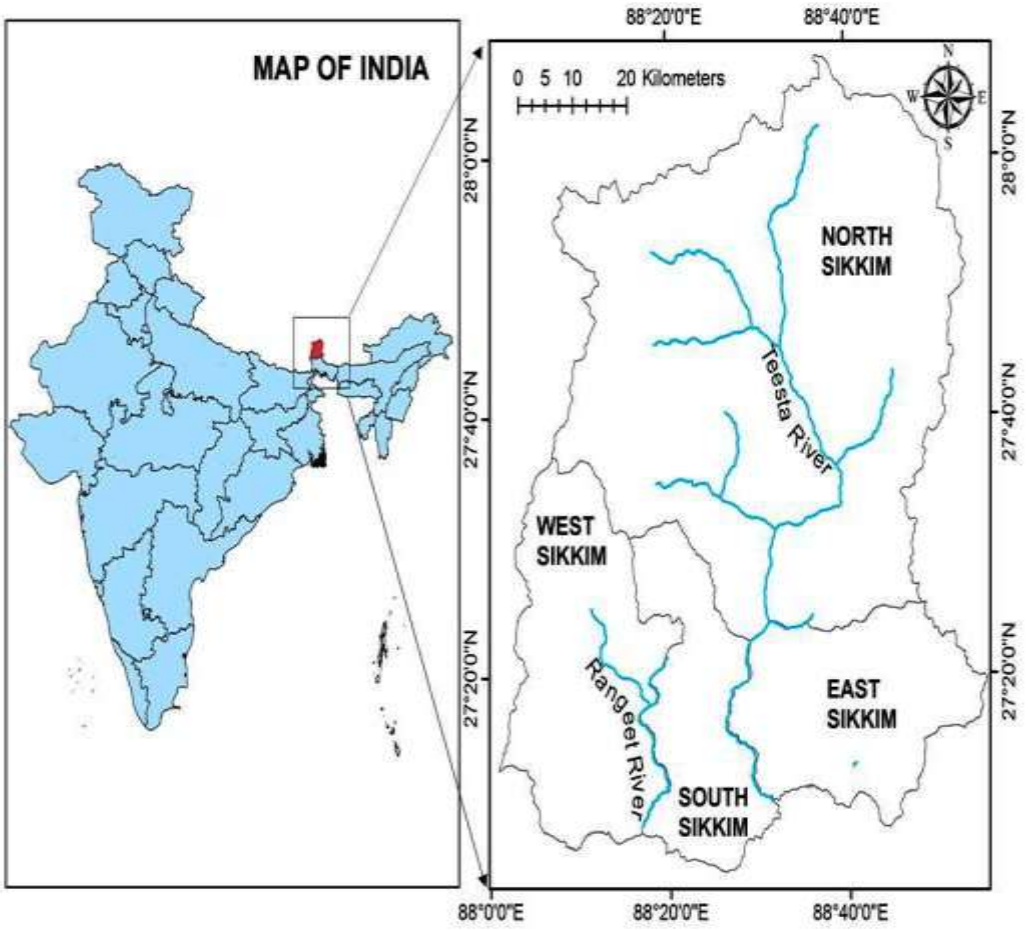

Figure 2: Map of Study area

3.8. *Botanical explorations in Sikkim*

The botanical uniqueness and diversity of Sikkim have attracted a large number of plant collectors, ecologists, and botanists from many countries of the world. Griffith & McClelland (1847) carried out the first floristic diversity study in Sikkim in1843 and published the first comprehensive report of Sikkim flora in 1847. Sir J.D Hooker arrived in Darjeeling in 1848 and explored the west and northern regions of Sikkim during the year 1848-49 that made the foundation of Indian flora published with George Bentham in 1872-1897. Other distinguished botanists who subsequently collected plants from Sikkim and made a significant contribution were Gamble (1875, 1896) studied Sikkim's trees and climbers of Sikkim, Clarke (1876, 1885), King &

Pantling (1898) studied the Orchids of Sikkim Himalaya, Smith (1913), Bruhl (1926), Professor Hara of Tokyo University (1966, 1971) and Ohashi of Tokyo University (1975).

Sikkim was an independent state and merged with India in 1975. After the merger floristic exploration was continued by Grierson & Long (1983), Pradhan & Lachungpa (1990), Noltie (1994, 2000), Rai & Rai (1994), Hajra & Verma (1996), and Lucksom (2008) and published the pictorial guide to a large number of plants.

Kholia (2010, 2014) studied the lower plant group especially the ferns and ferns allies of Sikkim in collaboration with the Sikkim state biodiversity board.

3.9. *Economy and Administration set up*

The economy of Sikkim is dependent on agriculture where more than 80% of the population is dependent on it. Rice is one of the major crops cultivated up to 1800m in the terrace where many indigenous varieties are also grown. Maize, wheat, finger millet, buckwheat, mustard, pulses like rajma, pea, cowpea, cluster bean, rice bean, soya bean are some of the important crops cultivated for staple food. Ginger, large cardamom, cherry chilly, mandarin, potato, and tea are some of the important cash crops cultivated on a small to medium scale. *Seremna* variety of large cardamom and *Dallay Khorsany* (cherry chilly) are enlisted in the status of geographical indication.

Sikkim initiated the organic state involving about 60,000 farming families with 50,000 hectares of agricultural land and banned the use of synthetic agriculture input since 2003 (Bhutia, 2015). Sikkim got the status of a completely organic state of India in the year 2017 and it is the first complete organic state in the country.

Tourism is one of the income-generating avenues in Sikkim. The state government has initiated eco-tourism and village tourism involving the local people which are popular in some of the remote areas of Sikkim. The small population of Sikkim is also

involved in floriculture, beekeeping, poultry, dairy, traditional art and craft, and adventure sports for income generation.

Sikkim has the capacity to generate 8,000 MW of hydroelectricity and the electricity demand is increasing annually. 36 hydropower stations generate more than 5000 MW of electricity.

New Industrial Policy of Sikkim was announced in 1996 and identified agro-based industries, floriculture, animal husbandry, and dairy products, minor forest-based products, handloom, handicrafts and village industries, tourism, precision-oriented high-value low volume products, electronic and computer software, hydel power, and tea. In the year 2000, Sikkim Industrial Promotion and Incentive Act were enacted to attract investments in the state and amended in 2007. Sikkim is included under the North East Industrial and Investment Policy 2007 with various incentives for the investors. Most of the industrial units are micro, small, and medium. The prospect of opening up the border trade through Nathula poses both challenges and opportunities for the state.

The population of Sikkim is 6, 58,019 (India census.net 2021), for administrative convenience, Sikkim has four districts namely East, West, North, and South. There are 40 Block Development Offices and 176 Gram Panchyat Unit. Gangtok is the capital of Sikkim connected with National Highway 10 by road from the nearest West Bengal state and also connected by air route recently.

Chapter 4

Plant-based traditional knowledge of Sikkim Himalaya against rheumatoid arthritis (RA)

4.1. INTRODUCTION

Since prehistoric times, traditional medicine systems have been used by people to take care of their health, particularly in the treatment of diseases based on their practices, experiences, and beliefs. Traditional systems of medicine used by herbal practitioners provide substantial contributions to human health at the community level and globally maintain its popularity among the people (WHO, 2008). The majority of the populations especially in the developing countries as well as underdeveloped countries are still dependent on the traditional system of medicines for their primary health care (Sofowora, 1982; Hegde, 2003). Most of the countries of the old and new world have deep-rooted societies on traditional medicine system and it is very common among the people of India, China, Japan, Thailand, Sri Lanka, Pakistan, and Korea (Park *et al*., 2012). In India, popularly used traditional medicine system includes Ayurveda, Siddha, Unani, Sowa-rigpa, and Folk medicines confined to particular communities. Among all systems of medicine, Ayurveda is known as the most advanced and extensively used in India (Jaiswal & Williams, 2017). In India, around 8000 plant species are used in folk medicines and about 25,000 effective plant-based therapies are used for primary healthcare by the ethnic communities in rural areas (Sen & Chakraborty, 2015). About 2500 species of plants that are used in India as herbal medicine are used either directly as folk medicines or indirectly as modern pharmaceuticals (Choudhary *et al*., 2015). The traditional medicine system

provides the scientific base for the development of new drugs. Hence, the conventional system of medicines might be useful in discovering new potent and cost-effective remedies for treating diseases (Kalita *et al*., 2012).

Folk medicines based on traditional knowledge play an important role among the people belonging to indigenous groups for their health care and treatment of diseases. Such kind of knowledge is not documented properly and is transmitted from one generation to the following. Understanding the importance of traditional knowledge on the health care system, even World Health Organization is involved in the documentation of traditional medicines used by the indigenous communities from various regions of many countries (Buragohain, 2011).

With the advancement of science and technology in the field of medicines, the application of traditional knowledge has been decreasing and the dependency of the indigenous people on modern medicines has been increased. On the contrary, pharmaceutical companies are exploring the possibility of finding new molecules useful for the development of drugs based on traditional knowledge. Traditional knowledge of most of the communities is not documented properly and it is transferred from one generation to the next generation orally. Therefore, these people are not aware of the importance of their traditional knowledge.

Documentation of traditional knowledge on uses of ethnomedicinal plants is very important for the preservation of indigenous knowledge from being lost from our existing generation and subsequent generations (Mahwasane *et al*., 2013), also important for the sustainability of health care systems in rural and far-flung areas. Furthermore, such traditional knowledge is depleting due to the accessibility of modern medicines along with other modern facilities. Traditional knowledge provides

the basis for the search for noble drug molecules with the least side effects and cost-effective medicines on the manufacture for the treatment of human illnesses.

Rheumatoid arthritis (RA) is one of the chronic inflammatory diseases that exist as one of the major health problems globally. Even though there are various available modern medicines for treating RA, but their continuous uses may result in unavoidable side effects such as gastrointestinal problems including peptic ulcers. Therefore, there is a critical need to have the remedy to cure rheumatoid arthritis preferably without side effects to the patients.

In India, scientific investigation regarding the treatment of rheumatoid arthritis patients utilizing various ethnomedicinal plants by the indigenous communities has been reported (Pawar & Patil, 2006; Naidu *et al*., 2008; Sutha *et al*., 2010; Manjula *et al*., 2013). In Sikkim, there are no prior reports on the documentation of ethnomedicinal plants utilized by ethnic inhabitants of the Sikkim Himalaya for treating rheumatoid inflammation. Therefore, it is very important to study and document the Sikkim Himalayan ethnomedicinal plants that are utilized by the ethnic people of Sikkim for the treatment of rheumatoid arthritis.

Sikkim Himalayan region harbors the diverse vegetational wealth in the country. This region has been explored by many botanists who contributed the documentation of floristic data of the country. However, the plant-based traditional knowledge of this region remains hitherto unexplored and no comprehensive account is readily available about the use of plants for the treatment of rheumatoid arthritis. Only a few subsidiary notes on the uses of plants as a remedy for rheumatoid arthritis are available in the floristic account of some of the authors combined with Darjeeling Himalayan region

and Bhutan (Biswas, 1956; Bejoy, 2002; Singh *et al*., 2002; Maity *et al*., 2004; Chhetri, 2005; Sharma & Sharma, 2010; Panda & Misra, 2010).

In the Sikkim Himalayan region, three major communities (Lepchas, Bhutias, and Nepalis) have been practicing their traditional medicine system since time immemorial and they use diverse ethnomedicinal plants for curing various health-related ailments (Singh *et al*., 2002). The ethnic tribes of Sikkim have an enormous belief in the traditional medicine system that depends on the experience of experimentation consistently acquired from age to age (Panda & Misra, 2010 but their common purpose is to promote human health. Several traditional medicine systems of the people depend on the existing environment and ethnic expression of the area (Bhasin, 2007).

In Sikkim Himalaya, geographical factors are not only responsible for this, but also influence the interaction of ethnic people with other advanced traditional medicine systems and these circumstances have led them to establish their own health culture (Panda & Misra, 2010. In Sikkim, there are folk healers from different ethnic communities and they practice traditional medicine to take care of various health problems in the community. These practices are not properly documented and are passed verbally from one generation to another (Kala, 2003). Although the ethnic people of Sikkim Himalaya are well aware of the healing properties of their traditional native medicinal plants, however, their documentation is rare. Studies on some of the folk medicinal plants of Sikkim Himalaya for their curing properties on various health-related ailments has been done in general (Singh *et al*., 2002; Pradhan & Badola, 2008; Panda & Misra, 2010; Das *et al*., 2012; Tamang *et al*., 2017), however, no specific work has been performed on the documentation of ethnomedicinal plants for treating rheumatoid arthritis. Therefore this study was

undertaken to document traditionally used medicinal plants for treating rheumatoid arthritis by the ethnic communities of Sikkim Himalaya that are enumerated in this chapter.

4.2. MATERIALS AND METHODS

4.2.1. *Ethnobotanical field survey*

Information on plant uses for the treatment of rheumatoid arthritis were collected from the available literature and made a docket for the field survey. Detailed information on traditional herbal healers in the state was also collected from publications of the State Medicinal Plants Board, Government of Sikkim (2009). A standard format was prepared for the collection of the relevant information required for the present work.

A field survey was conducted after carefully designing the research plan to collect ethnobotanical information particularly for healing rheumatoid arthritis (RA) in the Sikkim Himalayan region. For the collection of information, respondents having sound knowledge of medicinal plants and their application in the local traditional treatment were identified by consulting with the old people of the villages and the village ward panchayat member from all four districts. In Sikkim, different communities have their own traditional herbal or folk healers such as *Bijuwa/Mangpa* (Rai community), *Phedangma* (Limbu community), *Lama* (Bhutia community), *Boongthing* (Lepcha community), *Jhakri*, *Baidhya* (Nepali community). The ethnobotanical information was recorded through interviews. Most of the resourceful persons were shy and reluctant in most of the cases but after developing the colloquial attitude and familiarization acknowledging their contribution to the society and also

mentioning the importance of traditional knowledge documentation, they were ready to disseminate the information on plant uses.

4.2.2. *Plant collection and authentification*

Healthy parts of the plant were collected from various places of the Sikkim Himalayan region preferring the flowering and fruiting period for the preparation of herbarium and phytochemical analysis in the laboratory. Discussions were held on the doubtful specimens and materials and also in the case of local names, which varied from place to place, but these were corrected afterward in the laboratory and herbaria. Each plant was collected in triplicate, tagged with the field number, and recorded in the field notebook. Phytochemical analysis and Identification of plants were done in the Department of Botany, Sikkim University, and authentication of the botanical name was done from the Botanical Survey of India, Eastern Himalayan Circle (Sikkim). Herbarium of each plant is deposited in the Department of Botany, Sikkim University bearing accession number with acronym SUH (Sikkim University Herbaria).

4.2.3. *Relative Frequency of Citation (RFC)*

In the present work relative frequency of citation (RFC) was measured to determine the most useful plant species in the Sikkim Himalayan region as one of the objectives needed to quantify the use of plants for the treatment of rheumatoid arthritis. It is calculated by dividing a frequency of citation (FC) (the number of informants who mention the use of the species) by a total number of informants in the survey (N) (Tardío & Pardo-de-Santayana, 2008). Relative frequency of citation (RFC) is an index of salience that does not consider the use category.

RFC=FC / N

RFC ranges from 0-1. The value is 0 when nobody refers to the plant as useful and 1 when all the informants mention the use of the species.

4.2.4. *Data analysis*

Information obtained from the field survey is summarized in **Table 4** with the following parameters: Botanical name with Accession number, local name (Nepali, Bhutia, Lepcha), family, short description, habitats, flowering and fruiting time, parts used for treating rheumatoid arthritis, place of collection, distribution and relative frequency of citation.

4.3. RESULTS

4.3.1. *Ethnobotanical field study for the documentation of medicinal plants*

The **s**tudy was conducted among 87 participants (66 male and 21 female) from all the four districts of Sikkim for the collection of information on ethnomedicinal plants used for treating RA. Among 87 participants, 7 participants were aged between 30-40 years (5 male and 2 female), 17 were aged between 40-50 years (12 male and 5 female), 28 were aged between 50-60 years (20 male and 8 female) and 35 of the participants were aged of above 61 years (29 male and 6 female). A bar histogram is presented in **Figure 3**. It is worth mentioning that most of the participants who contributed information had gained their skill in traditional medicine from their forefathers and close relatives.

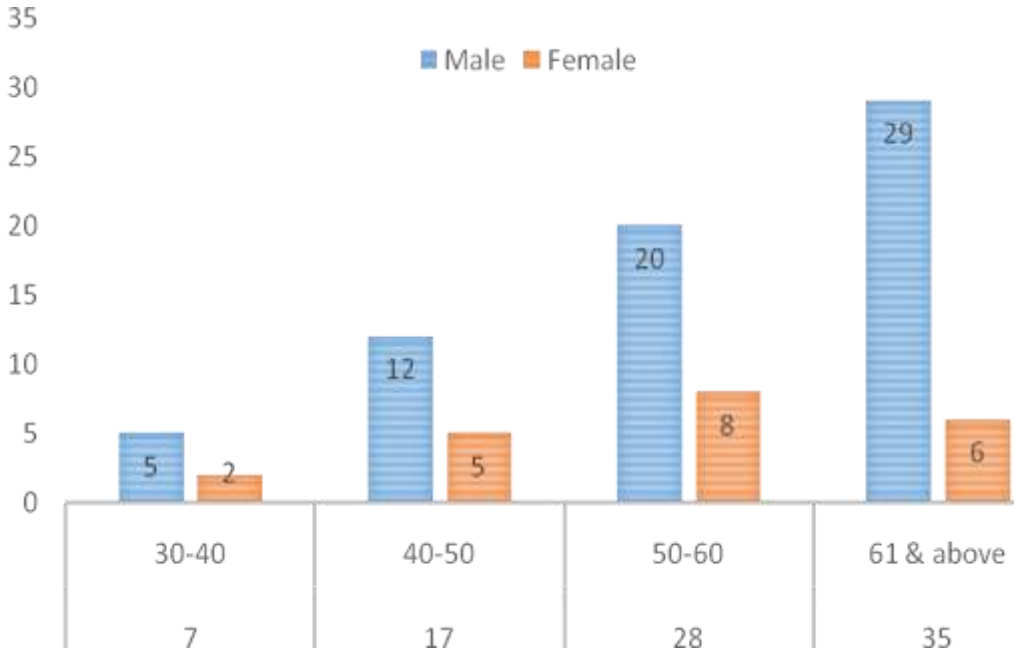

Figure 3: Demographic representation of respondents

Table 4: Data obtained from the ethnobotanical survey of the medicinal plants used in the treatment of RA in Sikkim Himalaya

Sl. no	Scientific name (Voucher No.)	Local name	Family	Descriptions	Habitats	Flowering and Fruiting time	Part used	Place of collection	Distributions	RFC
1.	*Acorus calamus* L. (SUH0236)	*Bojho* (Nepali); *Riklok* (Lepcha); *Sho-tako, Sudag* (Bhutia)	Acoraceae	Perennial herb. Sword-shaped leaves, flat and narrow, tapering into a long, acute point, and have parallel veins. Flowers spadix densely crowded with tiny greenish-yellow flowers. Fruits small, berry-filled with mucus.	Edges of small lakes, ponds and rivers, marshes, swamps, and wetlands.	Fl: From early to late summer depending on the latitude. Fr: July	Rhizomes	9th Mile, Namli, E.Sikkim	India (found throughout the Indian Himalayas up to 6000 m), central Asia, southern Russia and Siberia, and Europe. In Sikkim, it is commonly distributed in the tropical and temperate regions.	0.091
2.	*Asparagus racemosus* Willd. (SUH0237)	*Kurilo* (Nepali); *Neusiri* (Bhutia)	Liliaceae	Woody climber growing to 1-2 m in height. Leaves are like pine needles, small and uniform. Flowers minutes, white on short, spiky stems. Fruits blackish-purple, globular berries.	Common at low altitudes in the shade and tropical climates.	Fl: July Fr: September	Rhizomes	9th Mile, Namli, E.Sikkim	Throughout Asia, Australia and Africa. It is prevalent in the tropical and subtropical regions of India particularly central India and is also found in the subtropical Himalayas up to an altitude of 1500 m. In Sikkim, it is found in sub-tropical to tropical	0.080

									regions.	
3.	*Astilbe rivularis* Buch. -Ham. ex D. Don (SUH0148)	*Budokhati* (Nepali); *Pango* (Lepcha); *Tongsarygugay* (Bhutia)	Saxifragaceae	Perennial herb. Leaves are compound, lower leaflets further divided. Flowers panicle with clusters of tiny yellow flowers. Fruit is an ovoid capsule.	Grows well in moist places of the temperate Himalaya n region.	Fl: June-September Fr: October-March	Rhizomes	Pangthang, E.Sikkim	Predominantly found in the Philippines, Myanmar, Laos, Nepal, Thailand, India, and Southern Tibet. In India, it is predominant in Darjeeling and Sikkim Himalayas. In Sikkim, it grows in a temperate forest in moist areas.	0.103
4.	*Bergenia ciliata* (Haw.) Sternb. (SUH0147)	*Pakhanbed* (Nepali); *Rockfoil* (Lepcha); *Hong-Lem* (Bhutia)	Saxifragaceae	Evergreen perennial herb. Leaves suborbicular which are rounded at the apex and base. Flowers are bisexual, white, pink, or purple with long cymose panicles 4-10 cm long. Fruit capsule and rounded in shape.	Grows on rocks, ledges, and cliffs.	Fl: February-April Fr: March-July	Rhizomes	Yuksom, W.Sikkim	Afghanistan, South Tibet, India, China, and Bhutan. In India, it is found in the Himalayas. In Sikkim, it is found in the temperate and sub-alpine regions.	0.114
5.	*Betula alnoides* Buch.-Ham.ex D. Don (SUH0096)	*Saur* (Nepali); *Sanglikung* (Lepcha);	Betulaceae	Deciduous tree. Leaves simple, alternate, ovate-lanceolate, or ovate-elliptic, rounded at base, cuspidate-acuminate at apex, margin irregularly incurved	Subtropic al forests at an altitude between 700-	Fl: March-April Fr: May-June	Stem bark	Ravangla, S.Sikkim	India, Bhutan, Nepal, Bangladesh, Myanmar, southern China, Vietnam, Laos, and Thailand. In Sikkim, it is found in sub-tropical	0.160

		Thakpa (Bhutia)		setiform serrate. Flowers male catkins, female inflorescences 3-5 in a raceme. Fruits spikes.	2100m.				to temperate forests.	
6.	*Costus speciosus* (J. Koenig) Sm. (SUH0160)	*Betlauri* (Nepali); *Ruyang* (Lepcha)	Costaceae	An erect herb. Leaves subsessile, spirally arranged, oblong or oblanceolate- oblong, acute, or acuminate. Flower white, numerous, in very dense spikes. Fruits globosely Capsules.	Grow in dry land in the subtropical- temperate region.	Fl: July-October Fr: October-December	Stem	9th Mile, Namli, E.Sikkim	Indo-Malayan region and Sri Lanka. In India, it is found throughout the foothills of the Himalayas, Central India, Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu, and Kerala. In Sikkim, it is found in subtropical-temperate forest areas.	0.080
7.	*Curcuma angustifolia* Roxb. (SUH0234)	*Haledo* (Nepali); *Salek-sying* (Lepcha)	Zingiberaceae	Rhizomatous perennial herb. Leaves are typically simple, green, glabrous, lanceolate, and entire. Flowers spike. Fruit capsule.	Prefers shady areas and grows best in moist soil that is sandy, pebbly, or loamy.	Fl: July-August Fr: September-October	Rhizome	9th Mile, Namli, E.Sikkim	India, Burma, Laos, Pakistan, and Nepal. In India, it is found in the northeast and western coastal plains and hills. In Sikkim, it is often found at the edges or in the clearings of forests.	0.091

8.	*Curcuma caesia* Roxb. (SUH0150)	*Kalohardi* (Nepali); *Gey-sying* (Lepcha); *Katsura* (Bhutia)	Zingiberaceae	An erect rhizomatous herb. Leaves broad, broadly lanceolate or oblong, glabrous, the lamina shows deep, farraginous purple clouds in the middle region. Flowers spike on the basal peduncle. Fruit capsule.	It grows in the ground cover of forest area in the subtropical-temperate region.	Fl: June-July Fr: September-October	Rhizomes	9th Mile, Namli, E.Sikkim	India and South-East Asia. In India, it is mostly found in Bengal, North East, and Central India, within the altitudinal range of 200–1000 m. In Sikkim, it is widely cultivated as a medicinal plant.	0.080
9.	*Datura metel* L. (SUH0095)	*Kalo Dhatura* (Nepali); *Rinchen-Nyongboou* (Lepcha)	Solanaceae	Short-lived shrub. Simple, alternate, petiolate, entire or deeply lobed, glabrous, showing unicostate reticulate venation and exstipulate. Flowers Solitary and axillary cyme. Fruits sparsely spinescent capsule.	Common in hills up to 1500m, occasional in plains. On wastelands, farms, roadsides.	Fl: Throughout the year Fr: Throughout the year	Young twig and leaf	9th Mile, Namli, E.Sikkim	Tropical and subtropical Asia and Africa, now widely cultivated in the warmer regions. In India, it is found in Assam, Sikkim, Bihar, Meghalaya, Odisha, Rajasthan, and Uttar Pradesh. In Sikkim, it is widely cultivated.	0.091
10.	*Diploknema butyracea* (Roxb.) J.F. Macbr (SUH0203)	*Chewri* (Nepali); *Yel-Poat* (Lepcha)	Sapotaceae	Tree up to 25m tall. Leaves are elliptic-oblong, ovate, or ovate-oblong. Flowers are borne in clusters in leaf axils. Fruits ovoid-globose to oblong, exocarp fleshy.	It grows mainly in the sub-Himalayan tracts on steep slopes,	Fl: November-January Fr: April-July	Bark	Namchebong, E.Sikkim	Throughout the Himalayan belt including Nepal, India, and Bhutan at an altitude of about 300–1500 m. In India, it is found in the Himalayas,	0.034

					ravines, and cliffs at elevations of 300 - 1500m.				at an altitude of 1600m, from Uttarakhand to Sikkim, also in Andaman & Nicobar. In Sikkim, it is found in wastelands near the villages.	
11.	*Equisetum diffusum* D. Don (SUH0094)	*Salibesali* (Nepali)	Equisetaceae	The perennial plant grows up to 10-25 inches, rich in silica. The main stem 4-10-ridged; each side of ridge raised and forming edges reaching lower sheath teeth; each edge with a row of tubercles reaching sheath teeth; sheath tubes long, narrow, grayish-green in the lower portion, blackish-brown in the upper portion, with a deep groove going through the back of the sheath. Strobilus terete, 1-9 cm, 4-8 mm in diam., apex blunt; stalk prolonged when mature and 1-3 cm. small to medium-sized.	Grows at open but shady, wet places, streamsides, mossy falls, wet meadows	Fl: - Fr: -	Whole plant	ICAR area, Tadong, E.Sikkim.	India, Bangladesh, China, Laos, Myanmar, Nepal, Pakistan, Thailand, Tibet, and Vietnam. In India, it is found in north-eastern states, western Himalayas, central Himalayas. In Sikkim, it is found at the sites of streams and rivers, along roadsides, wet meadows, and wet places.	0.103
12.	*Ficus benghalensis*	*Baar* (Nepali)*;*	Moraceae	Evergreen to the deciduous	Evergreen	Fl:	Bark	9th Mile,	Native to tropical Asia,	0.023

	L. (SUH0233)	*Kavigi* (Lepcha); *Nyagrodha* (Bhutia)		tree. Leaves are ovate or obovate to elliptic, glabrous above and finely pubescent beneath. Male flowers: numerous ostiolar, shortly pedicellate; female flowers: sessile, mixed with gall flowers; gall glowers numerous, pedicellate. Fruits globose to depressed-globose.	to deciduous forests; and cultivated around villages.	November-January Fr: November-January		Namli, E.Sikkim	from India to Myanmar, Thailand, Sri Lanka, and Malaysia. In India it is found in Andhra Pradesh, Assam, Bihar, Delhi, Goa, Gujarat, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Punjab, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, Uttarakhand, Sikkim, and West Bengal. It is cultivated around villages in Sikkim.	
13.	*Fraxinus floribunda* Wall. (SUH0154)	*Lankhuri* (Nepali); *Payjeu, Paizku* (Lepcha)	Oleaceae	Medium-sized deciduous tree. Leaves compound, opposite, pinnate, with 7-9 serrate leaflets. Flowers arranged in a many-flowered inflorescence, terminal, unisexual. Fruits nuts with a long narrow	Grows best on a shaded, moist locality mostly slopes rather than river	Fl: April-May Fr: July-December	Bark	Namche ybong, E.Sikkim	Afghanistan, Pakistan, India, Nepal, southern China, southern Japan, Myanmar, Thailand, Laos, Vietnam. In India, it is found in Eastern Himalayas and Khasi hills. In Sikkim, it is found in forest areas	0.114

				wing.	terraces.				from tropical to temperate regions.	
14.	*Juglans regia* L. (SUH0205)	*Okhar* (Nepali)*;* *Kanola* (Lepcha); *Starga* (Bhutia)	Juglandaceae	Deciduous tree. Leaves are alternately arranged, odd-pinnate with 5-9 leaflets. Male flowers are in drooping catkins; female flowers are terminal, in clusters of 2-5. Fruits green, semi-fleshy husk, and a brown, corrugated nut.	Grows in the temperate Himalayas from 1,000 to 3,000 m altitude.	Fl: April – May Fr: August - October	Bark	Yuksom, W.Sikkim	Native to the Carpathian Mountains of eastern Europe, but often found growing wild eastward to the Himalayas, India, Nepal, and China. In India, it is found in Western Himalayas, eastern and north-eastern regions. In Sikkim, it is found in Broad-leaves forests, also planted near habitation, 1800-2500 m.	0.023
15.	*Kaempferia rotunda* L. (SUH0204)	*Bhuichampa* (Nepali)*;* *Ribirip* (Lepcha)	Zingiberaceae	Tuberous rooted herb. Leaves simple, ovate-lanceolate, long, purple color. Flowers purple, aromatic, spike. Fruits oblong, capsule.	Monsoon forests and open grassland at elevations from around sea level	Fl: March-May Fr: March-May	Tuber	9th Mile, Namli, E.Sikkim	Southern China, India, Sri Lanka, Myanmar, Thailand, Vietnam, Malaysia, Indonesia. In India primarily found in the north-eastern part and along the western ghats. In Sikkim, it is found in forest areas of	0.080

					to 2,600 meters.				low hills.	
16.	*Litsea cubeba* (Lour.) Pers. (SUH0100)	*Siltimur* (Nepali)*;* *Tanghaecher-Kung* (Lepcha)	Lauraceae	Small evergreen tree, unisexual, dioecious, with aromatic leaves. Leaves are simple, alternate, exstipulate, lanceolate, caudate-acuminate at apex, entire along the margin, membraneous, bright green, glabrous. Flowers are borne in solitary or clustered, 4-6 flowered umbels. Fruits berry, globose.	Sunny slopes, thickets, sparse forests, roadsides, watersides at elevations of 300 - 3,200 meters.	Fl: November-March Fr: February-July	Fruit	9th Mile, Namli, E.Sikkim	Southeast Asian countries, including India, China, Bhutan, Nepal, Myanmar, Vietnam, Korea, Taiwan, and Indonesia. In India, it grows abundantly in the Himalayas and is reported from Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Uttaranchal, Uttar Pradesh, and West Bengal. In Sikkim, it is found in Subtropical forest slopes, 300-1500 m.	0.172
17.	*Litsea glutinosa* (Lour.) C.B. Rob. (SUH0092)	*Awley harchur* (Nepali); *Sunyok-Kung* (Lepcha)	Lauraceae	Dioecious tree. Leaves are simple, alternate, stalked. Leaf lamina is elliptic, leathery, shiny dark green, the apex is rounded, the	Found in forest margins, streamsides, sparse	Fl: March-June Fr: September-	Stem bark	9th Mile, Namli, E.Sikkim	Native to India, Southern China, Malaysia, Australia, and the Western Pacific islands. It is found	0.103

				base is broadly wedged, slightly asymmetrical, the margin is entire. The inflorescence umbels contain many small yellowish flowers, the male flowers are tubulate and have 15 or more stamens and reduced pistil. The female flowers have 15 reduced staminodes, a filiform style topped with a peltate stigma. Fruits berry globose.	forests, or thickets at elevations of 500-1,900 meters.	October			throughout Asia, including several regions of China, India, Bhutan, Myanmar, Nepal, the Philippines, Thailand, and Vietnam. In Sikkim, it is found in warmed-broad leaf forests, 900-1700m.	
18.	*Morus macroura* Miq. (SUH0239)	*Thulokimbu* (Nepali)*;* *Mekrap-Kung* (Lepcha); *Singtok* (Bhutia)	Moraceae	Deciduous, dioecious tree. Leaves are distichously arranged, ovate shaped, with the dark green upper surface and pale green underneath. Leaf base is rounded to cordate with acuminate apex and toothed margin. Hairs in the veins are present. The male inflorescence is axillary and paired in the form of catkins, and the female inflorescence is cylindrical.	It grows in montane and submontane forests, ranging in altitude from 400 to 2500 m.	Fl: April-May Fr: July-August	Stem bark	9th Mile, Namli, E.Sikkim	India, Bhutan, Nepal, South China, Myanmar, Cambodia, Thailand, Sumatra, and Java. It is widely distributed throughout India (including, Assam, Bihar, Himachal Pradesh, Karnataka, Madhya Pradesh, Meghalaya, Rajasthan, *Sikkim*, Uttar Pradesh, Uttarakhand). In Sikkim, it is found in	0.023

				Fruits aggregate, syncarp.					areas of altitude ranging from 200-2000m.	
19.	*Pentapanax leschenaultii* (DC.) Seem. (SUH0159)	*Chinde* (Nepali)	Araliaceae	Small trees. Leaves simple pinnate or digitate; leaflets ovate-acuminate, glabrous, highly reticulate. Inflorescence terminal, umbels. Flowers plenty; calyx lobes 5, acute; petals 5, yellow; stamens 5; ovary inferior; styles connate. Fruits drupe.	It grows in shady and moist areas.	Fl: April-June Fr: June-November	Stem bark	Dzongu, N.Sikkim	Widely distributed in Asia, especially in China, India, Nepal, Myanmar, and Sri Lanka. In India, it is found in Uttarakhand, West Bengal, *Sikkim*, and Tamil Nadu. In Sikkim, it is found in shady and moist areas.	0.091
20.	*Piper longum* L. (SUH0158)	*Pipli* (Nepali); *Kautim Poat* (Lepcha); *Pipilin* (Bhutia)	Piperaceae	Dioecious climber. Stems are flexuous, pubescent, and terete. The leaves are simple, ovate, papery, the base cordate and asymmetrical, the apex acuminate and with two or three pairs of secondary nerves arising from the base. The inflorescence is a cylindrical and slightly curved spike and opposite the leaves. Fruits drupe, globose.	It grows in tropical and subtropical regions.	Fl: October-January Fr: October-January	Fruit	Dzongu, N.Sikkim	India, Malaysia, Nepal, Bhutan, Sri Lanka, and Vietnam. In India, it grows in tropical and subtropical regions. In Sikkim, it is found in the lower hill forest.	0.045

21.	*Prunus cerasoides* D. Don (SUH0155)	*Payung* (Nepali); *Kongki-Kung* (Lepcha); *Khamber* (Bhutia)	Rosaceae	Deciduous tree. Leaves are ovate, acuminate, doubly serrate, and glabrous. Flowers are hermaphrodite and are pinkish-white in color. Fruits ovoid yellow that turns red as it ripens.	It grows in the temperate forest from 1200-2400m.	Fl: October-December Fr: December-February	Stem bark	Soreng, W.Sikkim	India, Nepal, Bhutan, Myanmar, West China, and Thailand. In India, it is found in the Himalayas from Himachal Pradesh in North-central India to Sikkim and is restricted to sub-montane and montane Himalayas ranging from 1500-2400 m. In Sikkim, it grows in the temperate forest.	0.045
22.	*Quercus lamellosa* Sm. (SUH0097)	*Buk* (Nepali)	Fagaceae	Evergreen tree. Leaves are spirally arranged, ovate-elliptic, with a sharply saw-toothed margin. The flowers are catkins. Female flowers mature into large, broad acorns, set in a deep cup with concentric rings of woody scales. Fruits a single-seeded nut set in a woody cupule.	It grows in the Himalayan oak forest between 1600-2800m.	Fl: April-May Fr: November	Bark	Soreng, W.Sikkim	It grows in Eastern Himalayas, from central Nepal to Sikkim, Bhutan, and southwest China, at altitudes of 1600-2800m. In Sikkim, it is found in the temperate forest.	0.114
23.	*Quercus thomsoniana*	*Phalath* (Nepali)	Fagaceae	Evergreen tree. Leaves lanceolate, pointed apex,	It grows in the	Fl: April-May	Bark	Soreng, W.Sikki	India, Bhutan, Nepal, Bangladesh, Myanmar.	0.023

	A.DC. (SUH0238)			asymmetrical, base rounded, margin minutely toothed at the apical, shiny green. Male flowers long catkins, female flowers solitary, and terminal. Fruits acorn, flattened, apex depressed, cup half-round, sessile.	Himalaya n oak forest between 1600-2400m.	Fr: September-October		m	In India, it is found in Eastern Himalayas and North-eastern Himalayas. In Sikkim, it grows in an evergreen oak forest.	
24.	*Rheum australe* D. Don (SUH0151)	*Khokim* (Nepali)	Polygonaceae	Perennial herb. Leaves are radical, orbicular, or broadly ovate, very large with long petioles. Flowers small, dark purple, or pale red in axillary panicles. Fruits ovoid-oblong, wings narrower than disk, the notch at both ends.	It grows in grassy or rocky slopes, forest margins, crevices, and moraines.	Fl: June-July Fr: August-September	Rhizomat ous root	Nathang valley, E.Sikkim	India, Nepal, Myanmar, China, Pakistan. In India, it occurs in the temperate Himalayas, from Kashmir to Sikkim, at an elevation of 2800–4000m. In Sikkim, it grows on alpine slopes.	0.068
25.	*Rheum nobile* Hook.f. & Thoms (SUH0152)	*Padamchal/ Padmaguru* (Nepali); *Tchuka* (Lepcha); *Kenju* (Bhutia)	Polygonaceae	Perennial herbs with a conical tower of delicate, straw-colored, shining, translucent, regularly overlapping bracts; the higher ones have pink edges. Leaves large, radical, glossy, green in color, with red petioles and	It grows in rock ledges, open slopes in the alpine zone at 4000-4800m	Fl: June-July Fr: August-September	Rhizomat ous root		Native to the Himalayas, from northeastern Afghanistan, east through northern Pakistan and Nepal, Sikkim (in India), Bhutan, and Tibet to Myanmar, occurring in	0.137

				nerves, form a broad base to the plant. Flowers are short branched panicles of diminutive green. Fruits panicles of deep brown pendulous.	altitude.				the alpine zone at 4000-4800m altitude.	
26.	*Rhododendron arboreum* Sm. (SUH0235)	*Laligurans* (Nepali)*;* *Aetok-Kong, Kaon-Ke-Kong* (Lepcha); *Ka-ra-baka* (Bhutia)	Ericaceae	Evergreen shrub or small tree, with reddish-brown bark. Leaves are oblong to lanceolate, with grooved mid-vein and lateral veins deeply impressed above, glossy green undersides with thin or thick felted hairs. Flowers are bell-shaped, blood-red, or pink to white. Fruits capsules.	It grows over a wide range of altitudes.	Fl: March-April Fr: June-September	Flower	Barsey, W.Sikkim	Bhutan, China, India, Myanmar, Nepal, Sri Lanka, Pakistan, and Thailand. In India, it is *R. arboreum* is widely distributed from the western to the eastern Himalayan region. In Sikkim, it occurs in Evergreen oak and Blue Pine forests, 1200-3000m.	0.091
27.	*Ricinus communis* L. (SUH0156)	Dalda/*Reri* (Nepali); *Raklop* (Lepcha)	Euphorbiaceae	Fast-growing, evergreen, and monoecious shrub. Leaves are glossy, long-stalked, alternate, and palmate with five to twelve deep lobes with coarsely toothed segments. Male flowers are numerous, yellowish-green with prominent creamy	It usually grows on wasteland in tropical regions.	Fl: Almost all the year Fr: Almost all the year	Young twig	Jorethang, S.Sikkim	Indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout the tropical regions. In Sikkim, it is found in waste ground in towns and villages.	0.023

				stamens; the female flowers, borne at the tips of the spikes, lie within the immature spiny capsules Flowers. Fruits are spiny capsules.						
28.	*Stephania glabra (Roxb.)* Miers. (SUH0098)	*Tamarkey* (Nepali)*;* *Mungkyo Rik* (Lepcha)	Menispermaceae	Herbaceous, perennial, climbing herb. Roots are tuberous. Leaves are ovate or round, blunt at tip, hairless, leaf-stalked long, thickened at the base. Male flowers are borne in leaf axils, in umbel-like cymes; female flowers oblong-ovoid. Fruits are drupes.	It grows at altitudes of 1000-2500m.	Fl: April-May Fr: May-November	Rhizomatous tuber	9th Mile, Namli E.Sikkim	India, China, Nepal, Bangladesh, Myanmar, and Thailand. In India, it is found in Arunachal Pradesh, Assam, Himachal Pradesh, Jharkhand, Madhya Pradesh, Sikkim, Meghalaya, Mizoram, Nagaland, Uttar Pradesh, Uttarakhand, West Bengal. In Sikkim, it often grows near streams, in Subtropical & Warm broad-leaved forests.	0.149
29.	*Urtica dioica* L. (SUH0153)	*Patley Sisnu* (Nepali)*;* *Sarong* (Lepcha)	Urticaceae	Dioecious, perennial herb. Leaves are borne oppositely on an erect, wiry, green stem, and have a strongly serrated margin, a cordate	It grows in roadsides, pastures, fields,	Fl: June-August Fr: August-September	Root	9th Mile, Namli, E.Sikkim	Native to Europe, much of temperate Asia and western North Africa and naturalized widely in other temperate	0.080

				base, and an acuminate tip. The leaves and stems are very hairy with non-stinging hairs. Flowers numerous in dense axillary inflorescences. Fruits elliptic, flat, dull, yellowish-brown, achene protected by tepals.	waste ground, logging clearings, shore, and stream-side broad-leaved forests.				regions. In India, it is found in the Himalayas, at altitudes of 1000-2500m. In Sikkim, it grows around habitations, roadsides, and on disturbed ground.	
30.	*Viscum nepalense* Sprengel (SUH0149)	*Harchur* (Nepali); *Singthutmu* (Lepcha)	Loranthaceae	Parasitic herbs. branches usually pendulous, sometimes whorled, yellowish-green; basal internodes often rounded; succeeding internodes decussately flattened. Flowers subtended by two coalescent bracteoles, originating at the nodes; female flowers often solitary, but common; staminate flowers smaller and less prevalent. Fruit suborbicular.	It grows on trees in the lower and middle hills.	Fl: February-April Fr: September	Whole plant	Uttarey, W.Sikkim	China, India, Australia, Java, Taiwan, Peninsular Malaysia, Vietnam, the Philippines, Thailand, Myanmar, Sri Lanka, Nepal, Laos, Sumatra, Borneo, Thailand, and Bhutan. In India, it is found in Himachal Pradesh, Uttar Pradesh, Sikkim, Arunachal Pradesh. In Sikkim, it grows on trees in the temperate forest.	0.114
31.	*Vitex negundo* L. (SUH0093)	*Sewali* (Nepali)*;*	Verbenaceae	Small tree. Leaves 3-5-foliolate, leaflets narrowly	It commonly	Fl: April-August	Bark	9th Mile, Namli,	Afghanistan, Bangladesh, Bhutan,	0.034

		Nirguntha (Bhutia)		oblong or elliptic to lanceolate, base acute, apex acuminate. Flowers numerous borne in panicles. Fruits succulent drupes.	grows near water bodies, recently disturbed land, grasslands, and mixed open forests.	Fr: September-February		E.Sikkim	Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam, Kenya, Madagascar, Mozambique, Tanzania; Australasia, and North America. In India, it is found almost everywhere though mostly on wastelands from the seashore to an altitude of about 1500m. In Sikkim, it is found in waste places around villages, moist localities, and in the deciduous forests.	
32.	*Zingiber capitatum* Roxb. (SUH0157)	*Jungaley aduwa* (Nepali); *Pungyok Heng* (Lepcha)	Zingiberaceae	Herbaceous perennial plant. Roots are tuberous rhizomes. Leaves are stalkless, narrowly lance-shaped, smooth above and velvety beneath. Flowers spike. Fruits capsules.	It grows in tropical semi-evergreen forests, moist conditions, along	Fl: August-September Fr: September-October	Rhizome	9th Mile, Namli, E.Sikkim	India, Bangladesh, Vietnam, and Nepal. In India, it is found in the Himalayan region (Kumaun to Sikkim), Assam, Madhya Pradesh, and many other parts of India. In	0.045

					streams.				Sikkim, it grows in the tropical semi-evergreen forest.	
33.	*Zingiber zerumbet* (L.) Roscoe ex Smith. (SUH0099)	*Phachang* (Nepali); *Salek* (Lepcha)	Zingiberaceae	Perennial rhizomatous herb. Leaves are oblong or oblong-lanceolate, base obtuse, apex acuminate, pubescent below. Flowers spikes. Fruits capsules.	It grows in moist places, hilly slopes, and is widely cultivated in tropical and subtropical regions around the world.	Fl: August-September Fr: September-November	Rhizome	9th Mile, Namli, E.Sikkim	It is native to Southeast Asia but has now been widely cultivated around the world in tropical and subtropical regions. It is distributed primarily in India, Bangladesh, Sri Lanka, Malaysia, and Nepal. In Sikkim, it is cultivated by the villagers in their farmland.	0.091

In the present work, it is found that a total of 33 plants are accounted to be utilized as medicinal plants from the study area particularly for treating rheumatoid arthritis, and are presented in **Table 4**. Out of 33 medicinal plants, 5 plants belong to the family Zingiberaceae, 2 plants each belong to the families Saxifragaceae, Moraceae, Lauraceae, Fagaceae, and Polygonaceae, and a single plant each belong to the families Acoraceae, Liliaceae, Betulaceae, Costaceae, Equisetacea, Piperaceae, Loranthaceae, Solanaceae, Oleaceae, Juglandaceae, Araliaceae, Rosaceae, Ericaceae, Euphorbiaceae, Menispermaceae, Urticaceae, Loranthaceae and Verbenaceae **(Figure 4)**. Most commonly used plant parts are rhizomes (32%), stem bark (28%), leaf (7%), fruit (6%), whole plant part (6%), tuber (6%), latex (6%), flower (3%), root (3%) and stem (3%) **(Figure 5)**.

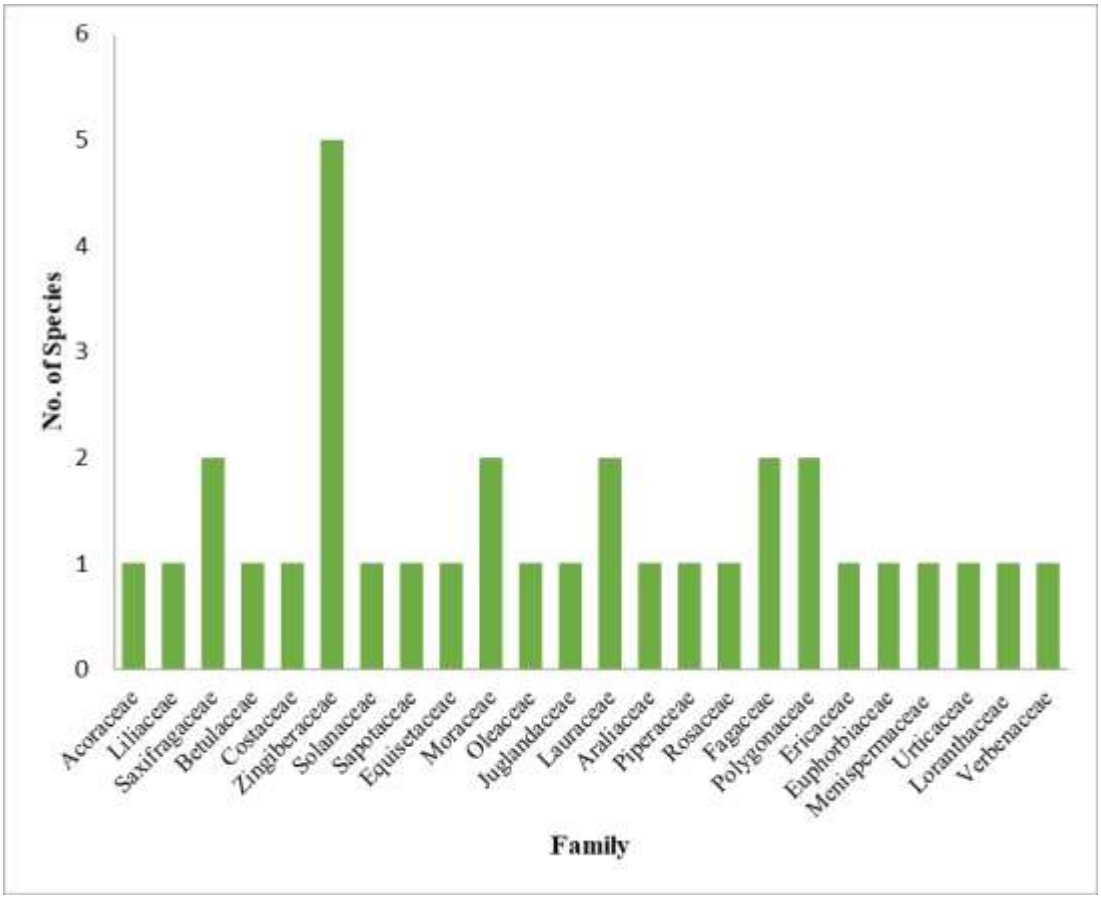

Figure 4: Relative no. of species per family used for the treatment of RA.

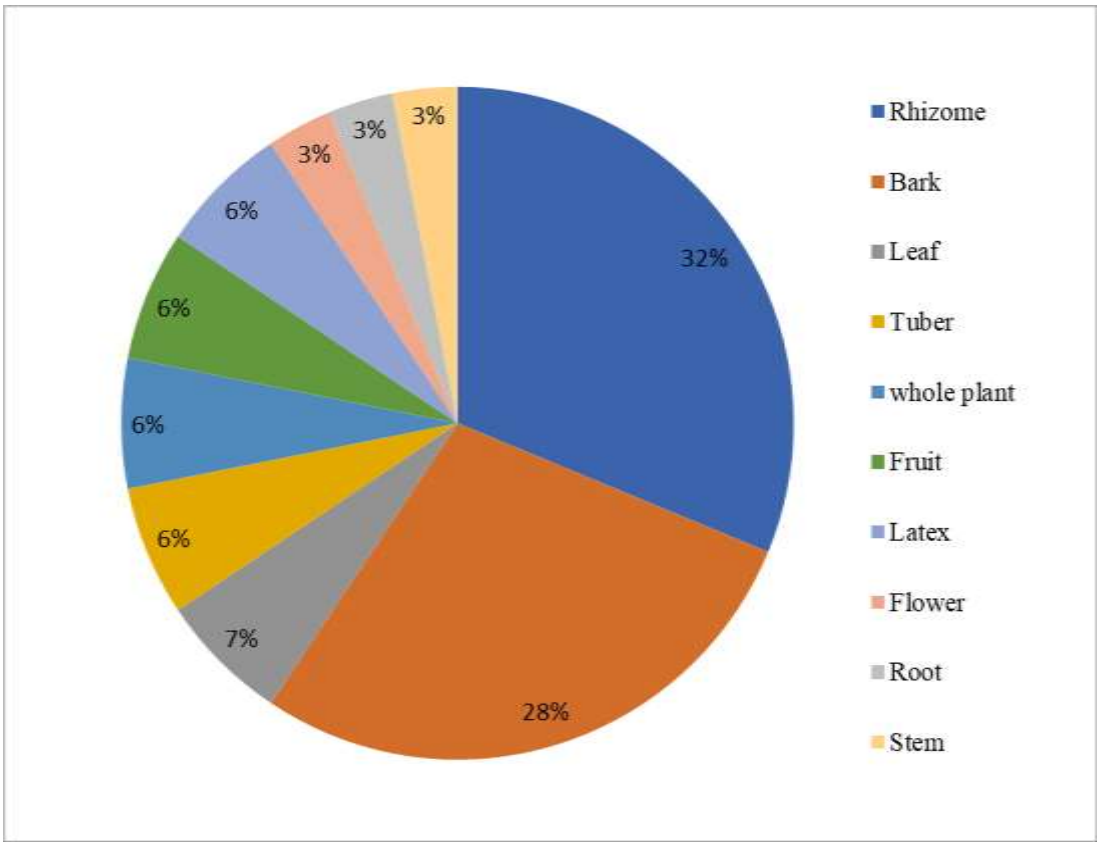

Figure 5: Percentage of different parts used in medicinal plants.

In the present work, use of plants with the highest number of recommendations by the informants during the fieldwork are *Astilbe rivularis* (9), *Bergenia ciliata* (10), *Betula alnoides* (14), *Equisetum diffusum* (9), *Fraxinus floribunda* (10), *Litsea cubeba* (15), *Litsea glutinosa* (9), *Quercus lamellosa* (10), *Rheum nobile* (12), *Stephania glabra* (13), and *Viscum nepalense* (10).

Plants with moderate or frequent recommendations in the field by the informants are *Acorus calamus* (8), *Asparagus racemosus* (7), *Costus speciosus* (7), *Curcuma angustifolia* (8), *Curcuma caesia* (7), *Datura metel* (8), *Kaempferia rotunda* (7), *Pentapanax leschenaultii* (8), *Rhododendron arboreum* (8), *Urtica dioica* (7), and Zingiber *zerumbet* (8).

Plants with less number recommendation are *Diploknema butyraceae* (3), *Ficus benghalensis* (2), *Juglans regia* (2), *Morus macroura* (2), *Piper longum* (4), *Prunus cerasoides* (4), *Quercus thomsoniana* (3), *Rheum australe* (6), *Ricinus communis* (2), *Vitex negundo* (3) and *Zingiber capitatum* (4) **(Figure 6)**.). Plant habit wise percentage of uses of reported plants are herbs (40 %), followed by trees (39%), shrubs (9%), climbers (9%), and parasitic herb (3%) **(Figure 7)**.

Out of 33 plants collected, 11 plants were found to have high scoring relative frequently consensus (RFC) mentioned for treating RA which are *Rheum nobile* (0.137), *Fraxinus floribunda* (0.114), *Equisetum diffusum* (0.103), *Astilbe rivularis* (0.103), *Bergenia ciliata* (0.114), *Litsea cubeba* (0.172), *Quercus lamellosa* (0.114), *Betula alnoides* (0.160), *Viscum nepalense* (0.114), *Litsea glutinosa* (0.103), and *Stephania glabra* (0.149). The remaining 22 plants had an RCF score below 0.1 **(Table 4)**.

It is found that the plants are mostly utilized in the dry form as medicines, and fleshy plants are additionally valuable in the herbal formulation. The folk healers accounted that the mature plants are usually preferred for medicines and they are harvested any time of the year as they are accessible and when required. Populations of important medicinal plants are scarce especially during the dry season and are collected when their population is plentiful. Collected medicinal plants are generally dried under the shade away from the direct sunlight and stored in air-tight containers.

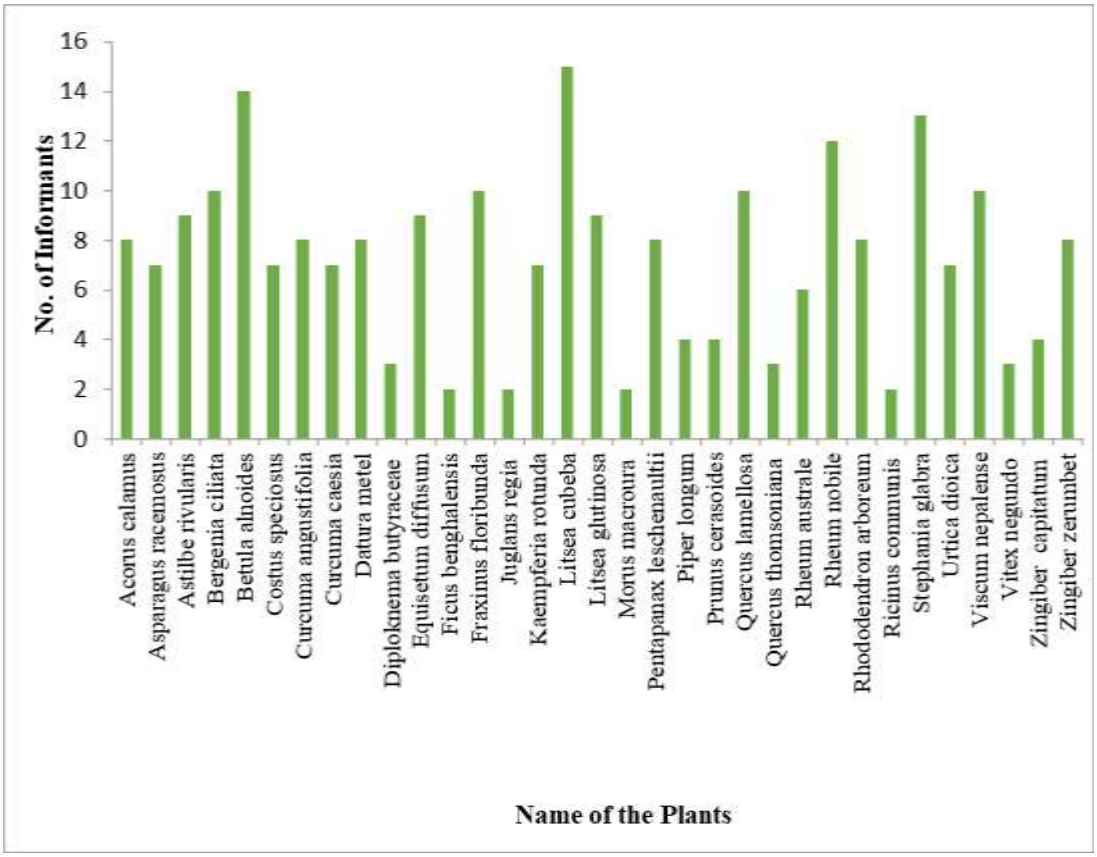

Figure 6: Bar graph showing the number of times the respondents recommended a particular plant species.

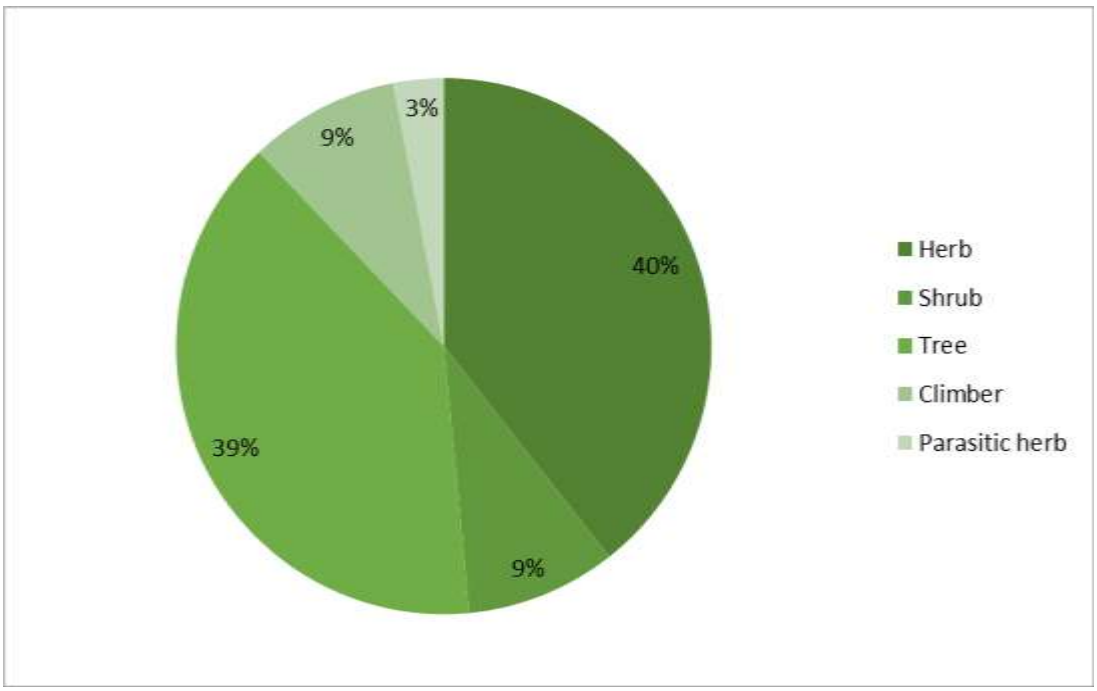

Figure 7: Percentage of plants habits.

4.3.2. *Methods of preparation of plants used for treating RA*

In the study area, various kinds of approaches were utilized in the treatment of RA joint pain. Various plants were accounted for as being used in the prescriptions and all the folk healers utilized either single plant or mixes of multiple plants in their remedies. A few informants reported the use of non-plant materials (honey, milk, egg, and terpene oil) in their medicinal plant preparation to improve the responsiveness of herbal remedies. The mainly utilized methods of preparation include decoction, infusion, paste, a poultice of the required plant parts, and the items were either regulated orally or by applying the medicinal preparations topically on the affected parts of the body until patients recover. Twenty-one (21) most commonly prepared recipes from the plants along with non-plant materials were recorded as depicted by the informants. Single plant recipes were *Stephania glabra* (dry powder 1-2 teaspoonfuls is mixed water and take 3 times/day orally), *Litsea glutinosa* (poultice from the barks is applied externally over affected joints), *Litsea cubeba* (oil is extracted from the fruit's seed and applied topically over affected joints), *Equisetum diffusum* (hot water infusion of whole plants is taken 1glass/day orally), *Acorus calamus* (rhizomatous poultice is applied topically over affected areas), *Costus speciosus* (cold water infusion from stem is taken 1glass/day orally), *Juglans regia* (Bark poultice is applied over affected joints), *Ficus benghalensis* (bark latex is applied over affected areas), *Morus macroura* (bark latex is applied over affected areas), *Piper longum* (infusion of fruit is administered 1glass/day orally) and *Vitex negundo* (poultice of bark is applied topically on the affected joints), *Rhododendron arboreum* (infusion of flowers is administered orally), fruits of *Diploknema butyraceae* (bark poultice is applied on the affected joints to get relief from pains). Paste from the tuberous part of *Kaempferia rotunda* and roots of *Urtica dioica* is

applied topically over affected areas. Young twigs of *Ricinus communis* and *Datura metel* are ground along with coconut to make a paste and are applied topically over affected parts. Infusion from the barks of *Quercus lamellosa* and rhizomes of *Rheum nobile* and *Astilbe rivularis* is administered 1glass/day orally. An equal amount of *Asparagus racemosus* rhizomatous powder and barks powder of *Fraxinus floribunda* are mixed with terpene oil and are applied topically over affected joints. Dry powders of *Bergenia ciliata* rhizomes, *Astilbe rivularis* rhizomes, and *Viscum nepalense* are mixed with 1 glass of milk along with 2-4 drops of honey and administered orally for reducing joint pains. A decoction from the barks of *Quercus lamellosa* and rhizomes of *Rheum australe* is administered 1glass/day orally. A heavy infusion which comprises *Viscum nepalense, Kaempferia rotunda* rhizomes, *Ficus benghalensis* barks, *Fraxinus floribunda* barks, *Prunus cerasoides* barks, and *Betula alnoides* barks are mixed with honey, milk, egg yolk and is administered 1/2glass/day orally. Poultices made from equal quantities of rhizomes of *Curcuma caesia*, *Curcuma angustifolia,* and *Zingiber zerumbet* are applied topically over affected joints. An infusion made from the rhizome of *Zingiber capitatum*, the bark of *Pentapanax leschenaultii,* and bark of *Fraxinus floribunda* is administered orally as a tea 3 times a day. An artificial hot spring is prepared in a wooden bucket containing water by putting some hot stone along with medicinal herbs (*Viscum nepalense*, bark of *Fraxinus floribunda*, *Quercus lamellosa*, and *Quercus thomsoniana*) and the patients are allowed to stay in that artificial hot spring for 1-2 hours once in a week. It is found that some medicinal plants (*Fraxinus floribunda, Kaempferia rotunda, Astilbe rivularis, Quercus lamellosa,* and *Viscum nepalense*) are utilized by folk healers in preparation of numerous remedies for treating RA. Medicinal preparations are generally prepared just before use, but if needed, storage doesn't exceed 3-5 days.

However, powders are kept as long as needed but were always placed in a dry and safe environment away from direct sunlight. There were no side effects reported by the traditional healers upon the use of their herbal remedies. The herbal remedies are reported to alleviate the symptoms of RA as long as the remedies are continuously taken as prescribed and a few precautions must be taken, such as avoiding certain food items, especially sour and spicy items, bamboo shoots, and alcohol.

4.4. DISCUSSION

Over the years, people have been using various ethnomedicinal plants for the treatment and prevention of inflammatory diseases, particularly RA, and plants are considered safe and effective for treating diseases. Research has shown that people with RA suffer from chronic pain and those who are disappointed with current medical treatment are using alternative treatments, and about 60-90% of arthritic peoples are using complementary and alternative medicine (Rao *et al*., 1999). Ethnic people of India have been using ethnomedicinal plants for treating rheumatoid arthritis. Such studies on documentation of ethnomedicinal plants utilized by different tribes were done in different parts of India and reported various ethnomedicinal plants for treating rheumatoid arthritis (Pawar & Patil, 2006; Rathore *et al*., 2007; Naidu *et al*., 2008; Manjula *et al*., 2013; Choudhary *et al*., 2015; Kumar *et al*., 2015b; Swamy & Reddi, 2016; Shyamala *et al*., 2016). In Sikkim, few documentation studies were done on general ethnomedicinal plants used by ethnic tribes for curing different ailments as well as on ethnobotanical plants for other basic needs (Singh *et al*., 2002; Maity *et al*., 2004; Chhetri, 2005; Pradhan & Badola, 2008; Dash, 2009; Panda & Misra, 2010; Das *et al*., 2012; Panda, 2012; Singh *et al*., 2015; Sherpa *et al*., 2015;

Shrestha *et al*., 2015; Chamlagai & Singh, 2016; Aranya *et al*., 2016; Chakraborty *et al*., 2016; Mahendra *et al*., 2017; Tamang *et al*., 2020).

From the present study, it was revealed that the ethnic peoples of Sikkim Himalaya used various ethnomedicinal plants for treating RA. A total of 33 plant species belonging to 24 families were reported to be utilized as medicinal plants for treating RA in the study area. The reported plants are summarized in **Table 4**, with the botanical name of the plants, family, local name, and partly used. Mainly of the plants were from the Zingiberaceae family followed by Saxifragaceae, Moraceae, Lauraceae, Fagaceae, Polygonaceae, Acoraceae, Liliaceae, Betulaceae, Costaceae, Equisetacea, Piperaceae, Loranthaceae, Solanaceae, Oleaceae, Juglandaceae, Araliaceae, Rosaceae, Ericaceae, Euphorbiaceae, Menispermaceae, Urticaceae, Loranthaceae and Verbenaceae **(Figure 4)**. The obtained information denoted that the folk healers used different parts of therapeutic plants for the preparation of anti-RA remedies. However, rhizomes were mostly used followed by bark, fruits, leaves, whole plants, tuber, latex, flower, root, and stem **(Figure 5)**. The majority of informants prescribed the plants were *Astilbe rivularis*, *Bergenia ciliata*, *Betula alnoides*, *Equisetum diffusum*, *Fraxinus floribunda*, *Litsea cubeba*, *Litsea glutinosa*, *Quercus lamellosa*, *Rheum nobile*, *Stephania glabra*, and *Viscum nepalense*. Plants with moderate or frequent recommendations in the field by the informants were *Acorus calamus*, *Asparagus racemosus*, *Costus speciosus*, *Curcuma angustifolia*, *Curcuma caesia*, *Datura metel*, *Kaempferia rotunda*, *Pentapanax leschenaultii*, *Rhododendron arboreum*, *Urtica dioica*, and *Zingiber zerumbet*. Plants with lesser recommendation were *Diploknema butyraceae*, *Ficus benghalensis*, *Juglans regia*, *Morus macroura*, *Piper longum*, *Prunus cerasoides*, *Quercus thomsoniana*, *Rheum australe*, *Ricinus communis*, *Vitex negundo*, and *Zingiber capitatum* **(Figure 6)**. Amongst the documented species,

Litsea cubeba (0.172) was frequently used by the informants followed by *Betula alnoides* (0.160), *Stephania glabra* (0.149), *Rheum nobile* (0.137), *Fraxinus floribunda*, *Quercus lamellosa*, *Bergenia ciliata*, and *Viscum nepalense* (0.114), *Astilbe rivularis*, *Equisetum diffusum*, and *Litsea glutinosa* (0.103) which highlighted their importance in RA management.

The data obtained suggested that the traditional healers used different parts of the medicinal plants for the preparation of anti-RA remedies. However, rhizomes and barks were the key parts used by folk healers to prepare herbal remedies that can affect plant biodiversity, and it is an unsustainable processing practice that poses a threat to the conservation status of some rare native plants. Folk healers are also conscious of this and to mitigate these problems, they typically cultivated some medicinal plants in the vicinity of the residential area, an indication of the sensitivity of the ethnic community for the conservation and sustainable use of essential ethnomedicinal plants. Unlike other studies in which herbal remedies are administered exclusively through oral routes for the treatment of chronic joint pain (Wambugu *et al*., 2011), the respondents in this study reportedly indicated that their prescriptions for treating RA are mainly applied topically over damaged joints. Although, some of the folk healers additionally recommend oral administrations of the herbal remedies. The period of treatments was usually long or till recovery, likely due to chronic joint pain in rheumatoid arthritis, which is not entirely curable and the goal of the treatment is to reduce the outcomes of disease progression (Bullock *et al*., 2018).

Medicinal plant preparations for joint pain treatment and rheumatoid arthritis typically require a complex mixture of different plants, plant parts, and preparation methods (Wambugu *et al*., 2011). Combined plant extracts provide a broader range of

biological effects due to additive and synergistic effects, and therefore this may be the potential route for the production of efficient, safe, and cost-effective plant therapy worldwide (Yuan *et al*., 2017). Several conventional herbal medicinal products are available commercially licensed phytopharmaceutical (Yuan *et al*., 2016). Even so, the lack of adequate standardization, safety precautions, quality control, and adulteration with conventional medicines are a major challenge for the use of traditional phyto-remedies (Karunamoorthi *et al*., 2013). It is therefore recommended that all medicinal plants be scientifically validated for their reported effectiveness, safety, and toxicity (Sen & Chakraborty, 2017).

4.5. CONCLUSION

This study presents the ethnic knowledge of the people of Sikkim Himalaya regarding the use of medicinal plants against rheumatoid arthritis. It is concluded by documenting 33 medicinal plants which belong to 24 families and 29 genera. Till now, no such work has been done in Sikkim Himalaya on the documentation of ethnomedicinal plants for treating rheumatoid arthritis. Furthermore, it is crucial to scientifically assess the reported medicinal plants to ensure the wellbeing of the individuals consuming the traditional prescriptions and for the improvement of possible drugs against RA.

Chapter 5

Phytochemical Screening and Antioxidant Activity

5.1. INTRODUCTION

Phytochemicals or secondary metabolites are naturally occurring, chemical compounds in plants, which are used not only for their own resistance but also have great therapeutics potencies (Ogunmefun, 2018). Phytochemicals are usually found in different parts (leaves, fruits, flowers, fruits, seeds, rhizomes, roots, barks, etc.,) of plants to facilitate the protection from different diseases as well as herbivores. Most of the plants have a wide variety of phytochemicals including phenolic compounds (tannins, phenolic acid, flavonoids, lignans, quinones, stilbenes, coumarins), nitrogenous compounds (betalains, alkaloids, amines), vitamins (vitamin-A, C, D, E), and terpenoids (including carotenoids). All these phytochemicals are beneficial to humankind due to their potential therapeutic activities against various human diseases. Terpenoids are known for their wide variety of biological activities as anti-inflammatory, anti-microbial, anti-malarial, and anti-cancerous properties (Sülsen *et al*., 2017; Cox-Georgian *et al*., 2019, Yang *et al*., 2020). Alkaloids are known for having anti-microbial, insecticidal, anti-proliferative, anti-inflammatory, anti-metastatic and anesthetics activities (Shi *et al*., 2014; Sayhan *et al*., 2017). Phenolic compounds are potent antioxidants for scavenging free radicals (Cai *et al*., 2004; Martins *et al*., 2016; Santos-Sánchez *et al*., 2019). Flavonoids are one of the huge groups of phenolic compounds and they are known to have various therapeutic potentials, including anti-cancer, anti-microbial, anti-aging, anti-inflammatory, and anti-atherosclerotic properties (Braca *et al*., 2002; Cushnie & Lamb, 2005; Kaurinovic

& Vastag, 2019; Karak, 2019). Because of this, the phytochemicals are regarded as potential natural antioxidants and attain a considerable part in the advancement of current medication for sicknesses including cancer, hepatic diseases, diabetes, inflammation, and arthritis (Nair *et al*., 2013; Tabolacci *et al*., 2019; Forni *et al*., 2019). Therefore, in recent times, various researchers are concerned with the analysis of therapeutic plants for antioxidant phytochemicals, including flavonoids, tannins, and phenols due to their capability in preventing and managing various human ailments.

Free radicals are highly unbalanced compounds produced during the usual cellular metabolism of living organisms (Gupta *et al*., 2014). ROS (Reactive Oxygen Species) production in organisms is an unavoidable process. Generally, there is a counterbalance between a free radical or ROS generation and internal antioxidant defense systems, but in case if this balance is distorted, it can direct to oxidative stress which is harmful to all the important cellular components including proteins, DNA, and membrane lipids, which ultimately cause cell death (Mahajan & Tandon, 2004). In order to deal with oxidative stress, human beings have developed an antioxidant defense mechanism, including specific enzymes such as catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase, along with several low molecular weight antioxidants (cysteine, ascorbate, α-tocopherol, thioredoxin, glutathione, vitamins, etc.). But sometimes this antioxidant defense system may be affected by some pathological or environmental factors, resulting in the progression of various free radicals which affect the normal function of cells leading to human aging and development of chronic diseases such as autoimmune disease including, rheumatoid arthritis, cancer, etc. (Fridovich, 1997; Wei *et al*., 2001; Rahman *et al*., 2012; Kumar *et al*., 2016). So, there is a need for regular consumption of a diet having

antioxidant properties. Antioxidants are the most important substances which can stabilize free radicals, which help to inhibit the imbalance developed during oxidative stress, and plant products are considered as a significant source of natural antioxidants with high potential in treating inflammatory diseases like rheumatoid arthritis (Forni *et al*., 2019). T-cells and cytokines, along with reactive oxygen species like superoxide anions and hydrogen peroxide released by activated macrophages play an essential role in the development of rheumatoid arthritis (Feldmann *et al*., 1996). Some of the recent studies confirmed the role of free radicals or ROS in the development of rheumatoid arthritis (RA) (Goţia *et al*., 2001; De Leo *et al*., 2002; Knekt *et al*., 2000; Karatas *et al*., 2003; Kamanli *et al*., 2004). The proper supply of dietary antioxidant nutrients may reduce free radical development and improve the level of antioxidants in RA patients (Bae *et al*., 2003). In this way, antioxidants are related to RA, and the medicinal plants having various phytochemicals with potent antioxidant properties may be helpful in controlling the oxidative stress in RA patients. In this present chapter, an enumeration of phytochemical analysis and antioxidant activities of 22 ethnomedicinal plants which are utilized against RA by the ethnic people of the Sikkim Himalayan region has been presented.

5.2. MATERIALS AND METHODS

5.2.1. *Sample preparation and extraction*

Based on the recommendation of potential ethnomedicinal plants by the respondents used for the treatment of RA, 22 medicinal plants were selected out of 33 for further phytochemical analysis. A required part of the selected plants was collected from the natural habitat with prior permission obtained from the State Biodiversity Board, Government of Sikkim, and brought in the laboratory. It is then thoroughly washed by

tap water to wash away the debris and chopped into pieces. Further, it is dried at 40ºC in a thermostatically controlled oven until it attained a constant weight. The samples were then crushed into a fine powder using the mechanical grinding machine. 10 g powder of plant sample from each plant was extracted in 100 ml of three different solvents (methanol, acetone, and water) by using the Soxhlet apparatus for 24 hours at 30ºC. After 24 hours, the obtained extract was filtered and concentrated under vacuum using a rotatory evaporator (Heidolph, Schwabach, Germany) and dried further in the vacuum desiccators. After the complete dryness of the plant extract, the yield value was calculated and kept at 4ºC in the refrigerator. The extract was then reconstituted with the relevant solvents to obtain the concentrations needed for further study.

5.2.2. *Physicochemical analysis*

The powder prepared from the plant sample was evaluated for physicochemical analysis such as total ash content, the content of acid-insoluble ash, the content of water-soluble ash, loss on drying, and extractive value in three different solvents (methanol, acetone, and water) as per the standard method (Khandelwal, 2007).

a) *Determination of total ash value of plant samples*

In the pre-ignited crucible, two grams of plant sample in powdered form were taken and ignited steadily by heating to 500-600 ° C in the muffle furnace, until it became white. After that, it was cooled in a desiccator and weighed. The content of total ash in terms of percentage was calculated by using the following formula:

Total ash content=W_1-W_2 (W_1= Wt. of plant sample along with crucible weight, W_2= final Wt. of the sample)

Total ash value (%)= [total ash content/initial wt. of plant sample]×100

b) *Acid insoluble ash*

Total ash obtained was boiled lightly with 25ml dilute HCl (HCl 1:25) for 5 minutes. The insoluble matter was collected on ashless filter paper and rinsed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible and dried on a hot plate and ignited to constant weight. After that, the residue was allowed to cool in a desiccator for 30 minutes and weighed without delay. Acid insoluble ash content was determined based on the sample taken initially.

c) *Water-soluble ash*

The total ash obtained was boiled with 25ml of water for 5 minutes. Then the insoluble material was collected on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature, not beyond 450°C, then cooled in a desiccator and taken the weight immediately. Water-soluble ash was measured by the difference in the weight of the insoluble matter and the weight of total ash. The percentage of water-soluble ash was calculated with reference to the sample taken initially.

5.2.3. *Phytochemical analysis*

a) *Qualitative phytochemical analysis*

Qualitative phytochemical screening of selected plant samples was done using three different solvents (methanol, acetone, and water) following the standard methods (Harborne 1973, Sofowora 1993, Trease & Evans 1989) to determine the presence or absence of various phytochemical constituents especially alkaloids, flavonoids,

phenols, tannins, steroids, terpenoids, saponins, phlobatannins, anthraquinones, carbohydrates, and glycosides.

i. ***Alkaloids***: The sample extracts (0.5 ml) were mixed with 1% HCl (8 ml) then warmed and filtered. The filtrate (2 ml) was tested individually with both Mayer's and Dragendroff's reagents and noticed whether the alkaloids were present or not in the form of turbidity or precipitation.

ii. ***Flavonoids (Shinoda's test)***- The plant extracts (1 ml) were dissolved in distilled water (2 ml) and then warm in a water bath. Afterward, 4 drops of concentrated HCl were added along with a pinch of magnesium metal powder. The immediate appearance of orange color indicates the presence of flavones, red crimson color for flavonols, and pink magenta color for flavanones.

iii. ***Tannins***: The plant extracts (2.5 ml) were dissolved in 10 ml distilled water and filtered. The filtrates were treated with 1% neutral $FeCl_3$. The emergence of purple, intense green, blue or black color indicated the presence of tannins.

iv. ***Phenols***: To the plant extracts (1 ml), 2 ml of distilled water was added followed by the addition of 2-3 drops of 10% neutral ferric chloride ($FeCl_3$) solution. The emergence of blue or green color indicates the presence of phenols.

v. ***Phlobatannins***: Plant extracts (5 ml) were boiled with 2% HCl for 2-3 hours. The deposition of red precipitates at the bottom of the test tube was taken as affirmation for the existence of phlobatannins.

vi. ***Saponins***: Plant extracts (1 ml) were diluted to 5 ml with distilled water in a test tube and shaken vigorously, the formation of about 1 cm layer of froth specifies the presence of saponins.

vii. ***Terpenoids***: The plant extracts (5 ml) were mixed with chloroform (2 ml) followed by the addition of 3 ml concentrated H_2SO_4. A formation of a reddish-brown color layer at the crossing point shows the existence of terpenoids.

viii. ***Anthraquinones***: The plant extracts (5 ml) were hydrolyzed with dilute H_2SO_4 followed by the reaction with benzene (1 ml) and NH_3 (1 ml). The appearance of a rose pink color confirms the presence of anthraquinones.

ix. ***Steroids (Salkowski's test)*** - A few drops of concentrated H_2SO_4 acid were added by the wall of the test tube containing plant extracts. The appearance of golden yellow color signifies the existence of steroids.

x. ***Glycosides***: The plant extracts (5 ml) were hydrolyzed with concentrated HCl (5 ml) and boiled in a water bath for 1-2 hours. Then the hydrolysate extracts were dissolved in water (1 ml) followed by the addition of 10% aqueous NaOH. The formation of a yellow color denotes the existence of glycosides.

xi. ***Carbohydrates***: To the extracts (1ml) were added to H_2O (1ml) followed by the addition of 20 drops of boiling Fehling's reagents (A&B) in a test tube. The formation of a red-brick precipitate at the bottom of the test tube denotes the existence of carbohydrates.

b) ***Quantitative phytochemical estimation***

The total phenolic content (TPC) of the selected plant samples was determined by following the method as proposed by Lin *et al* (2011), with slight modifications. 1ml extract of plant samples was mixed with 5ml of 10 times diluted Folin-ciocalteu reagent along with 4 ml of Na_2CO_3 (7.5%). The mixture was placed at room temperature for 90 minutes and the absorbance was estimated at 760 nm using a UV-visible spectrophotometer (Thermo Fisher Scientific). The standard curve was

prepared by using gallic acid by serial dilution from 10-100 µg/ml. Results were expressed as mg GAE/g of dry extract weight.

The total flavonoid content (TFC) of the selected plant samples was measured by utilizing a standard method with some modifications as prescribed by Lin *et al* (2011). 5 ml of plant extract was mixed with 0.3 ml 5% sodium nitrite for 5 minutes in a test tube. After that 0.3ml of 10% aluminum chloride was put into it. After 6 minutes, 2 ml of NaOH was added to stop the reaction. The mixture was further diluted with distilled water till the volume reached 10 ml. The absorbance was instantly estimated at 510 nm utilizing a UV-visible spectrophotometer. Then a standard curve was prepared by utilizing rutin concentration ranging from 10-100 µg/ml. TFC was expressed as mg of RtE/g of dry extract weight.

Total Flavonol content was determined by using the standard method given by Kumaran & Karunakaran (2006) and utilizing Rutin as standard. 2ml of 2% aluminum trichloride and 3 ml sodium acetate solutions were added to 2ml of methanol extract. The solutions were kept at 20ºC for 2.5 hours and the absorbance was estimated at 440 nm using a UV-visible spectrophotometer. Results were expressed as mg RtE/g of dry extract weight.

The tannin content of plant extract was measured by using the standard method of Tambe & Bhamber (2014). 0.1ml of each extract was taken in a test tube containing 7.5ml distilled water followed by the adding up of 0.5ml Folin-Ciocalteu reagent along with 1ml of 35% Na_2CO_3 and it was further diluted to 10ml with distilled water. The blend was shaken well and kept for 30 minutes a room temperature. Absorbance was estimated at 725 nm by utilizing a UV-visible spectrophotometer. Results were expressed as mg GAE/g of dry extract weight.

5.2.4. *Evaluation of antioxidant activities*

Antioxidant activities of each selected medicinal plant in the methanolic extract were performed by using three free radicals scavenging assays i.e. DPPH radical scavenging assay, ferrous chelating activity, and ferric ion reducing assay to support the ethnomedicinal properties of selected plant species.

a) *DPPH free radical scavenging assay*

The free radical scavenging activities of selected plant extract were determined based on their scavenging activity of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical as per the standard method with slight modifications (Hatano *et al*., 1988). Different concentrations (100, 200, 300, 400, and 500 µg/ml) of the plant extract in methanol were prepared and 1 ml of each solution of different concentration was added to 3 ml of 0.04% methanolic DPPH free radical solution. After 30 minutes the absorbance of the solution was measured at 517 nm by using a UV-visible spectrophotometer and that was compared with the absorbance of standard ascorbic acid concentrations (100-500 µg/ml). Then the percentage inhibition was estimated by utilizing the following formula:

% inhibition= [(Absorbance of blank - absorbance of sample)]/(absorbance of blank) x 100

From the calibration curves obtained from different concentrations of plant extract, the IC_{50} (inhibitory concentration 50%) was determined. IC_{50} value signifies the concentration of plant sample that inhibits DPPH free radical by 50% (Gupta *et al*., 2003).

b) ***Ferrous chelating assay***

The chelating activities of selected plant extract for ferrous ion (Fe^{2+}) were estimated according to the standard method of Su *et al* with slight modifications (Su *et al*., 2008). Different concentrations (200, 400, 600, 800 and 1000 µg/ml) of plant extracts were prepared. To 0.4ml of extracts of different concentrations, 1.6 ml of methanol was diluted and mixed with 0.04 ml of $FeCl_3$ (2 mM) followed by the addition of 0.8 ml of ferrozine (5 mM). The mixture was shaken well and also incubated for 10 minutes at room temperature. The absorbance of mixtures was estimated at 562 nm in opposition to a blank. The EDTA was utilized as a positive control. The ability of chelating activity was estimated by utilizing the following formula:

% inhibition= [(Absorbance of blank-absorbance of sample)]/ (absorbance of blank) x 100

From the calibration curve obtained, the IC_{50} (µg/ml) value was determined.

c) ***Ferric ion reducing assay***

The reducing activity of plant extract for ferric ion (Fe^{3+}) was measured according to the standard method with slight modifications (Deng *et al*., 2010). Different concentrations of samples were (200, 400, 600, 800, 1000 µg/ml) prepared and 1 ml of each varied concentration of the sample was mixed with 2.5ml of Phosphate buffer saline solution (pH 6.6) along with 2.5ml potassium ferricyanide solution in the test tube and incubated at 50ºC in a water bath for 20 minutes. The reaction was stopped by adding 2.5 ml of 10% trichloroacetic acid and allowed to stand at room temperature for 10 minutes. 2.5ml of the upper portion of the solution was taken in a test tube and the same volume of distilled water along with 0.5ml of 0.1% $FeCl_3$ was

added and left in a room temperature for 20 minutes and absorbance was estimated at 700 nm. A considerable absorbance of the reaction mixture signifies greater reducing action. Ascorbic acid was utilized for standard control.

5.2.5. *Statistical analysis*

All the measurements were made in triplicate and the results obtained are expressed as the mean ± standard deviation (SD). To calculate the IC_{50}values, a linear regression investigation was performed by utilizing Microsoft Office Excel 2010.

5.3. RESULTS

5.3.1. *Physicochemical parameters and their extractive yield*

The analysis of physicochemical parameters including total content of ash, acid insoluble and water-soluble ash, extractive value in three different solvents (methanol, acetone, and aqueous), and loss on drying are enumerated in **Table 5**. Ash value is the amount of inorganic stuff in drug material that is used to determine the purity along with the quality of the drug (Sudha & Srinivasan, 2013). Extractive yield decides the number of important phytoconstituents extracted by a particular solvent from the medicinal plants (WHO, 1998) and it is useful in the evaluation of drug adulteration (Kumar *et al*., 2011). The percentage yield is based on the solubility of the phytoconstituents present in the plant sample into a desirable solvent (Patil *et al*., 2012b). Results showed that the methanolic extract of most of the plants in the present work gave a high yield as compared to extracts of acetone and aqueous.

Table 5: Physicochemical parameters and their extractive yield (% W/W)

Plant sample	Total ash	Water-soluble ash	Acid insoluble ash	Loss on drying	Extractive yield (%)	
Acorus calamus	15	9.9	3.15	11.26	Methanol	16.78
					Acetone	8.69
					Aqueous	8.48
Asparagus racemosus	14.2	6.1	2.9	9.8	Methanol	4.61
					Acetone	3.16
					Aqueous	0.78
Astilbe rivularis	12.3	6.3	2.8	12.05	Methanol	10.3
					Acetone	8.71
					Aqueous	6.2
Bergenia ciliata	10.55	5	1.8	19.15	Methanol	26.78
					Acetone	27.87
					Aqueous	17.8
Betula alnoides	5.35	2.75	0.95	16.8	Methanol	13.7
					Acetone	13.2
					Aqueous	11.9
Costus speciosus	11.35	4.9	2.1	10.51	Methanol	19.28
					Acetone	7.01
					Aqueous	5.31
Curcuma angustifolia	10.1	5.65	1.95	4.4	Methanol	9.94
					Acetone	11.3
					Aqueous	8.65
Curcuma caesia	5.85	4.3	2.6	9.52	Methanol	14.7
					Acetone	7.59
					Aqueous	2.91
Datura metel	9	4.4	1.75	4.7	Methanol	20.4
					Acetone	8.14
					Aqueous	7.87
Equisetum diffusum	13	7.8	4.15	8	Methanol	15.9
					Acetone	4.8
					Aqueous	27.9
Fraxinus floribunda	9	4.1	2.1	8.72	Methanol	9.26
					Acetone	7
					Aqueous	9.3
Kaempferia rotunda	5.7	1.6	0.5	6.62	Methanol	8.12
					Acetone	1.15
					Aqueous	3.91
					Methanol	51.5

Litsea cubeba	5.35	2.75	1.45	9.26	Acetone	36
					Aqueous	26.01
Litsea glutinosa	4.8	2.15	0.5	13.74	Methanol	15.1
					Acetone	3.21
					Aqueous	4.45
Pentapanax leschenaultii	9.6	4	2.05	13.55	Methanol	11.43
					Acetone	9.24
					Aqueous	7.35
Quercus lamellosa	10.35	4.65	2.05	13.95	Methanol	23
					Acetone	14.4
					Aqueous	9.6
Rheum nobile	9.35	4.1	2.05	19.48	Methanol	31.7
					Acetone	17.4
					Aqueous	9.87
Rhododendron arboreum	9.1	1.7	0.65	16.8	Methanol	46.4
					Acetone	15.3
					Aqueous	45.56
Stephania glabra	8	4.35	1.5	6.74	Methanol	21.1
					Acetone	11.2
					Aqueous	18.9
Urtica dioica	13.55	3.9	1.45	22.3	Methanol	10.74
					Acetone	5.52
					Aqueous	7.45
Viscum nepalense	6.45	1.8	1.05	9.44	Methanol	27.17
					Acetone	17
					Aqueous	12.56
Zingiber zerumbet	9.25	5.15	2.15	13.94	Methanol	8.35
					Acetone	5.39
					Aqueous	5.86

5.3.2. *Phytochemical analysis*

The preliminary phytochemical screening was performed on the 22 selected medicinal plants in three different solvents (methanol, acetone, and aqueous) and results revealed the existence of a range of phytoconstituents (viz. flavonoids, carbohydrates, terpenoids, phenols, tannins, saponins, anthraquinones, alkaloids, glycosides, and phlobatannins) **(Table 6)**.

Table 6: Preliminary phytochemical examination of selected medicinal plants

Phytochemicals	*Acorus calamus*			*Asparagus racemosus*		
	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	-	-	-	-	-	-
Phlobatannins	+	-	-	+	+	+
Terpenoids	+	+	-	-	-	-
Saponins	+	-	+	+	+	+
Anthraquinones	+	+	-	-	-	-
Glycosides	+	+	+	+	-	+
Carbohydrates	+	+	+	+	+	+
Phytochemicals	*Astilbe rivularis*			*Bergenia ciliata*		
	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	-	-	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Phlobatannins	-	-	-	+	+	+
Terpenoids	+	+	-	+	+	-
Saponins	+	-	+	+	-	+
Anthraquinones	+	+	-	+	+	-
Glycosides	-	-	-	+	-	+
Carbohydrates	+	+	-	+	+	+
Phytochemicals	*Betula alnoides*			*Costus speciosus*		
	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	-	+	-	-	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	-	-	-	+	-	+
Phlobatannins	-	-	-	+	+	+
Terpenoids	+	+	+	-	-	-
Saponins	+	+	+	+	+	+
Anthraquinones	+	-	+	-	-	-
Glycosides	-	-	-	+	-	+
Carbohydrates	+	+	+	+	+	+
	Curcuma angustifolia			*Curcuma caesia*		

Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	-	-	-	-	-	-
Phlobatannins	+	+	-	+	+	-
Terpenoids	+	+	+	+	+	+
Saponins	-	-	+	-	-	+
Anthraquinones	+	-	-	+	-	-
Glycosides	-	+	-	-	+	-
Carbohydrates	+	+	+	+	+	+
	Datura metel			*Equisetum diffusum*		
Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	+	-	-	+	+	+
Phlobatannins	-	-	-	+	+	-
Terpenoids	-	-	-	-	-	-
Saponins	+	-	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Glycosides	+	+	-	+	+	-
Carbohydrates	+	+	-	+	+	+
	Fraxinus floribunda			*Kaempferia rotunda*		
Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	-	-	-
Phenols	+	+	+	+	+	+
Steroids	+	+	-	-	-	-
Phlobatannins	-	-	-	+	-	-
Terpenoids	+	+	-	+	+	-
Saponins	-	-	+	+	-	+
Anthraquinones	-	-	-	+	+	-
Glycosides	-	+	-	-	+	-
Carbohydrates	+	+	+	+	+	+
	Litsea cubeba			*Litsea glutinosa*		
Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	+	-	+	-	-
Flavonoids	+	+	+	+	+	+

Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	-	-	+	+	+	-
Phlobatannins	-	-	-	+	+	+
Terpenoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Glycosides	+	+	+	+	-	-
Carbohydrates	+	-	+	+	-	+
	Pentapanax leschenaultii			*Quercus lamellosa*		
Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	-	+	+	-	-	+
Phlobatannins	+	-	+	+	+	+
Terpenoids	+	+	+	+	+	-
Saponins	+	+	+	+	+	+
Anthraquinones	-	-	-	+	+	+
Glycosides	-	+	-	-	-	-
Carbohydrates	+	+	+	+	+	+
	Rheum nobile			*Rhododendron arboretum*		
Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	+	-	-	-	-
Flavonoids	+	+	-	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	+	+	-	-	-	-
Phlobatannins	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	-
Saponins	+	-	+	-	-	+
Anthraquinones	-	-	-	+	+	-
Glycosides	+	+	+	-	-	-
Carbohydrates	+	-	-	+	+	+
	Stephania glabra			*Urtica dioica*		
Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Steroids	-	-	+	+	+	+

Phlobatannins	-	-	-	+	-	-
Terpenoids	+	-	-	+	+	+
Saponins	-	-	+	+	-	+
Anthraquinones	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+
Carbohydrates	+	-	+	+	+	+
	Viscum nepalense			*Zingiber zerumbet*		
Phytochemicals	Methanol	Acetone	Aqueous	Methanol	Acetone	Aqueous
Alkaloids	-	-	-	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	-	-
Phenols	+	+	+	+	+	+
Steroids	-	-	+	-	-	-
Phlobatannins	-	-	-	+	+	-
Terpenoids	-	-	+	+	+	+
Saponins	+	-	+	-	-	+
Anthraquinones	-	-	-	+	+	-
Glycosides	+	-	+	-	+	-
Carbohydrates	+	+	+	+	+	+

+ For presence and – for absence.

Based on the results of the preliminary phytochemical test, quantitative estimation was performed for some major phytoconstituents viz. phenol, flavonoid, flavonol, and tannin. Comparative studies of total phenol content were articulated as mg GAE/g of dry extract weight and are presented in **Table 7**. Among the selected plant samples analyzed in three different solvents for total phenols content, methanolic extract of *Litsea cubeba* contained the maximum (71.72±0.022 mgGAE/g dry extract wt.) and the aqueous extract of *Kaempferia rotunda* contained the minimum (9.08±0.021mgGAE/g dry extract wt.). Total flavonoid contents expressed as rutin equivalent (RE) per gram dry extract weight and are presented in **Table 7**. The flavonoid content varied among the selected plants in which the methanolic extract of *Zingiber zerumbet* contained maximum (83.5±0.022 dry extract wt.) and aqueous extract of *Litsea cubeba* contained the minimum (3.17±0.01mgRE/g dry extract wt.) flavonoid content.

Total flavonol contents were also expressed as rutin equivalent (RE) per gram dry extract weight which are represented in **Table 7**. Among the selected plant samples analyzed, the methanolic extract of *Equisetum diffusum* contained a maximum (92±0.038mgRE/g) dry extract weight) and water extract of *Datura metel* contained the minimum (22.9±0.003mgRE/g dry extract wt.) flavonol content.

Total tannin contents were expressed as gallic acid equivalent (GAE) per gram dry extract weight and are presented in **Table 7**. In the selected plant species, tannin content was found to be maximum in methanolic extract of *Curcuma caesia* (72.3±0.026mgGAE/g dry extract wt.) and minimum in aqueous extract of *Pentapanax leschenaultii* (16.29±0.006mgGAE/g dry extract wt.).

Table 7: Quantitative phytochemical estimation of selected medicinal plants in three different solvents

Plants	Solvents	Total phenol mgGAE/	Total flavonoids mgRE/g	Total flavonol mgRE/g	Total tannin mgGAE/g
Acorus calamus	Methanol	48.03±0.067	82.75±0.034	86.7±0.028	57.8±0.028
	Acetone	32.69±0.045	62.15±0.036	66.26±0.031	37.42±0.027
	Aqueous	22.3±0.039	41.32±0.043	51.98±0.035	35.8±0.012
Asparagus racemosus	Methanol	24.9±0.058	54.67±0.039	64.5±0.014	45.2±0.019
	Acetone	19.26±0.049	42.26±0.046	56.2±0.048	32±0.006
	Aqueous	12.68±0.028	35.7±0.052	45.8±0.065	30.12±0.015
Astilbe rivularis	Methanol	55.46±0.089	74.79±0.024	84±0.050	58.6±0.019
	Acetone	37.85±0.064	60.1±0.019	63.27±0.011	48.2±0.008
	Aqueous	30.14±0.053	54.4±0.011	69.7±0.048	40.49±0.004
Bergenia ciliata	Methanol	46.41±0.059	80.86±0.019	60.62±0.025	59.7±0.022
	Acetone	37.78±0.047	63.5±0.038	49.9±0.033	42.4±0.009
	Aqueous	32.62±0.032	49±0.027	52.69±0.032	34.3±0.0096

Betula alnoides	Methanol	34.25±0.14	22.83±0.005	62.2±0.031	55.8±0.067
	Acetone	30.57±0.014	15.2±0.052	52.4±0.040	55.86±0.20
	Aqueous	26.3±0.019	22.59±0.015	31.53±0.049	46.2±0.006
Costus speciosus	Methanol	29.16±0.051	54.65±0.039	67.32±0.031	57.78±0.036
	Acetone	22.08±0.054	40.6±0.041	55.8±0.027	43.18±0.015
	Aqueous	12.54±0.064	36.17±0.035	33.65±0.035	35.5±0.010
Curcuma angustifolia	Methanol	25.41±0.049	62.8±0.017	63±0.048	69.3±0.007
	Acetone	31.06±0.039	57.9±0.029	50.4±0.038	63.16±0.012
	Aqueous	25.9±0.023	20.7±0.028	50.57±0.013	43.9±0.008
Curcuma caesia	Methanol	39.62±0.016	71.74±0.01	62.38±0.010	72.3±0.026
	Acetone	34.32±0.073	57.2±0.004	55.51±0.027	62±0.069
	Aqueous	33.32±0.073	22.36±0.012	49.34±0.030	40.49±0.010
Datura metel	Methanol	33.19±0.038	64.48±0.008	45.64±0.074	63.6±0.002
	Acetone	22.72±0.038	47.17±0.005	23.9±0.003	60±0.003
	Aqueous	32.83±0.039	63.5±0.079	22.9±0.003	61.6±0.003
	Methanol	40.4±0.049	39.7±0.049	92±0.038	55.48±0.036

Equisetum diffusum	Acetone	17.49±0.061	16.9±0.054	51.8±0.060	35.2±0.015
	Aqueous	33.61±0.039	6.9±0.017	47.2±0.043	37.8±0.051
Fraxinus floribunda	Methanol	32.83±0.025	40.6±0.019	50±0.029	45.2±0.006
	Acetone	31.56±0.079	36.8±0.021	33.47±0.034	25.2±0.004
	Aqueous	26.47±0.067	33.36±0.029	26.8±0.016	22±0.005
Kaempferia rotunda	Methanol	33.04±0.039	53.7±0.016	81.95±0.012	51.25±0.030
	Acetone	17.7±0.045	17.9±0.011	45.46±0.007	43.18±0.010
	Aqueous	9.08±0.021	16.9±0.054	28.9±0.015	39.34±0.011
Litsea cubeba	Methanol	71.72±0.022	53.95±0.012	65±0.018	53.94±0.008
	Acetone	40.89±0.192	3.87±0.043	53.9±0.048	42±0.045
	Aqueous	62.53±0.057	3.17±0.01	31.53±0.015	35.5±0.053
Litsea glutinosa	Methanol	50.15±0.212	56.7±0.128	83.5±0.134	52±0.003
	Acetone	32.76±0.021	56±0.043	66.5±0.007	32.8±0.007
	Aqueous	33.19±0.072	36.7±0.051	30.3±0.006	23.59±0.027
Pentapanax	Methanol	64.01±0.025	48.3±0.001	81.78±0.014	49.4±0.015
	Acetone	60.48±0.032	33.5±0.034	73.67±0.026	32.8±0.009

leschenaultii	Aqueous	12.61±0.057	13.2±0.019	63.79±0.112	16.29±0.006
Quercus lamellosa	Methanol	32.62±0.059	50.4±0.032	67.5±0.058	59.32±0.031
	Acetone	30.92±0.028	24±0.041	42.46±0.078	58.6±0.013
	Aqueous	29.08±0.076	30.1±0.011	37.7±0.073	54.4±0.004
Rheum nobile	Methanol	39.2±0.072	71±0.068	83.24±0.025	62.4±0.039
	Acetone	21.73±0.0153	64.9±0.044	70.85±0.023	41.26±0.015
	Aqueous	27.81±0.011	44.8±0.024	69.6±0.038	33.96±0.017
Rhododendron arboreum	Methanol	16.2±0.023	36.6±0.03	54.27±0.038	48.18±0.015
	Acetone	14.73±0.008	29.6±0.021	51.9±0.011	43.9±0.013
	Aqueous	13.9±0.012	17.9±0.010	50±0.067	38.9±0.013
Stephania glabra	Methanol	22.3±0.035	54.2±0.007	56.39±0.038	53.2±0.048
	Acetone	18.13±0.0026	21.6±0.013	42.46±0.038	43.5±0.009
	Aqueous	18.34±0.029	15.3±0.018	31.53±0.047	42±0.017
Urtica dioica	Methanol	23.9±0.041	38.5±0.023	37.35±0.050	39.34±0.010
	Acetone	19.19±0.047	35.4±0.018	33.65±0.031	31.2±0.012
	Aqueous	13.6±0.031	21.4±0.042	28±0.012	22.8±0.003

Viscum nepalense	Methanol	52.2±0.032	63.8±0.027	49.5±0.028	62.4±0.023
	Acetone	44.29±0.021	49.9±0.014	37.9±0.043	46.2±0.008
	Aqueous	43.15±0.013	39.2±0.042	34.9±0.027	38.5±0.010
Zingiber zerumbet	Methanol	48.67±0.036	83.5±0.022	65.38±0.097	55.48±0.029
	Acetone	22.93±0.013	67.5±0.014	40±0.029	44.34±0.013
	Aqueous	23.7±0.034	46.9±0.006	47.3±0.014	42±0.052

Values are manifested as mean ± SD, n=3, GAE is Gallic acid equivalents, and RE is Rutin equivalents

In reference to the results shown in **Table 7**, it is confirmed that methanolic extract of maximum plants contains a high content of Phytoconstituents as compared to acetone and aqueous extract. The box plot **(Figure 8)** depicted that among the methanolic extracts of different plants Flavonoid content was found to be high in *Zingiber zerumbet*, *Litsea glutinosa* content the highest amount of flavonol, while the high phenolic content was in *Litsea cubeba* and high Tannin content was found in *Curcuma caesia*. Therefore, out of the three extracts, the only methanolic extract is considered for the antioxidant test as well as for further analysis.

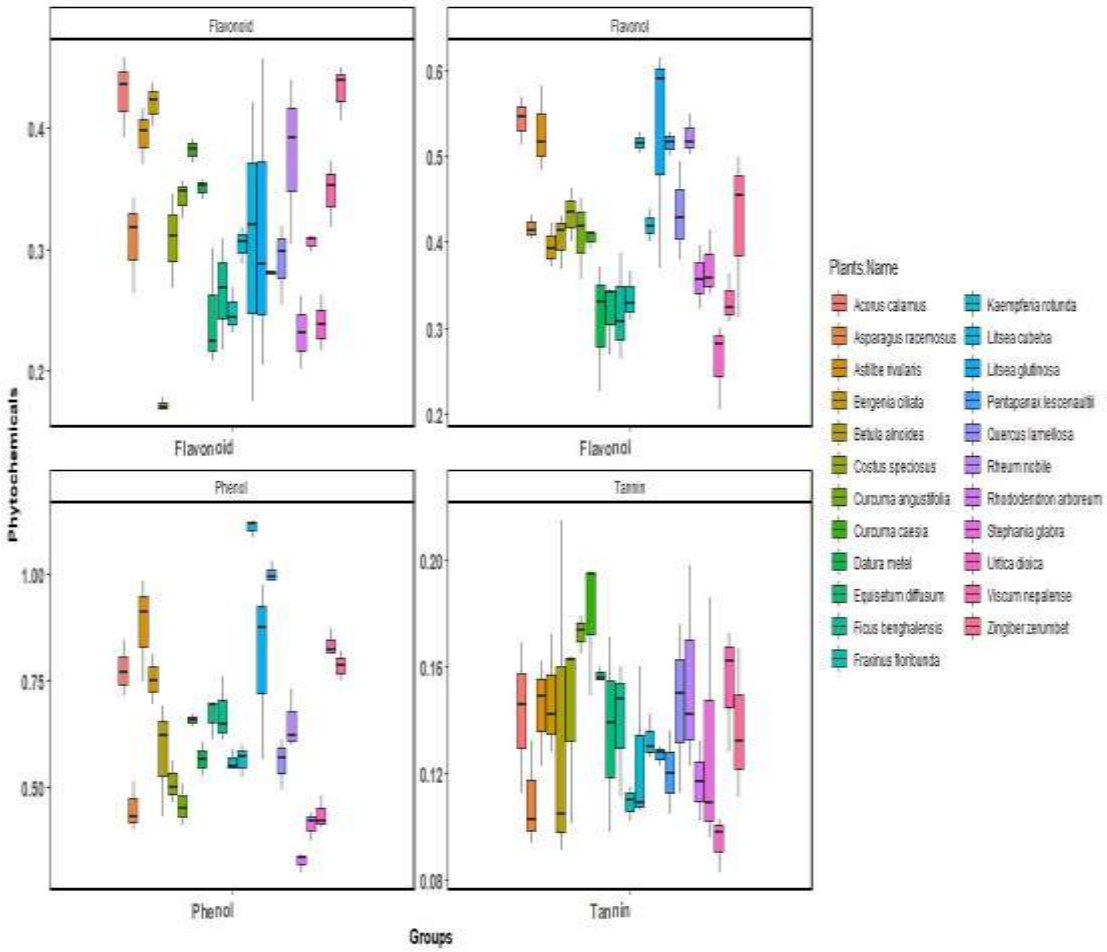

Figure 8: Phytochemicals content of selected medicinal plants in methanolic extract.

5.3.3. *Evaluation of Antioxidant Activities*

Antioxidant activities of the methanolic extract of each selected medicinal plant were examined by using DPPH radical scavenging assay, ferrous chelating activity, and ferric ion reducing assay to support the medicinal values of selected plant species.

a) *The DPPH (2,2-diphenyl-picrylhydrazyl) scavenging assay*

The doses dependent curve of the DPPH radical scavenging property of methanolic extract of each medicinal plant is given in **Figure 9**. It showed that all selected plants in methanolic extract on higher concentration acquired a better antioxidant property in comparison to ascorbic acid. The antioxidant property in terms of IC_{50} value was also calculated and presented in **Figure 10**. Maximum antioxidant activity was shown by *Kaempferia rotunda* at the concentration of 132 µg/ml, followed by *Quercus lamellosa* at the concentration of 152 µg/ml, *Stephania glabra* at the concentration of 180 µg/ml and *Fraxinus floribunda* 186.2 µg/ml among the selected plants, which were found to be more effective as compared to standard ascorbic acid (189 µg/ml), followed by *Betula alnoides* (196.9 µg/ml), *Equisetum diffusum* (199.5 µg/ml), *Curcuma caesia* (205 µg/ml) *Litsea glutinosa* (207 µg/ml), *Acorus calamus* (234.2 µg/ml), *Bergenia ciliata* (244.9 µg/ml), *Costus speciosus* (246.5 µg/ml), *Viscum nepalense* (248 µg/ml), *Rhododendron arboreum* (250 µg/ml), *Datura metel* (252 µg/ml), *Astilbe rivularis* (256.6 µg/ml), *Asparagus racemosus* (287 µg/ml), *Litsea cubeba* (287.6 µg/ml), *Zingiber zerumbet* (296 µg/ml), *Urtica dioica* (297 µg/ml), *Pentapanax leschenaultii* (299.8 µg/ml), and Rheum nobile (308 µg/ml).

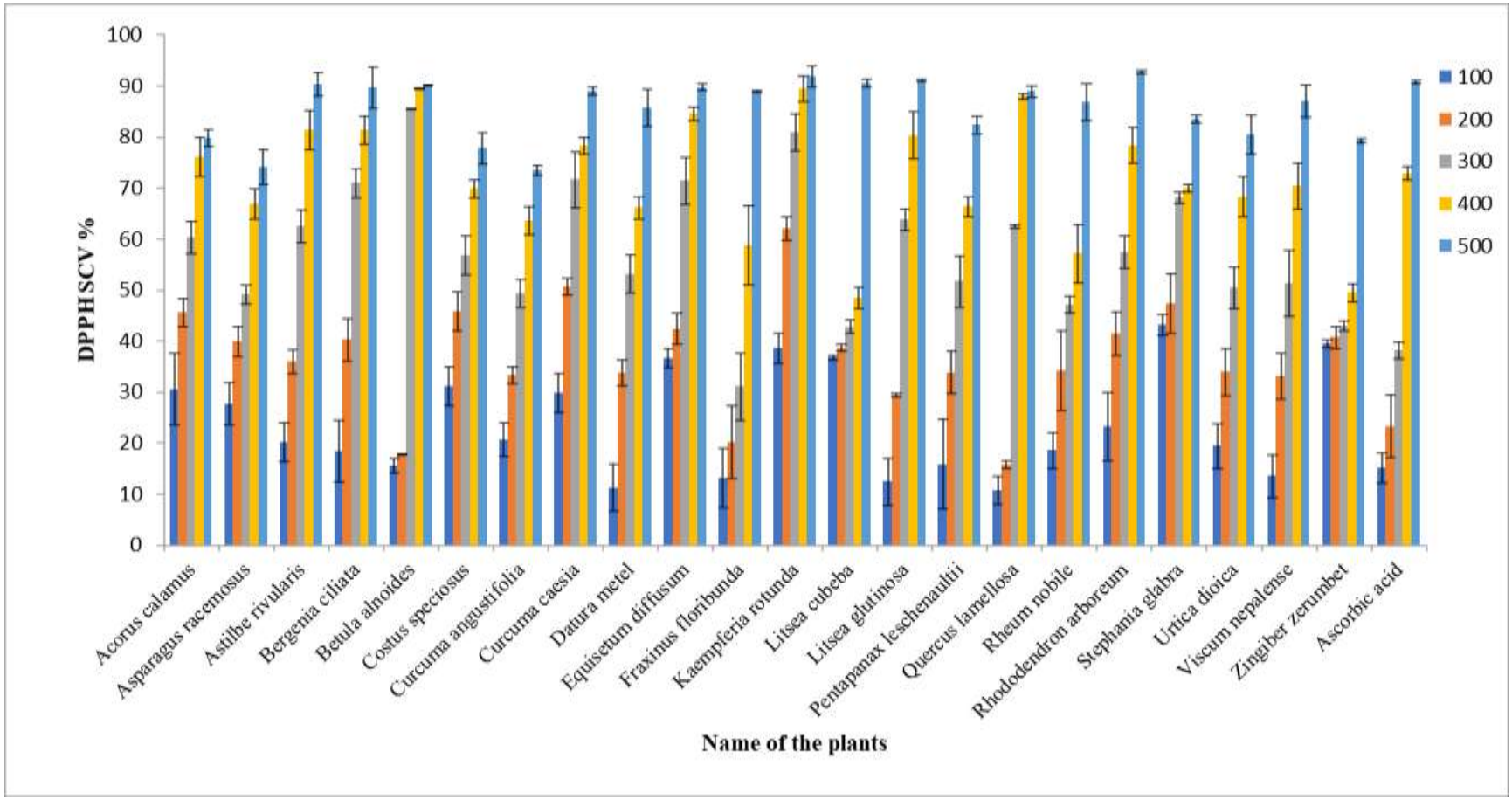

Figure 9: DPPH free radical scavenging activity of selected medicinal plants in comparison with standard Ascorbic acid. Values are expressed as mean value±standard deviation (n=3) and the concentration of the extract is provided in terms of µg/ml.

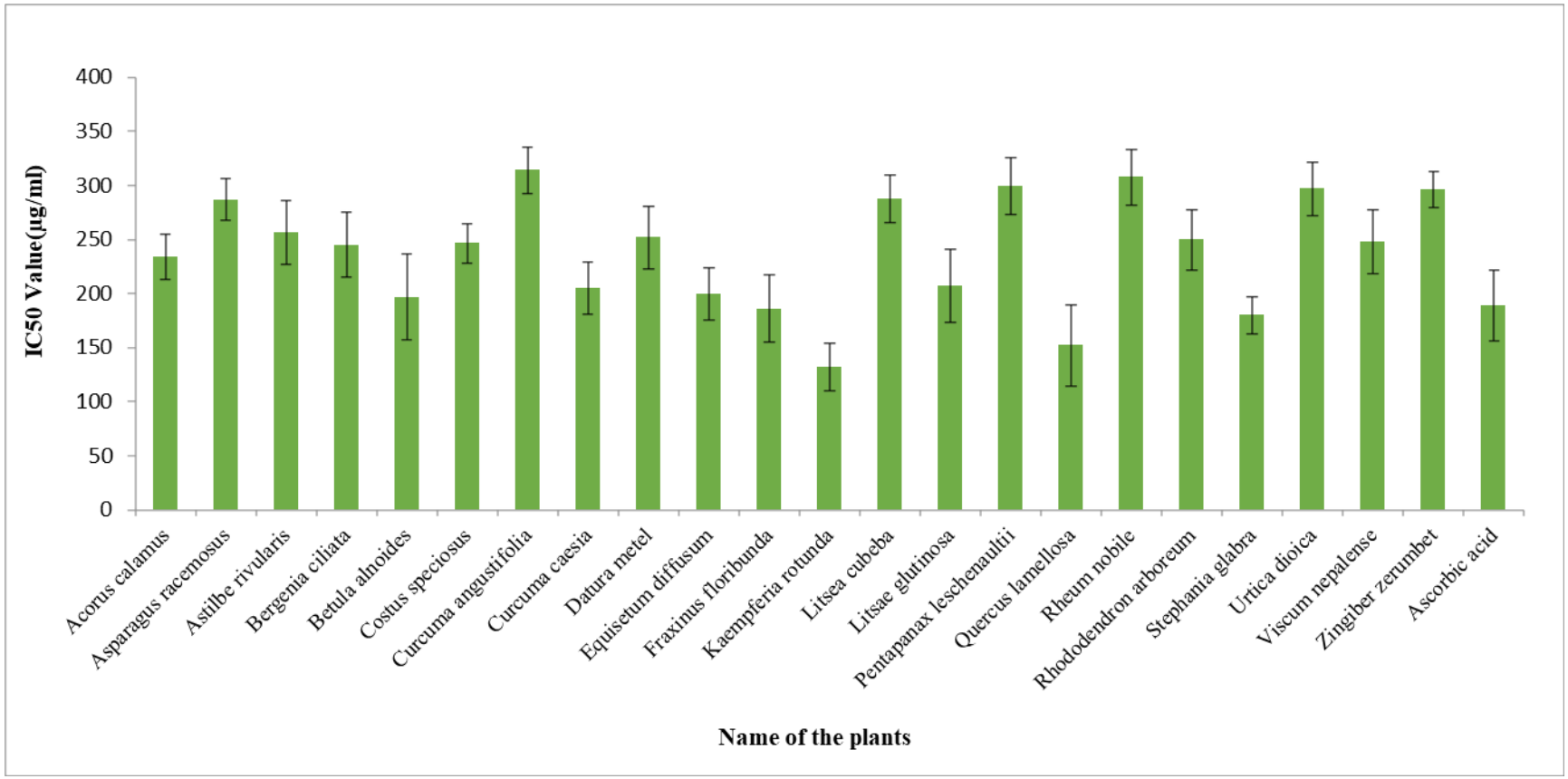

Figure 10: DPPH activity of selected medicinal plants and standard ascorbic acid with their respective IC_{50} value. IC_{50} values are expressed as mean ± S.D.

b) ***Ferrous chelating assay***

The ferrous chelating assay also served as a significant method for the evaluation of the antioxidant activity. The reducing ability of all the selected plants in the methanolic extract by ferrous ion binding ability is given in **Figure 11**. It showed that, with the increase in their concentration, the reducing ability of all plants increases. Reducing activity in terms of IC_{50} value was also determined and presented in **Figure 12**. Maximum reducing activity was shown by *Equisetum diffusum* (439.74 µg/ml), *Zingiber zerumbet* (456.7 µg/ml), *Betula alnoides* (464.8 µg/ml), *Rheum nobile* (523.1 µg/ml), *Fraxinus floribunda* (525.6 µg/ml) and *Astilbe rivularis* (613.7 µg/ml) were found to be effective as standard EDTA (614.4 µg/ml), followed by *Kaempferia rotunda* (620.3 µg/ml), *Stephania glabra* (638.1 µg/ml), *Acorus calamus* (647.9 µg/ml), *Litsea cubeba* (663.2 µg/ml), *Curcuma caesia* (666.1 µg/ml), *Urtica dioica* (685.1 µg/ml), *Asparagus racemosus* (687. 5 µg/ml), *Datura metel* (699 µg/ml), *Viscum nepalense* (707.6 µg/ml), *Rhododendron arboreum* (708.5 µg/ml), *Quercus lamellosa* (724 µg/ml), *Litsea glutinosa* (727.4 µg/ml), *Pentapanax leschenaultii* (735.4 µg/ml), *Curcuma angustifolia* (735.4 µg/ml), *Bergenia ciliata* (744.8 µg/ml), and *Costusspeciosus* (833.6µg/ml).

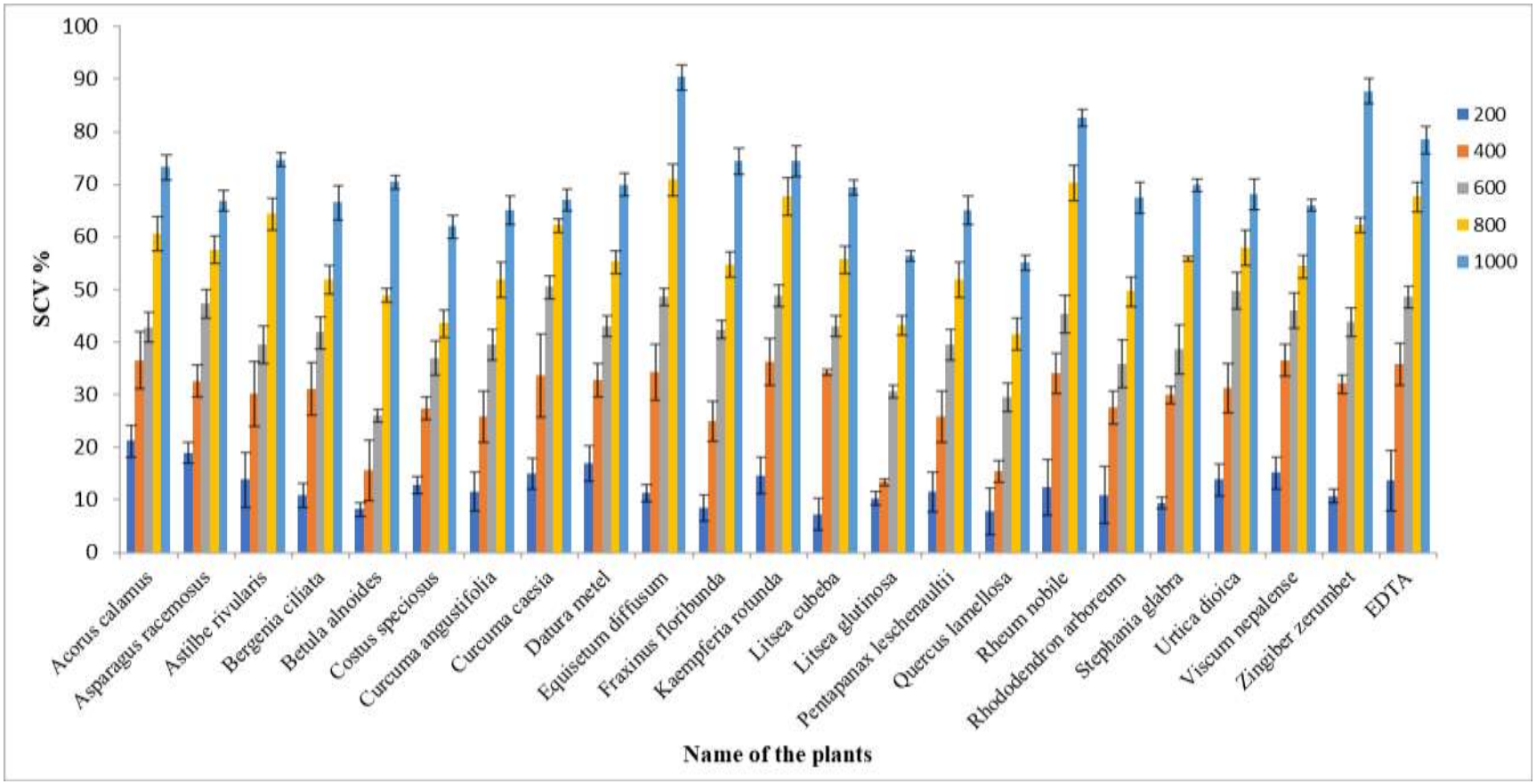

Figure 11: Ferrous chelating free radical scavenging activity of selected medicinal plants in comparison with standard EDTA. Values are expressed as mean value±standard deviation (n=3) and the concentration of the extract is provided in terms of µg/ml.

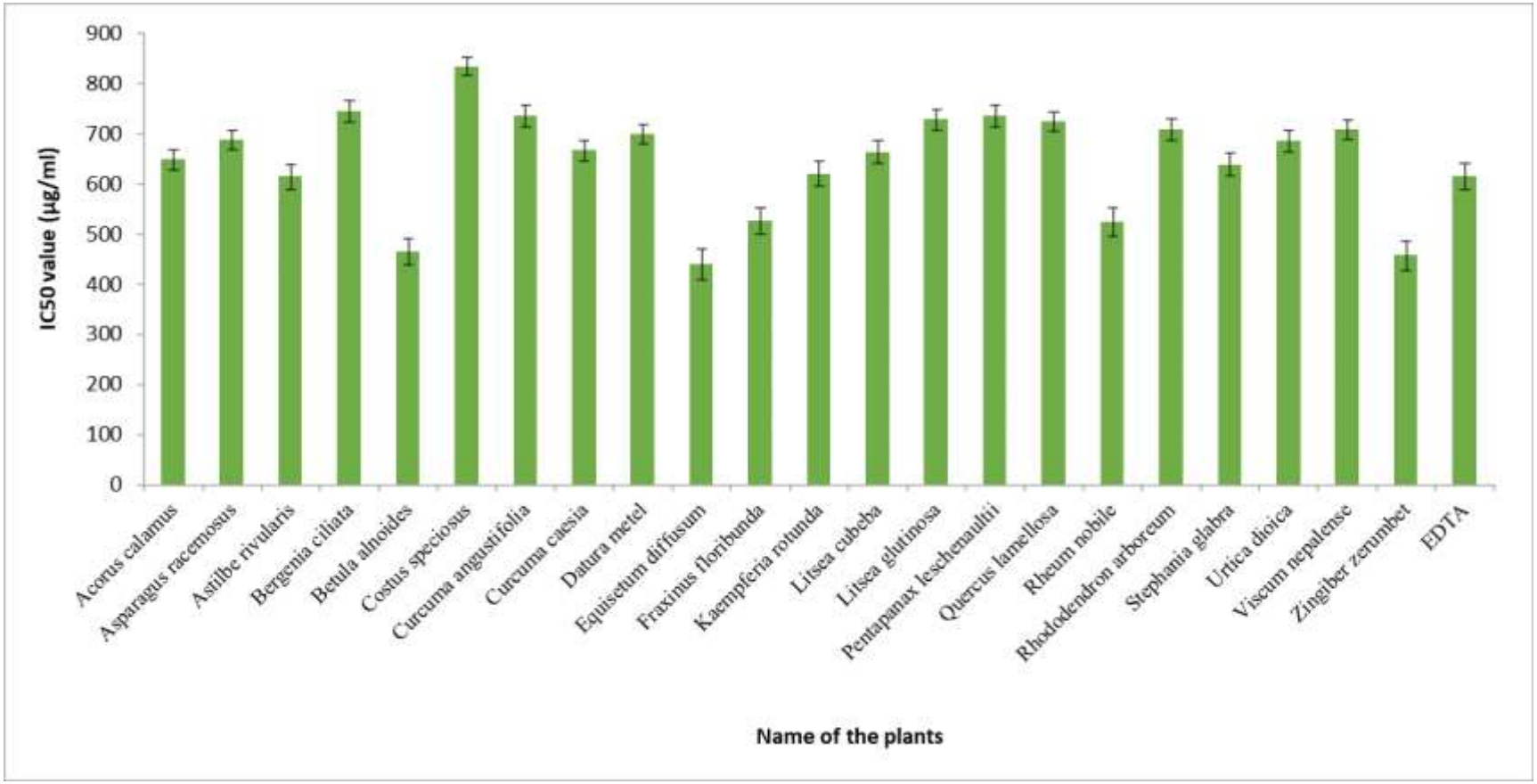

Figure 12: Ferrous chelating assay of selected medicinal plants and EDTA with their respective IC_{50} value. IC_{50} values are expressed as mean ± S.D.

c) *Ferric ion reducing assay*

In this assay, there is a conversion of complex ferricyanide to ferrous form due to the reducing capability of the plant sample. The formation of Pearl's Prussian blue color was estimated at 700 nm by using a UV-visible spectrophotometer, to determine the concentration of ferrous ions. Rising absorbance of the solution indicated the increased reducing activity of the plant extracts. The dose-dependent curves of all plants are given in **Figure 13.** It showed that the reducing ability of all the plants increased with the increase in their concentration. At the maximum concentration of 1000 µg/ml, the highest reducing activity was shown by *Stephania glabra* with absorbance value 3.162, *Litsea cubeba* (3.134), *Zingiber zerumbet* (3.097), *Astilbe rivularis* (3.04), *Quercus lamellosa* (2.94), and *Rheum nobile* (2.92) which were found to be more effective in comparison to standard ascorbic acid with absorbance value 2.869, followed by *Rhododendron arboreum* (2.859), *Curcuma caesia* (2.796), *Betula alnoides* (2.67), *Viscum nepalense* (2.63), *Fraxinus floribunda* (2.521), *Litsea glutinosa* (2.47), *Bergenia ciliata* (2.21), *Datura metel* (1.923), *Urtica dioica* (1.919), *Pentapanax leschenaultii* (1.87), *Curcuma angustifolia* (1.845), *Costus speciosus* (1.783), *Acorus calamus* (1.682), *Asparagus racemosus* (1.615), *Kaempferia rotunda* (1.42), and *Equisetum diffusum* (1.28). Absorbance value showed that the selected 22 plant samples possess the potent reducing ability in comparison with a standard (ascorbic acid).

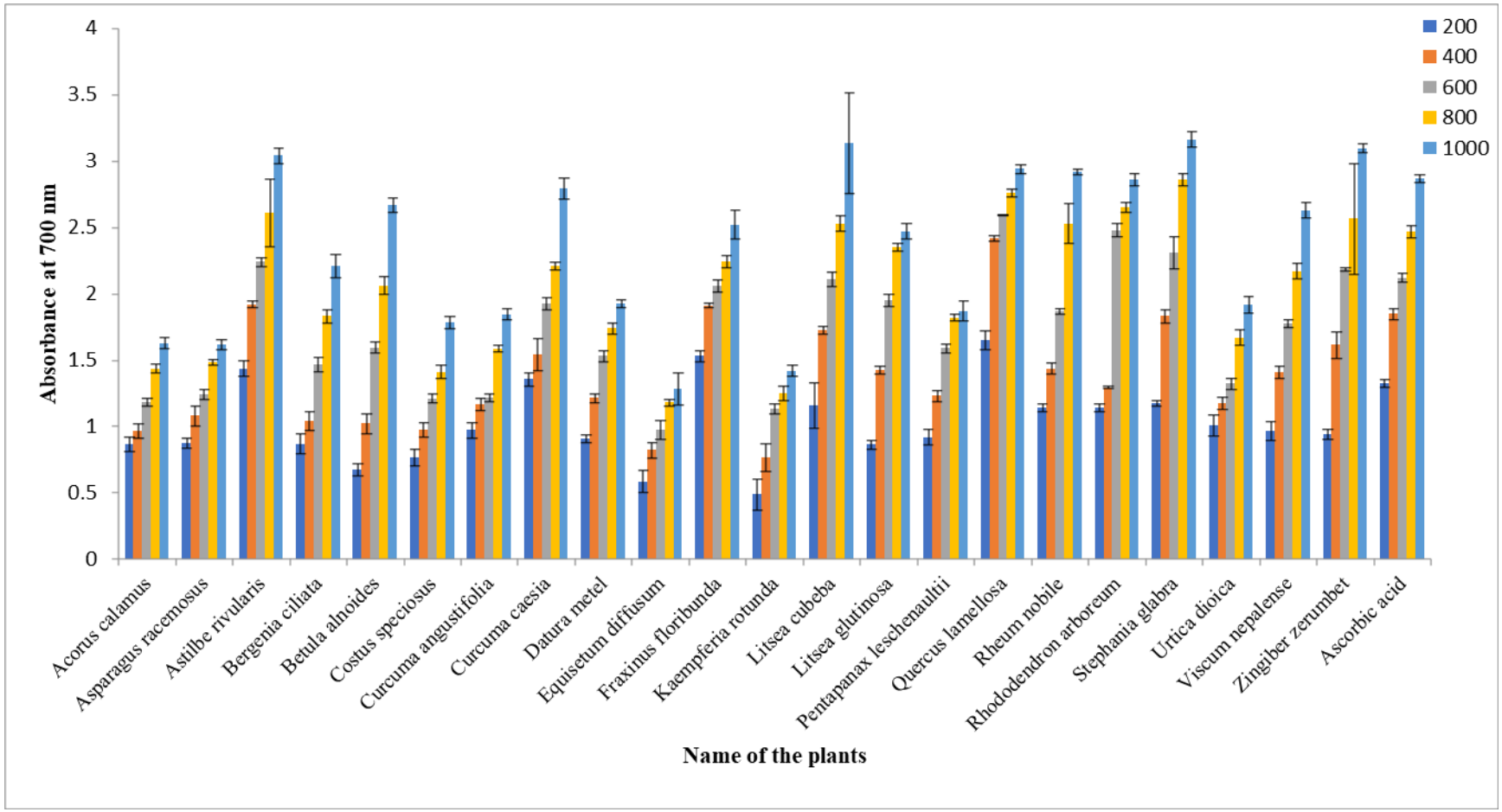

Figure 13: Ferric ion reducing ability of selected medicinal plants in comparison with standard Ascorbic acid. Values are expressed as mean value±standard deviation (n=3) and the concentration of the extract is provided in terms of µg/ml.

5.4. DISCUSSION

Plants are significant sources as antioxidants, anti-inflammatory, and antiarthritic medium (Zubair *et al*., 2012; Atawodi *et al*., 2013). Free radicals are the main mediators that stimulate the inflammation process, and the antioxidants can neutralize such free radicals, which will lead to a decrease in the effects of inflammation (Conforti *et al*., 2008). The antioxidant activity of a plant depends on the reaction system and is strongly associated with the composite nature of phytochemicals (Zou *et al*., 2011). The pharmacological potential of therapeutic plant extracts is endowed with the content of various phytochemicals, including flavonoids, terpenoids, phenols, flavonols, tannins, proanthocyanidins, vitamins, and nitrogenous compounds (Saeed *et al*., 2012; Dzoyem *et al*., 2014). Phytochemical estimation of medicinal plants in terms of qualitative as well as quantitative is regarded as an important step in the field related to medicinal plant research (Kokate, 1994). Phenols are the most significant phytoconstituents in plants with many health benefits and it shows the correlation with antioxidant property due to the scavenging activity of phenolic hydroxyl groups (Vinson *et al*., 2001). Phenols are found to be efficient hydrogen donors with strong antioxidant potency (Yen *et al*., 1993). Therefore, it is logical to determine the phenols (qualitatively as well as quantitatively) in the selected plant species. Studies were performed to evaluate the association of total phenols of the plants and their antioxidant activity, in which some authors found the association between total phenols content and antioxidant properties (Javanmardi *et al*., 2003; Paixao *et al*., 2007; Biglari *et al*., 2008), while others have found no association between them (Kähkönen *et al*., 1999; Ismail *et al*., 2004; Terpinc *et al*., 2012). From this study, it can be explained that the results do not show any correlation between the total phenolic content and the antioxidant activity. Moreover, the extracts with higher

antioxidant activity did not contain high phenolics, suggesting that the types of phenolics are more essential than their quantity for certain activities. The total phenol content of the selected ethnomedicinal plants ranged from the methanolic extract of *Litsea cubeba* (71.72±0.022 mgGAE/g dry extract wt.) to aqueous extract of *Kaempferia rotunda* (9.08±0.021mgGAE/g dry extract wt.). The polyphenols mainly flavonoids are assorted into different groups (flavonols, flavanones, flavones, catechins, chalcones, and anthocyanidins) based on the chemical structures and pharmacological properties, which are associated with their functional groups. Flavonoids act as an antioxidant by scavenging oxidizing species like hydroxyl radical, superoxide anion, and quenchers of singlet oxygen (Ratty & Das, 1988). Moreover, flavonoid exerts protection to the cell structures through numerous mechanisms by producing a high amount of glutathione, which is a strong antioxidant as studied by researchers (O'Byrne *et al*., 2002). Among the selected plant samples, there was variation in the total content of flavonoids ranging from methanolic extract of *Zingiber zerumbet* (83.5±0.022 dry extract wt.) to aqueous extract of *Litsea cubeba* (3.17±0.01mgRE/g dry extract wt). Similarly, total flavonol content also varied ranging from methanolic extract of *Equisetum diffusum* (92±0.038mgRE/g dry extract wt.) to the aqueous extract of *Datura metel* (22.9±0.003mgRE/g dry extract wt.). Some of the recent studies suggested that flavonoids and associated polyphenols are responsible for anti-inflammatory and antioxidant properties (Luo *et al*., 2002; Okoli & Akah, 2004; Figueirinha *et al*., 2010; Diaz *et al*., 2012). Foodstuffs rich in tannins have numerous health benefits including immunomodulatory, antioxidant, anticancer, radical scavenging, cardioprotective, anti-inflammatory, vaso-dilating, UV-protective, and antithrombotic properties (Dixon *et al*., 2005; Sharma *et al*., 2007). Among the chosen plant species, the results showed the variation in total tannin content, ranging

from methanolic extract of *Curcuma caesia* (72.3±0.026mgGAE/g dry extract wt.) to the aqueous extract of *Pentapanax leschenaultii* (16.29±0.006mgGAE/g dry extract wt.). From this study, it may be generalized that methanol is the most efficient solvent for plant extraction, which contain the highest amount of Phytoconstituents as compared to acetone and aqueous solvent **(Table 7)**. Recently, numerous works have been done in the exploration of natural antioxidants from medicinal plants having antioxidant as well as anti-inflammatory properties used for scavenging oxidizing species (De las Heras *et al*., 1998; Desmarchelier *et al*., 2000; Schinella *et al*., 2002; VanderJagt *et al*., 2002). There are various phytochemicals derived from medicinal plants that have significant antioxidant and therapeutic properties, such as alkaloids, terpenoids, flavonoids, phenolic compounds, lignans, tannins, quinones, coumarins, etc. (Kaur *et al*., 2011). Findings of the present study revealed that in three different scavenging assays, all selected plants possess potential antioxidant activities, which were found to increase with increasing concentrations **(Figure 9, 11, 13)**.

5.5. CONCLUSION

In this study, 22 plants selected on the recommendation of the informants during the fieldwork treating rheumatoid arthritis showed the existence of numerous phytochemicals (Alkaloids, flavonoids, phenols, terpenoids, tannins, anthraquinones, etc.) The quantitative analysis of four phytochemicals namely phenols, tannins, flavonols, and flavonoids was performed. These plants also showed the potential antioxidant activities in three different scavenging assays which were found to increase with increasing concentrations. This may be due to the existence of various phytochemicals (phenols, tannins, terpenoids, flavonoids, etc.) and these results are in consonance with earlier findings and confirm the medicinal significance of selected plant species.

Chapter 6

In-vitro anti-RA activity

6.1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease that leads to progressive destruction of multiple synovial joints (Guo *et al*., 2018). As per World Health Organization, 0.3-1% of the total populace is affected by RA and among them, females are more progressively inclined to the ailment when compared with males (Tripathy *et al*., 2010). The prime symptoms of RA comprise soreness, swelling, and destruction of ligament and bone because of which perpetual inability happens. Despite specific etiology being obscure, it is mentioned that it is activated by the combination of hereditary inclinations as well as exposure to ecological variables including viruses (Babushetty & Sultanpur, 2012). The specific pathophysiology is as yet obscure but the liberation of free radicals such as superoxide and nitrous oxide radicals may activate the development of tumor necrosis factor and interleukins from T-cells, which eventually influence the development of growth factors, adhesive molecules, and cytokines on immune cells as factors that cause inflammation and damage of tissues (Kasper & Harrison, 2005). Pathological modifications in RA are penetration of inflammatory cells, hyperplasia of synovial layer, and neovascularization, which lead to the erosion of cartilage and articular demolition (Chunxia *et al*., 2011).

The aim of the treatment of RA patients is to reduce symptoms, slow down disease development, and upgrade the quality of life (Ngoc *et al*., 2005). In this manner, before beginning the treatment of RA certain objectives must be considered, for example, alleviation of pain, a decrease of inflammation, defense of articular

composition, protection of function, and control of general involvement (Kasper & Harrison, 2005). Currently, for the management of RA, approaches have changed from usually used Non-steroidal anti-inflammatory drugs (NSAIDs) or Disease-modifying anti-rheumatic drugs (DMARDs) to new biological agents including TNF (tumor necrosis factor) monoclonal antibody (Choudhary *et al*., 2015). Clinically, RA treatments include five methods: the premier one is the utilization of NSAIDs followed by low doses of glucocorticoids to limit the signs of inflammation along with disease progression; DMARDs including sulfasalazine, methotrexate, gold salts, and D-penicillamine are used in the management of chronic patients; while TNF-α counterbalance agents such as etanercept, infliximab, etc. are used in particular cases; anakinra is used to neutralize IL-1; and medications that obstruct the T-cell initiation, such as abatacept can also used to treat chronic cases; eventually, immunosuppressive and cytotoxic medications including azathioprine, cyclosporine, and cyclophosphamide are utilized to treat chronic patients (Kasper & Harrison, 2005; Rajkapoor *et al*., 2007; Mazumder *et al*., 2012). The aforementioned therapeutic approaches minimize the inflammation and joint damage. However, their long-standing risks are as yet obscure. Nonetheless, the long-term risk of medication consists of complications in the cardiovascular system, gastrointestinal ulcers, hematologic toxicity, pulmonary toxicity, nephrotoxicity, myelosuppression, stomatitis, cirrhosis, hepatic fibrosis, immune reactions, diarrhea, and narrow injection-site reactions. Furthermore, greater expenses and side effects which consist of high risks of infections along with malignancies required continuous monitoring (Chitme & Patel, 2009).

Herbal remedy for the management of RA: Herbal remedies are being utilized for the treatment or healing of numerous ailments since prehistoric periods and it's not an

overstatement to say that the utilization of herbal medications is as old as humanity (Tandon & Gupta, 2004). Herbal medicines are adapted from the age-old healing skill of working physicians of traditional medicine systems (Vispute & Khopade, 2011). Nowadays, in light of the fact that the presently accessible medications either contain certain side effects or are exceptionally costly, scientists show a great concern for those therapeutic agents that are derivative of plants (Badami *et al*., 2004). As a source for healing agents for the prevention and healing of various ailments, nature has favored us with an immense abundance of medicinal plants that are widely distributed throughout the world (Kalaria *et al*., 2012). Almost 80% of the world's population living in the developing world uses herbal medicines to fulfill their basic health needs (Ekor, 2014). Herbal medications can work as packages of human culture to combat sickness from the beginning of civilization (Sudha & Mathanghi, 2012). Phytoconstituents presence in significant parts of these therapeutic herbal plants is responsible for their desired therapeutic activity in the human body (Singh *et al*., 2011a).

Since ancient times, India is using herbal medicines of alternative and conventional systems in the form of Ayurveda, Sidha, Unani, Naturopathy, and Homeopathy (Kiran *et al*., 2011). There are about 2500 species of plant which are presently utilized as herbal remedies and are utilized either directly in the form of folk medicine or indirectly in form of modern pharmaceuticals for more than 3000 years (Thirumal *et al*., 2012). Therefore, from the information on traditional plants, one may be able to find novel effective, and less expensive medications (Kalita *et al*., 2012). Till date, numerous plants have been appeared to acquire considerable anti-RA activity thus providing potential means in developing new medicines with the least side effects. Various plants of traditional medicines and their products have been used in the

prevention of RA disease and studies are done to scientifically validate the potency of plants exhibiting anti-RA activity (Agarwal & Rangari, 2003; Amresh *et al*., 2007; Kikuchi *et al*., 2009; Gupta *et al*., 2009; Bhalerao *et al*., 2011; Chippada & Vangalapati, 2011; Fan *et al*., 2012; Endale *et al*., 2013; Farinon *et al*., 2013; Bhangale & Acharya, 2014; Choudhary *et al*., 2015; Sharma *et al*., 2018). This chapter lists the *in vitro* RA activity of 11 ethnomedicinal plants used by the ethnic people of Sikkim Himalaya.

6.2. MATERIALS AND METHODS

6.2.1. *Selection of plants for the determination of in-vitro anti-RA activity*

Selection of plants for *in-vitro* anti-RA activity was done based on the RFC value that is the number of recommendations by the informant for a particular plant species. A total of 11 plants having high RFC value, phytochemical contents, and antioxidant activity was further selected for the *in-vitro* anti-RA activity. Methanolic extract of the selected plants was used to determine the anti-RA activity by protein denaturation method and HRBC membrane stabilization method.

6.2.1.1. *HRBC membrane stabilization method*

In the analysis of the anti-inflammatory activity of the methanolic extract of selected plant species, the HRBC membrane stabilization method was used. Several studies reported the association between inflammation and RA (Del Rincón *et al*., 2003; Rho *et al*., 2009; Kokkonen *et al*., 2010; Ricciotti & FitzGerald, 2011; Hughes-Austin *et al*., 2013). The therapy of RA involves a wide range of agents that exert anti-inflammatory activity such as *NSAIDs*, *Glucocorticoids*, etc. Thus, in the present

investigation anti-RA activity of selected plants was evaluated through anti-inflammatory activity.

***Preparation of stock solution*:**

The extract solution of the plant sample was prepared by adding 80mg methanolic plant extract in 1ml distilled water to make the concentration of 80mg/ml and the solution was further diluted to the concentration of 10mg/ml. Similarly, a stock solution of the standard drug (diclofenac sodium) was also prepared. Then this stock solution of plant sample, as well as drug, was used to make appropriate concentrations with specific dilution for an *in-vitro* experiment.

Collection of blood samples:

5 ml of fresh blood was collected intravenously from the author of this work taking utmost care where NSAIDs have not been taken for 15 days. The blood was collected in the test tube under the standard laboratory condition and coagulation of the blood was prevented by using an equal volume of Alsever's solution.

Preparation of erythrocyte suspension:

5ml of collected blood sample was transferred to the centrifuge tube containing anti-coagulant Alsevers's solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%) and PBS (Phosphate buffer saline). After that, the solution is centrifuged for about 10 minutes at 3000 rpm, and then the supernatant was expelled. After that normal saline which is equal volume to the supernatant was used to dissolve the RBC pellets washed three times and the excess normal saline was discarded after wash. The amount of dissolved RBC pellets was estimated and recreated as 10% (v/v)

suspension along with isotonic buffer solution (pH=7.2). This reconstituted RBC will be the stock suspension and will be used for the experiment.

***Hypotonicity Induced Hemolysis*:**

For this test, the standard protocol of Manvar & Desai (2014) was followed with some modifications. The different concentration gradients of methanolic extract of both plant and standard (diclofenac sodium) drug were prepared (1000-5000 µg/ml) in distilled water. Reaction solution 4.5 ml containing 2 ml of hyposaline, 1 ml of phosphate buffer (pH 7.4), 1 ml of plant extract and standard of various concentrations, and 0.5 ml of 10% HRBC suspension. Similarly, control was also prepared by replacing the extract with distilled water. Subsequently, the reaction blends were incubated at 37 ℃ for 30 minutes furthermore, centrifuged at 2500 rpm in a room temperature for 5 minutes. The supernatant was accumulated carefully and the content of haemoglobin in the supernatant was estimated at 560 nm by using a spectrophotometer. The haemolysis percentage in control was assumed as 100% and the percentage of protection was calculated by the following formula:

Inhibition %= $100-[(OD_1/OD_2) \times 100]$

Where OD_1 is the Optical density of the test sample, OD_2 is the Optical density of the control sample.

6.2.1.2. *Protein denaturation method*

Protein denaturation is one of the factors causing rheumatoid arthritis and autoantigen production in certain arthritic diseases (Mizushima, 1966; Brown & Mackey, 1968; Singh *et al*., 2011b; Arya & Patni, 2013; Elisha *et al.*, 2016). Therefore, *in vitro* anti-

RA activity of the plant extract was examined through the protein denaturation method by using the method of Williams *et al* (2008) with minor modifications. Plant extracts of different concentrations varying from 1000-5000 µg/ml were prepared in methanol. 0.5 ml of each solution of distinct concentrations (1000, 2000, 3000, 4000, and 5000 µg/ml) of methanol extract was transferred to Eppendorf tube with the help of micropipette followed by the addition of 5ml 0.2% BSA solution (bovine serum albumin in Tris-buffer saline, pH=6.8). Similarly, a standard solution of Diclofenac sodium in a different concentration ranging from 1000-5000 µg/ml was also prepared. Since this drug is usually used for treating inflammatory diseases. A mixture of 0.5 ml of methanol and 5 ml 0.2% BSA were used as a control. Then the solution mixtures were incubated at 37ºC for 20 minutes and then at 72ºC for 5 minutes. Finally, the sample extract, standard, and control solutions were cooled for 5 minutes, and then the absorbance was estimated at 660 nm by utilizing a UV-visible spectrophotometer. The percentage inhibition was calculated by utilizing the formula:

% inhibition= [(Absorbance of blank – absorbance of sample)]/ (absorbance of blank) x 100

6.2.2. *Statistical analysis*

All measurements were made in triplicate and the results obtained were expressed as mean ± standard deviation (SD). Statistical analysis for *in-vitro* RA activity was done by using IBM SPSS version 23 (IBM Crop.), t-test was performed to compare the mean difference. P-value ≤0.05 was considered significant.

6.3. RESULTS

6.3.1. *In-vitro* anti-Rheumatoid arthritis activity

The *in-vitro* anti-RA activity of selected medicinal plants in the methanolic extract was determined by using two methods i.e. HRBC membrane stabilization and inhibition of protein denaturation methods.

6.3.1.1. *HRBC Membrane stabilization*

The results of HRBC membrane stabilization are shown in **Table 8** & **Figure 14**. It showed the significant stabilization towards the HRBC membrane which was found to be increased with an increasing concentration. The maximum percentage of stabilization was exhibited at the concentration of 5000µg/ml by all the selected plants such as *Astilbe rivularis* (85.73±0.345), *Bergenia ciliata* (92.29±0.053), *Betula alnoides* (68.46±0.244), *Equisetum diffusum* (77.79±0.138), *Fraxinus floribunda* (73.96±0.203), *Litsea cubeba* (96.80±0.045), *Litsea glutinosa* (87.41±0.036), *Quercus lamellosa* (80.15±0.032), *Rheum nobile* (73.11±0.062), *Stephania glabra* (84.58±0.259), and *Viscum nepalense* (72.29±0.380) which were considered to be effective and as good as standard drug diclofenac (77.98±0.082). Unlike the other plant samples anti-RA activity of *L. cubeba* was significantly different (p=0.004, $P<0.05$) which indicates that the activity of plant extract is more potent or effective than the standard drug.

Table 8: *In-vitro* anti-RA activity of selected medicinal plants in comparison with standard drug (Diclofenac sodium) by HRBC membrane stabilization method.

Name of the plants	Protection % in different concentration (µg/ml)					P-value
	1000	2000	3000	4000	5000	
Astilbe rivularis	56.87±0.182	68.17±0.270	78.64±0.300	81.95±0.329	85.73±0.345	0.532
Bergenia ciliata	58.29±0.021	61.88±0.059	73.16±0.060	83.16±0.018	92.29±0.053	0.643
Betula alnoides	49.84±0.082	54.61±0.116	58.61±0.173	62.60±0.215	68.46±0.244	0.026
Equisetum diffusum	60.12±0.056	64.58±0.084	69.57±0.083	72.92±0.117	77.79±0.138	0.743
Fraxinus floribunda	63.81±0.148	66.42±0.156	69.02±0.156	71.39±0.175	73.96±0.203	0.663
Litsea cubeba	75.92±0.039	86.75±0.101	93.57±0.042	95.59±0.044	96.80±0.045	0.004
Litsea glutinosa	52.64±0.032	65.70±0.083	77.75±0.072	82.40±0.036	87.41±0.036	0.696
Quercus lamellosa	50.32±0.080	58.88±0.050	65.53±0.042	75.49±0.052	80.15±0.032	0.496
Rheum nobile	54.87±0.088	59.37±0.035	63.24±0.060	67.87±0.038	73.11±0.062	0.150
Stephania glabra	71.12±0.296	72.03±0.325	74.58±0.229	82.44±0.270	84.58±0.259	0.132
Viscum nepalense	60.58±0.206	64.28±0.229	66.57±0.229	68.97±0.342	72.29±0.380	0.289
Diclofenac sodium	63.18±0.136	65.26±0.152	71.18±0.152	74.43±0.111	77.98±0.082	-

Values are expressed as mean ± SD, n=3 in each concentration

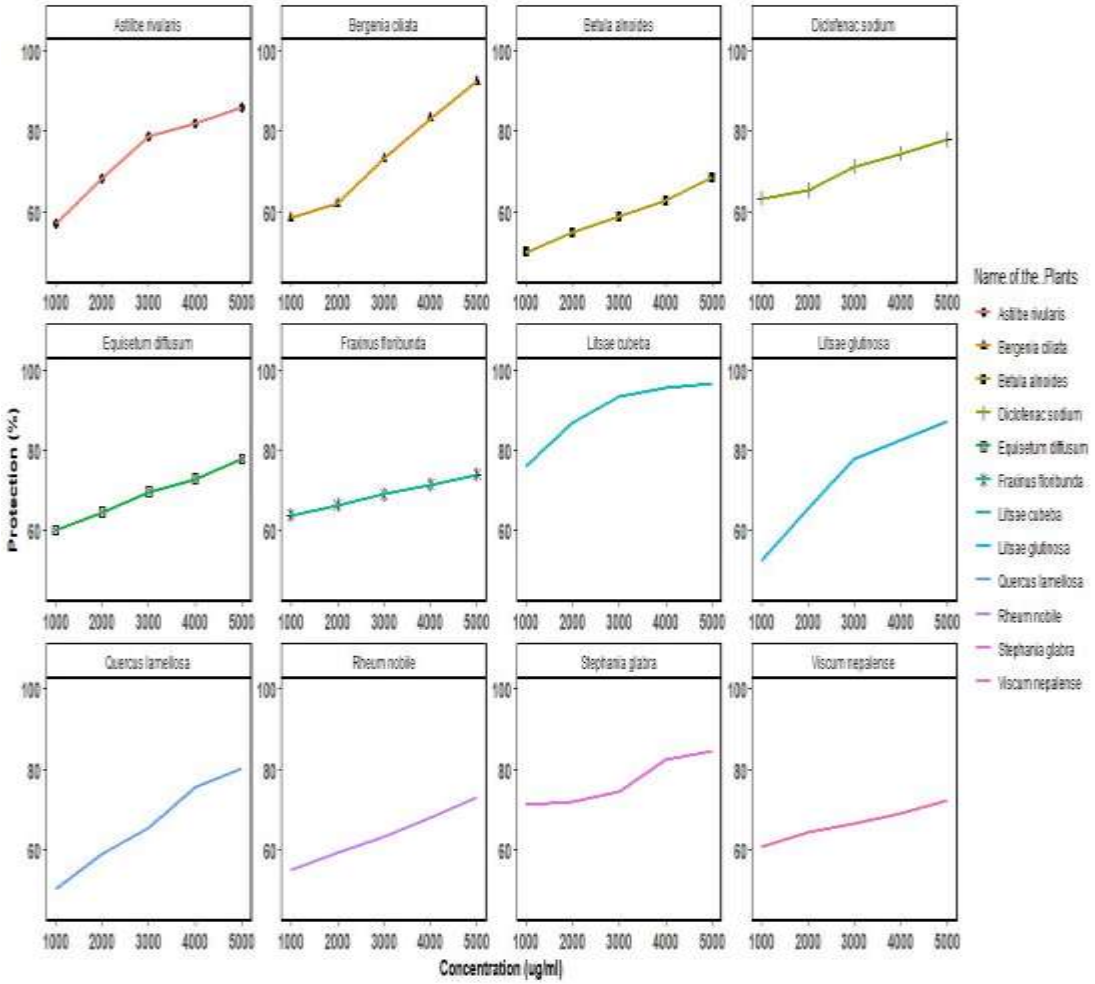

Figure 14: Graph showing *in-vitro* anti-RA activity of selected medicinal plants in comparison with standard drug (Diclofenac sodium) by HRBC membrane stabilization method.

6.3.1.2. *Protein denaturation*

The findings of anti-RA activity of selected medicinal plants in methanolic extract in comparison with Diclofenac sodium which was used as a standard drug are presented in **Table 9** & **Figure 15** and it showed an increase in inhibition property with increasing concentration. At 5000 µg/ml, the inhibition percentage of medicinal plants were *Astilbe rivularis* (73.98±0.037), *Bergenia ciliata* (70.70±0.053), *Betula alnoides* (79.05±0.037), *Equisetum diffusum* (74.14±0.048), *Fraxinus floribunda*

(63.29±0.038), *Litsea cubeba* (81.80±0.078), *Litsea glutinosa* (77.57±0.087), *Quercus lamellosa* (68.59±0.025), *Rheum nobile* (79.98±0.037), *Stephania glabra* (76.90±0.055), and *Viscum nepalense* (78.50±0.086) which were seen to be effective and as good as diclofenac sodium (83.83±0.054). The statistical analysis showed that the anti-RA activity of plants extracts was not significantly different than the activity of the drug, indicating that the activity of selected plants is comparable to the standard drug used.

Table 9: *In-vitro* anti-RA activity of selected medicinal plants in comparison with standard drug (Diclofenac sodium) by protein denaturation method.

Name of the plants	**Protection % in different concentration (µg/ml)**					**P-value**
	1000	2000	3000	4000	5000	
Astilbe rivularis	30.12±0.094	42.95±0.075	53.60±0.065	69.39±0.045	73.98±0.037	0.809
Bergenia ciliata	36.60±0.048	46.35±0.046	55.11±0.044	63.49±0.081	70.70±0.053	0.764
Betula alnoides	36.41±0.059	45.71±0.046	54.18±0.050	68.81±0.055	79.05±0.037	0.649
Equisetum diffusum	28.71±0.100	34.13±0.051	49.56±0.054	60.37±0.054	74.14±0.048	0.924
Fraxinus floribunda	35.73±0.058	44.97±0.048	47.86±0.038	52.00±0.076	63.29±0.038	0.870
Litsea cubeba	29.22±0.068	41.38±0.046	59.70±0.044	72.08±0.083	81.80±0.078	0.678
Litsea glutinosa	30.22±0.136	36.12±0.042	48.95±0.073	64.77±0.049	77.57±0.087	0.953
Quercus lamellosa	30.38±0.104	32.21±0.020	39.55±0.049	53.83±0.074	68.59±0.025	0.661
Rheum nobile	21.88±0.038	32.49±0.073	41.06±0.055	57.61±0.075	79.98±0.037	0.786
Stephania glabra	30.12±0.103	41.61±0.031	53.96±0.046	69.13±0.040	76.90±0.055	0.794
Viscum nepalense	32.27±0.128	41.09±0.093	56.40±0.049	69.61±0.107	78.50±0.086	0.728
Diclofenac sodium	25.76±0.045	34.77±0.056	45.01±0.063	64.13±0.051	83.83±0.054	-

Values are expressed as mean ± SD, n=3 in each concentration

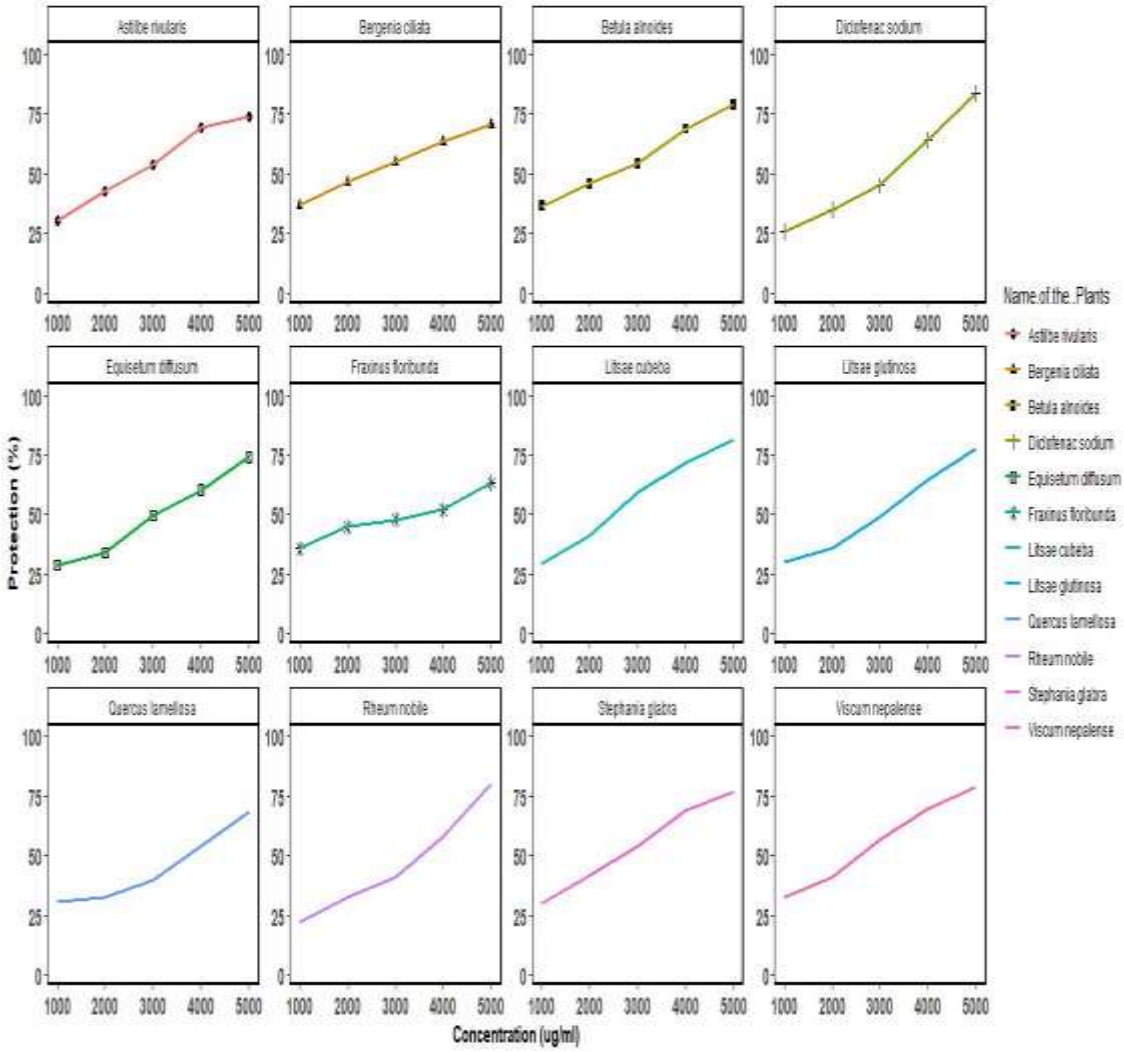

Figure 15: Graph showing *in-vitro* anti-RA activity of selected medicinal plants in comparison with standard drug (Diclofenac sodium) by protein denaturation method.

6.4. DISCUSSION

In the traditional medicinal system, various plants and their products are used for the treatment of RA and various studies have been performed to scientifically validate the potency of plants exhibiting anti-RA activity (Agarwal & Rangari, 2003; Kikuchi *et al*., 2009; Farinon *et al*., 2013; Sharma *et al*., 2018). In the present investigation, the anti-RA activity in methanolic extracts of selected ethnomedicinal plants was determined by *in-vitro* methods viz. stabilization of HRBC membrane along with inhibition of protein denaturation.

The membrane of the RBC is comparable to the membrane of the lysosome. The lysosomal degranulation is a major step in triggering an inflammatory response. Therefore, homology can be drawn that if a plant extract has a stabilizing effect on the RBC membrane it may also stabilize the lysosomal membrane thus inhibiting degranulation and limiting the inflammatory responses (Nagaharika & Rasheed, 2013; Alamgeer *et al*., 2015). Stabilizing impact on heat and saline causes erythrocyte lysis which is an excellent indicator of anti-inflammatory action along with anti-arthritic activity (Shilpa *et al*., 2018). Since RA is an inflammatory disorder, therefore the inhibition of RBC hemolysis in a hypotonic medium may provide evidence of the anti-RA effect of the plant's extract. Present results suggested that all the selected plants (*Astilbe rivularis*, *Bergenia ciliata, Betula alnoides*, *Equisetum diffusum*, *Fraxinus floribunda, Litsea cubeba*, *Litsea glutinosa*, *Quercus lamellosa, Rheum nobile, Stephania glabra,* and *Viscum nepalense*) exhibited a potent anti-RA activity on dose-dependent manner, which increased with increasing concentrations and maximum protection was provided at 5000µg/ml **(Figure 14)**. Unlike the other plant samples anti-RA activity of *Litsea cubeba* was significantly different (p=0.004, $P<0.05$), suggesting that the activity of plant extract is more effective than the standard drug **(Table 8)**.

The protein denaturation process was employed for investigating the anti-RA activity of selected medicinal plant species by utilizing bovine serum albumin. When heat is given to bovine serum albumin it goes through denaturation and expresses the type-III hypersensitivity response antigens which are related to the ailments such as serum sickness, systemic lupus erythematosus, rheumatoid arthritis, and glomerulonephritis (Agarwal & Shanmugam, 2019). This protein denaturation is likely to be related to the breakdown of hydrogen, electrostatic, disulphide, and hydrophobic bond of protein

structures (Arya *et al*., 2014) and is very well recorded as a factor for inflammatory conditions in RA, cancer, and diabetes (Shilpa *et al*., 2018). Protein denaturation is one of the factors causing RA and autoantigen production in arthritic diseases (Mizushima, 1966; Brown & Mackey, 1968; Singh *et al*., 2011b; Arya & Patni, 2013; Elisha *et al*., 2016). Therefore, the plants extract with inhibition property of protein denaturation may be helpful in preventing the development of RA. In the present analysis, the inhibition property of protein denaturation was exhibited by all the selected plants and may help in preventing the development of RA. Present results suggested that selected plants (*Astilbe rivularis*, *Bergenia ciliata*, *Betula alnoides*, *Equisetum diffusum*, *Fraxinus floribunda*, *Litsea cubeba*, *Litsea glutinosa*, *Quercus lamellosa*, *Rheum nobile*, *Stephania glabra*, and *Viscum nepalense*) were capable of controlling the development of autoantigen as well as inhibits the protein denaturation in arthritic condition. All selected plants possess the anti-RA activity in a dose-dependent way **(Figure 15)** and the t-test revealed that the anti-RA activity of selected plant extracts was not significantly different from that of the standard drug **(Table 9)**. Indicating that the activity of selected plant species was comparable to that of standard drug utilized. The results are suggestive of the potential of selected plants in regulating the production of autoantigen and inhibiting the protein denaturation in RA disease.

The anti-RA activity of plants extract was possibly due to the occurrence of diverse phytochemical constituents (Phenol, terpenoids, flavonoids, flavonols, tannin, etc). Phenolic compounds work in a comparable way as NSAIDs do, and some of them inhibit other pro-inflammatory mediators by inhibiting their activity (Ambriz-Pérez *et al*., 2016). The flavonoids smoothly inhibit free radical production and prevent the development of pro-inflammatory mediators (Pan *et al*., 2010). Plants containing

flavonoid or polyphenol are a good choice to use as anti-RA treatment which is based on their three properties: antioxidant, anti-apoptotic, and anti-inflammatory (Sung *et al*., 2019). Terpene can be effective in the treatment of chronic pain due to inflammatory causes and can adjust the immune system and destructive tissues that cause the clinical appearance and development of arthritis (Carvalho *et al*., 2019).

6.5. CONCLUSION

One of the benefits of anti-inflammatory activity is the avoidance of lysosomal membrane lysis. In the analysis, all the selected plants (*Astilbe rivularis*, *Bergenia ciliata, Betula alnoides*, *Equisetum diffusum, Fraxinus floribunda, Litsea cubeba*, *Litsea glutinosa, Quercus lamellosa*, *Rheum nobile, Stephania glabra,* and *Viscum nepalense*) extract showed a protective effect against RBC membrane lysis in different concentrations caused by heat and hypotonicity. It can therefore be concluded that the plant extract can also stabilize the lysosomal membrane, which is comparable to the RBC membrane, and thereby cause anti-inflammatory effects. One of the primary causes of RA is the formation of autoantigens due to the denaturation of protein. In the method of protein denaturation inhibition, *Astilbe rivularis, Bergenia ciliata, Betula alnoides, Equisetum diffusum, Fraxinus floribunda, Litsea cubeba, Litsea glutinosa, Quercus lamellosa, Rheum nobile, Stephania glabra,* and *Viscum nepalense* extracts displayed concentration-dependent protein denaturation inhibition in the low to high concentration range. From the results, it can be inferred that by preventing protein denaturation, the selected plant's extract can regulate the development of autoantigens. The finding of *in vitro* model suggested that the selected plants have potent anti-RA potential.

Chapter 7

GC-MS analysis of bioactive phytoconstituents

7.1. INTRODUCTION

Phenols are a diverse group of compounds generated by plants' secondary metabolism. Phenolic compounds are aromatic or aliphatic compounds with at least one aromatic ring and one or more hydroxyl groups. Flavonoids and non-flavonoids are two types of phenolic compounds (Bravo, 1998). In flavonoids, an oxygen heterocycle connects two aromatic rings. They may be categorized as flavonols, flavones, isoflavones, anthocyanins, flavanones, and so on, depending on the degree of hydrogenation and the heterocycle substitution. In nature, glycosides are the most common form of flavonoids. The two key representative compounds of the non-flavonoid group, also known as phenolic acids, are benzoic and cinnamic acid. Lignins, tannins, and stilbenes are a few examples of phenolic acids. Phenolic-rich foods are reported to be beneficial for the well-being of people suffering from chronic diseases such as cardiovascular disease, diabetes, Parkinson's disease, Alzheimer's disease, and inflammation-related diseases (Vo *et al*., 2017). Therefore, for the benefit of human health, phenolic compounds have recently increased the interest of scientists and the food industry. Many phenolic compounds affect molecular and cellular signaling processes in addition to their antioxidant properties. Polyphenols can suppress the expression of many pro-inflammatory cytokines thus polyphenols can be considered anti-inflammatory in nature (Christman & Gu, 2020). Traditional medicines including plants with high phenolics are used for the treatment of inflammation-related diseases which have minimal side effects as compared to

conventional medicine (Ambriz-Pérez *et al*., 2016). Food with high phenolic content reduces the oxidative stress in the body which ultimately decreases inflammation (Garg *et al*., 2007; Al-Okbi, 2014). Dietary polyphenolic compounds have been extensively investigated *in vitro* and *in vivo* for their effects on rheumatoid arthritis (Yoon *et al*., 2013; Pašková *et al*., 2016; Neog *et al*., 2017; Sung *et al*., 2019; Christman & Gu, 2020). So, it must be of great importance to study the phenolic constituents in plants that are documented for treating RA. For the purpose of separation and determination of phenolic compounds, one of the most efficient analytical methods is Gas chromatography-Mass spectrometry (GC-MS).

GC-MS is a system that homogenizes the attributes of gas-liquid chromatography along with mass spectrometry for the identification of different components within the test samples. However, its primary area of utilization is the examination and separation of multi-constituent mixtures like essential oils, solvents 1-3, and hydrocarbons. Basically, with the utilization of flame ionization indicator and electron detain locator (which has high sensitivities) GC-MS can quantitatively decide the materials present at extremely low concentration. The second most significant application areas of GC-MS are in forensic work, pollution analysis, and general trace investigation. GC-MS is one of the most important methods in chemistry because of its effortlessness, affectability, and adequacy in isolating components of mixtures. It is generally utilized for quantitative examination of mixture along with qualitative analysis, compounds purification, and analysis of such thermo-element constants like heat of mixture with vaporization, and activity coefficients (Vyas, 1999; Milne & Beamish, 1999; Kaushik *et al*., 2002; Lal & Verma, 2006; De Fátima *et al*., 2006; Marston, 2007).

GC-MS has been recognized as an essential technological area for profiling and isolating secondary metabolites in both plants and non-plants materials (Lytovchenko *et al*., 2009; Kanthal *et al*., 2014; Fatima *et al*., 2019). Information on the chemical components of plants is not only desirable for finding of agents with therapeutic potential but it also enlightens the users with new knowledge that might be of extraordinary incentive in revealing a new basis of phytoconstituents. This may help further to add economic value for the production of complex chemical materials and to find out the actual importance of folkloric remedies (Al-Rubaye *et al*., 2017). Plants are considered as the basis of bioactive compounds that play a leading role in the protection of human health. Information accessible on plants indicates a reservoir of efficient chemotherapeutics which are non-phytotoxic, easily biodegradable, and more systemic (Sermakkani & Thangapandian, 2012; Yamuna *et al*., 2017; Hadi & Hameed, 2017). Consequently, an intensive validation of herbal remedies has risen as a novel science that emphasizes and prioritizes the standardization of herbal drugs and natural products due to the fact that numerous phytochemicals have complimentary as well as overlapping systems of action. Lately, GC-MS analysis has been progressively applied for the investigation of therapeutic plants, as this method has proved for being a significant technique for the investigation of non-polar constituents, essential volatile oil, lipids, fatty acids, and alkaloids (Al-Tameme *et al*., 2015; Sosa *et al*., 2016). The present study has been designed to explore the possible bioactive phytoconstituents from selected medicinal plants which are being used for the treatment of rheumatoid arthritis by ethnic people of Sikkim Himalayan region.

7.2. MATERIALS AND METHODS

Selection of plants

11 medicinal plants selected for the study of anti-RA activity through the *in-vitro* protein denaturation method and HRBC membrane stabilization method were further selected for GC-MS study.

GC-MS analysis

The GC-MS analysis of selected plant samples was performed at Aakaar Biotechnologies Private Limited by utilizing Shimadzu QP-2010 Plus with Thermal Desorption System TD 20.

Derivatization of plant extracts: Plant extracts were derivatized by taking 100 µg of concentrated plant sample in a separating funnel and shaken by adding 10 ml of water and ethyl acetate in the proportion of 1:4, and then the upper layer was collected and concentrated to 1ml in the rotary evaporator. Concentrated samples were further diluted by adding 50 µl of BSTFA+TMCS (N, O-Bis (trimethylsilyl) trifluoroacetamide and trimethylchlorosilane) followed by the addition of 10 µl of pyridine. Samples were further heated at 60ºC for 30 minutes and after that samples were transferred in GC vial and dried using nitrogen gas and finally samples were dissolved in methanol before GC-MS analysis. After that derivatized samples were exposed to GC-MS investigation.

GC condition: The injector temperature was put at 260℃, the column oven temperature was put at 60℃ with an increasing rate of 3℃/min to 300℃ (18 min) and with a flow rate of 1.21ml/min. The split ratio used was 10.0.

***MS condition*:** For MS, the ion source temperature was set at 230℃ and the detection voltage at +0.00 kV. The interface temperature was 270℃ with its solvent cut-off time of 3.50 minutes. The start time was set at 6.0 min and the end time was at 50.07 min.

Identification of Phytocompounds: The compounds were identified based on their retention time and mass spectra by comparing with the mass spectra of standard compounds from the National Institute of Standards and Technologies Library (NIST, 2008).

7.3. RESULTS

The results regarding the GC-MS investigation of 11 selected medicinal plants in methanol extract as per their retention time and peak area are enumerated below in **Table 10-20**. GC-MS chromatograms of methanolic extract of selected plants are given in **Figures 16-26**. It showed the existence of numerous phytoconstituents which were identified by mass spectrometry along with GC. The GC-MS spectra of all selected plants confirmed the presence of numerous phytoconstituents like essential oils, fatty acids, esters, alcohols, phenols, alkanes, steroids, and terpenes. However, the phenolic constituents viz. Phenol, 2,5-bis(1,1-dimethyl ethyl); *2,6*-Di-tert-butyl-*4-(*dimethylamino methyl)*phenol*; 3-Cyclohexene-1-Methanol, .alpha.,.alpha.,4; 1-naphthol and Phenol, 3,5-bis(1,1-dimethyl ethyl) were present in the plants namely, *Fraxinus floribunda, Litsea cubeba, Litsea glutinosa, Rheum nobile, Viscum nepalense, Astilbe rivularis, Bergenia ciliata, Betula alnoides,* and *Equisetum* diffusum. Moreover, other important phytoconstituents present in the selected plants were beta-sitosterol, stigmasterol, alpha-bisabolol, germacrene, beta-caryophyllene, chamazulene, eicosane, squalene, phytol, hexadecanoic acid methyl ester, tetradecanoic acid, 9-

octadecanoic acid, nondecanoic acid, betulin, alpha-humulene, heneicosane, spathulenol, alpha-copaene, alpha-curcumene, alpha terpinene, uvaol, viridiflorol, neophytadiene, isopulelegol, phthalic acid, coumarin, pamidronic acid, alpha-cadinol, elemol, alpha-bisabolene, etc, that has potent biological activity and are given in **Table 21**.

Table 10: Phytocomponents identified in the methanolic extract of *Astilbe rivularis* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	7.35	Limonene	Terpene	$C_{10}H_{16}$	136.2340	0.60
2	9.83	Benzene, 1,3-bis(1,1-dimethylethyl)	Phenylpropanes	$C_{14}H_{22}$	190.3245	0.49
3	10.05	3-Ethyl-3-methylheptane	Fatty acyls	$C_{10}H_{22}$	142.28	0.54
4	10.27	Cyclohexanemethanol, 2-(2-propenyl)-, trans	Terpene	$C_{10}H_{18}O$	154.25	1.86
5	10.82	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4	Terpineol	$C_{12}H_{20}O_2$	196.29	3.86
6	11.56	Bicyclo[7.2.0]Undec-4-ene,4,11,11-Trimethyl	Sesquiterpene	$C_{15}H_{24}$	204.35	3.06
7	12.25	Phenol, 3,5-bis(1,1-dimethylethyl)	Alkylated phenol	$C_{14}H_{22}O$	206.32	4.20
8	12.38	1-Chlorohexadecane	Halogen compound	$C_{16}H_{33}Cl$	260.9	1.03
9	12.99	Diethyl Phthalate	Phthalate ester	$C_{12}H_{14}O_4$	222.24	2.37
10	13.27	Tetrahydroionyl acetate	Tetrahydroionyl acetate	$C_{15}H_{28}O_2$	140.38	0.86
11	13.57	1-(4-Isopropylphenyl)-2-Methylpropyl acetate	Ester	$C_{11}H_{14}O_2$	178.23	1.33
12	13.92	Tetracosanoic Acid, Methyl ester	Fatty acid	$C_{25}H_{50}O_2$	382.7	1.38
13	14.08	2-Octanol, 2-methyl-6-methylene	Ester	$C_{11}H_{20}O_2$	184.27	1.13
14	14.45	Naphtho[2,1-b]furan,	Terpenoid	$C_{16}H_{28}O$	236.39	0.82

		dodecahydro-3a,6,6,9a-tetramethyl				
15	14.62	Isopropyl myristate	Ester	$C_{17}H_{34}O_2$	270.5	1.19
16	14.85	Cyclopentadecanone, 2-hydroxy	Volatile organic compound	$C_{15}H_{28}O_2$	240.38	4.22
17	15.02	Phthalic acid, di(trans-dec-3-enyl) ester	Fatty acid	$C_{22}H_{32}O_4$	360.5	1.46
18	15.34	Hexadecanoic Acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	5.60
19	15.53	Oxacycloheptadec-8-en-2-one, (8Z)	Cycloalkyl ester	$C_{16}H_{28}O_2$	252.39	1.19
20	15.64	n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256.43	2.45
21	16.48	9-Octadecenoic Acid (Z)-, methyl ester	Fatty acid	$C_{19}H_{36}O_2$	296.5	1.25
22	16.51	9-Octadecenoic acid, methyl ester, (E)	Fatty acid	$C_{19}H_{36}O_2$	296.5	0.64
23	16.63	Methyl stearate	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	1.40
24	19.02	Bis(2-ethylhexyl) phthalate	Phthalate ester	$C_{24}H_{38}O_4$	390.6	9.79
25	29.44	gamma.-Sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	9.30
26	31.56	Phenol, 2,4-bis (1,1-dimethylethyl)- phosphite (3:1)	Alkylbenzene	$C_{29}H_{50}O$	646.9	30.27
27	34.63	Betulinaldehyde	Triterpenoids	$C_{30}H_{48}O_2$	440.7	7.71

RT= Retention Time, MW= Molecular Weight

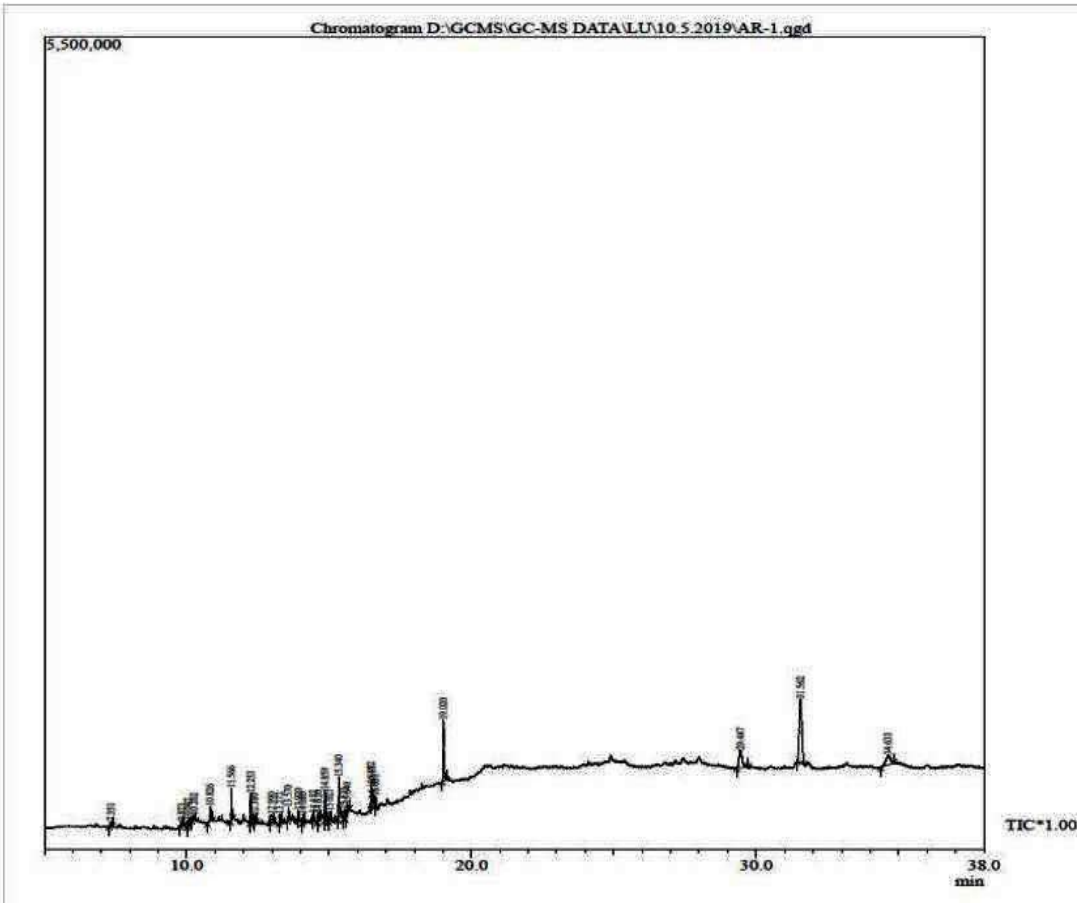

Figure 16: GC-MS chromatogram of *Astilbe rivularis* in methanolic extract

Table 11: Phytocomponents identified in the methanolic extract of *Bergenia ciliata* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	7.35	Limonene	Terpene	$C_{10}H_{16}$	136.234	0.60
2	7.62	3-Ethyl-3-methylheptane	Phenylpropanes	$C_{14}H_{22}$	190.324	0.49
3	9.82	Benzene, 1,3-bis(1,1-dimethylethyl)	Fatty acyls	$C_{10}H_{22}$	142.28	0.54
4	10.04	Nonane,5-(2-methylpropyl)	Terpene	$C_{10}H_{18}O$	154.25	1.86
5	10.15	Cyclohexene, 3-methyl-6-(1-methylethyl)	Terpineol	$C_{12}H_{20}O_2$	196.29	3.86

6	10.26	(2,6,6-Trimethylbicyclo [3.1.1]Hept-3-yl) heptan-3-amine	Sesquiterpene	$C_{15}H_{24}$	204.35	3.06
7	10.83	3-Cyclohexene-1-Methanol, .alpha.,.alpha.,4	Alkylated phenol	$C_{14}H_{22}O$	206.32	4.20
8	11.23	Cyclohexane, 1-Ethenyl-1-Methyl-2,4-Bis(1-Methylethenyl)	Halogen compound	$C_{16}H_{33}Cl$	260.9	1.03
9	11.56	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8methylene-,[1r-(1r*,4e,9s*)]	Phthalate ester	$C_{12}H_{14}O_4$	222.24	2.37
10	12.02	Dodecane, 2,6,11-trimethyl	Tetrahydroionyl acetate	$C_{15}H_{28}O_2$	140.38	0.86
11	12.25	Phenol, 3,5-bis(1,1-dimethylethyl)	Phenols	$C_{11}H_{14}O_2$	178.23	1.33
12	12.37	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	Fatty acid	$C_{25}H_{50}O_2$	382.7	1.38
13	12.65	5-Azulenemethanol, 1,2,3,3a,4,5,6,7-octahydro-.alpha.,.alpha	Ester	$C_{11}H_{20}O_2$	184.27	1.13
14	12.99	(-)-5-Oxatricyclo[8.2.0.0(4,6)]Dodecane,,12-Trim	Terpenoid	$C_{16}H_{28}O$	236.39	0.82
15	13.10	1,5-Heptadien-4-ol, 3,3,6-trimethyl	Ester	$C_{17}H_{34}O_2$	270.5	1.19
16	13.70	Tetradecane	Alkane hydrocarbon	$C_{14}H_{30}$	198.39	0.56
17	13.88	1h-Naphtho[2,1-B]pyran, 4a,5,6,6a,7,8,9,10,10a,10	Terpene	$C_{20}H_{32}O_2$	304.5	2.00
18	15.37	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	6.32
19	16.53	9-Octadecenoic acid	Fatty acid methyl	$C_{19}H_{36}O_2$	296.5	0.47

			ester			
20	16.64	Octadecanoic Acid	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	0.96
21	19.03	1,2-Benzenedicarboxylic acid	Aromatic dicarboxylic acid	$C_8H_6O_4$	166.13	1.04
22	24.90	Stigmast-5-en-3-ol, Oleat	Steroids	$C_{29}H_{50}O$	414.7	0.95
23	29.45	gamma.-Sitosterol	Phytosterol	$C_{29}H_{52}O_2$	432.7	5.36
24	31.56	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	19.48
25	38.32	5,11,17,23-Tetratert-Butylpentacyclo[19.3.1.1~	Ester	$C_{44}H_{56}$	584.9	29.58

RT= Retention Time, MW= Molecular Weight

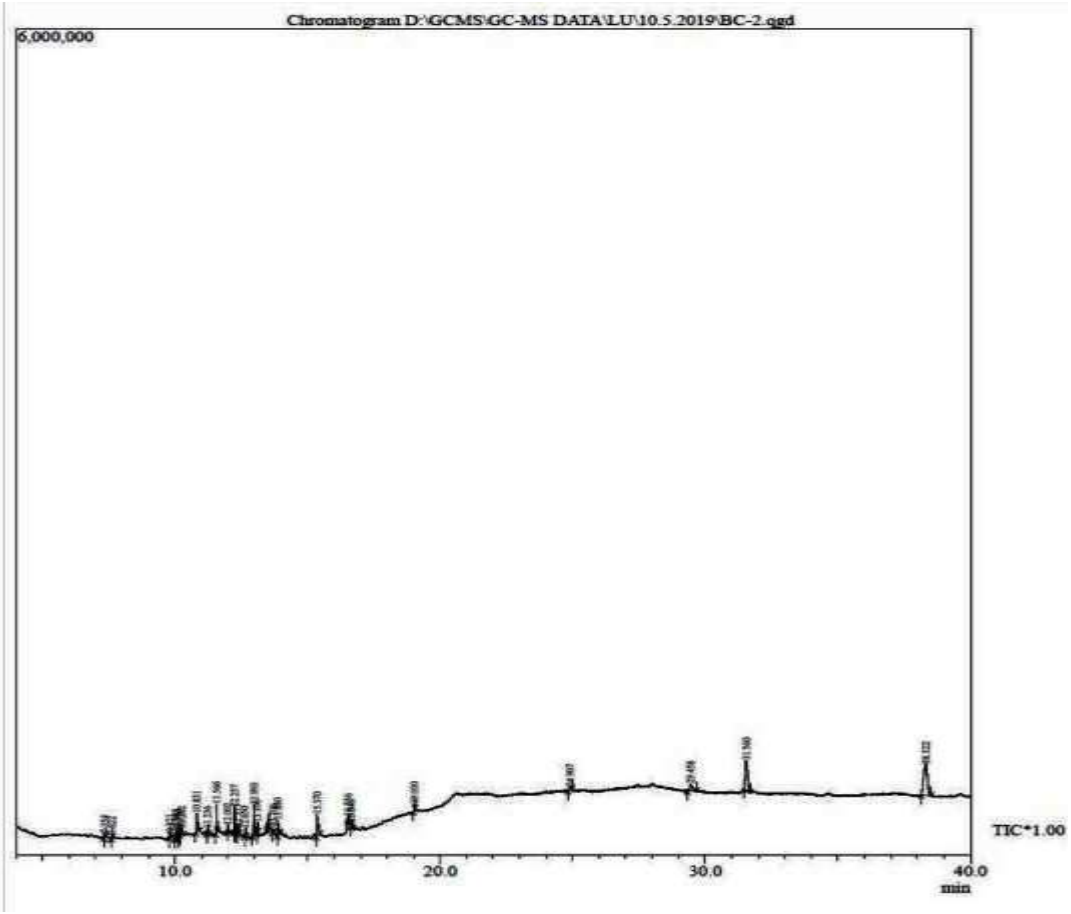

Figure 17: GC-MS chromatogram of *Bergenia ciliata* in methanolic extract

Table 12: Phytocomponents identified in the methanolic extract of *Betula alnoides* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	10.69	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4(1-methylethylidene)	Terpene	$C_{15}H_{24}$	204.35	0.17
2	10.79	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	Monoterpenoids	$C_{12}H_{20}O_2$	196.29	0.14
3	11.16	2,4-Diisopropenyl-1-methyl-1vinylcyclohexane	Terpene	$C_{15}H_{24}$	204.18	0.17
4	11.23	Cyclohexane, 1-Ethenyl-1-Methyl-2,4-bis(1methyletnenyl)	Terpene	$C_{15}H_{24}$	204.35	2.25
5	11.56	Bicyclo[7.2.0]Undec-4-Ene, 4,11,11-Trimethyl-8-	Sesquiterpene	$C_{15}H_{24}$	204.35	0.07
6	11.83	1-naphthol	Phenols	$C_{10}H_8O$	144.17	0.23
7	11.88	(1R,3S,4S)-1,3-Dimethyl-3-(4-methylpent-3-en-1-yl)-2oxab	Monoterpenoids	$C_{15}H_{24}O$	220.35	4.34
8	12.03	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3pentenyl)	Sesquiterpene	$C_{15}H_{24}$	204.35	0.20
9	12.09	Germacrene	Sesquiterpene	$C_{15}H_{24}$	204.35	3.99
10	12.21	Bicyclogermacrene	Sesquiterpene	$C_{15}H_{24}$	204.35	1.88
11	12.36	d-Cadinene	Sesquiterpene	$C_{15}H_{24}$	204.35	0.47
12	12.61	Acetamide, N-[2-oxo-1-(phenylmethyl)propyl]	Aromatic amine	$C_{12}H_{15}NO_2$	205.25	0.13
13	12.65	Nerolidol	Sesquiterpene	$C_{15}H_{26}O$	222.37	0.13

			alcohol			
14	12.74	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1propenyl-	Sesquiterpene	$C_{15}H_{24}$	204.35	0.17
15	12.84	β.-Elemene	Terpene	$C_{15}H_{24}$	204.35	1.39
16	12.92	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	Sesquiterpene	$C_{15}H_{24}O$	220.35	0.56
17	12.99	1h-Cycloprop[E]Azulene, decahydro-1,1,7tri	Terpene	$C_{15}H_{24}$	204.35	0.37
18	13.08	Cyclobutene, 4,4-dimethyl-1-(2,7 octadienyl)	Terpene	$C_{14}H_{22}$	190.32	0.39
19	13.18	7-Hydroxyfarnesen	Sesquiterpene	$C_{15}H_{24}O$	220.35	1.56
20	13.30	2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3cyc	Sesquiterpene	$C_{15}H_{26}O_2$	238.36	0.11
21	13.34	2-Heptanone, 6-methyl-6-[3-methyl-3-(1-methylethenyl)1-c	Sesquiterpene	$C_{15}H_{24}O$	220.35	0.28
22	13.49	2-Furanmethanol, tetrahydro.alpha.,.alpha., 5-trimethyl-5	Oxolanes	$C_{10}H_{18}O_2$	170.25	14.12
23	13.72	(S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3-en-1yl)dihyd	Sesquiterpene	$C_{15}H_{24}O_2$	236.25	34.64
24	13.88	Octadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	0.14
25	14.08	Espatulenol	Sesquiterpene	$C_{15}H_{24}O$	220.35	0.27
26	14.23	Cnidiol C	Terpenes	$C_{10}H_{18}O_2$	170.25	4.71
27	14.43	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	Sesquiterpene	$C_{15}H24O$	220.35	0.85
28	14.55	trans-p-Mentha-2,8-dienol	Monoterpenoids	$C_{10}H_{16}O$	152.23	0.50
29	14.67	(1R,3S,4S)-1,3-Dimethyl-3-(4-methylpent-	Cyclic ether	$C_{10}H_{16}O_2$	168.23	1.63

		3-en-1-yl)-2oxirane-2-carbaldehyde				
30	14.87	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8 hexahydrocy	Isochromene	$C_{18}H_{26}O$	258.4	0.16
31	14.99	3-Benzylidene-2,4-pentanedione	Ketones	$C_{12}H_{12}O_2$	188.22	0.64
32	15.08	2d-Methylhexanoic acid methyl ester	Fatty acid methyl ester	$C_8H_{16}O_2$	144.21	0.29
34	15.16	(Z)-2-(Hexa-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non-3-ene	Spiro-ether	$C_{13}H_{12}O_2$	200.23	19.34
35	15.30	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	0.36
36	15.67	(E)-2-(Hepta-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non3-e	Spiroether	$C_{14}H_{14}O_2$	214.26	1.18
37	15.74	Azuleno[4,5-b]furan-2(3H)-one, decahydro-3,6,9tris(methylene)-, [3aS(3a.alpha.,6a.alpha.,9a.alpha.,9b.beta.)]	Acyclic terpene alcohol	$C_{15}H_{22}O_4$	266.33	0.27
38	16.39	Isophytol	Terpenoid alcohol	$C_{20}H_{40}O$	296.5	0.12
39	16.47	9-Octadecenoic acid, methyl ester, (E)	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.11
40	16.56	Phytol isomer	Diterpenoid	$C_{20}H_{40}O$	296.5	0.39
41	17.65	Heneicosane	Acyclic alkanes	$C_{21}H_{44}$	296.6	0.12
42	18.78	Eicosane	Acyclic alkanes	$C_{20}H_{42}$	282.5	0.39
43	31.52	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	0.72

RT= Retention Time, MW= Molecular Weight

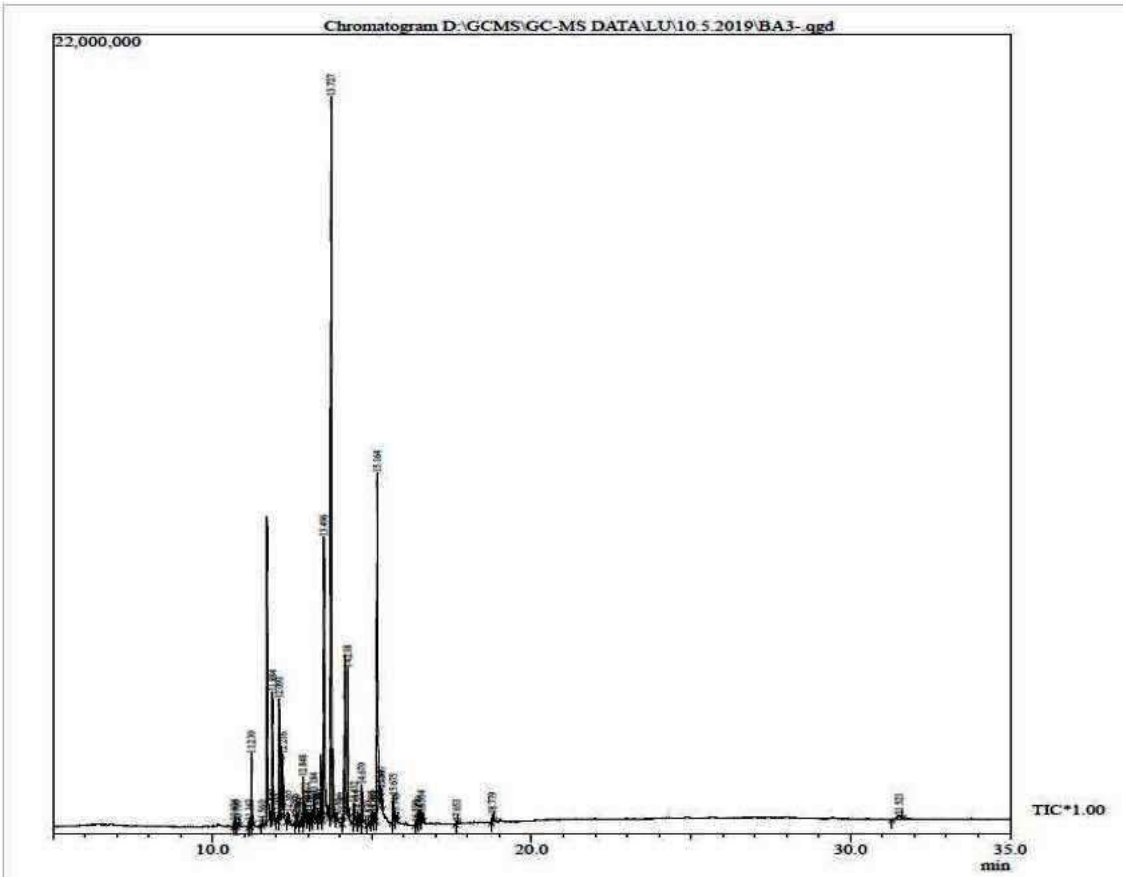

Figure 18: GC-MS chromatogram of *Betula alnoides* in methanolic extract

Table 13: Phytocomponents identified in the methanolic extract of *Equisetum diffusum* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	8.834	Isopulegol	Terpenes alkenes alcohols	$C_{10}H_{18}O$	154.25	2.23
2	9.19	1-Isopropyl-4-methyl-3-cyclohexen-1-ol	Terpenes alcohol	$C_{10}H_{18}O$	154.24	0.29
3	9.34	alpha-Terpineol	Monoterpene alcohol	$C_{10}H_{18}0$	154.25	0.23
4	9.62	6-Octen-1-ol, 3,7-dimethyl	Acyclic	$C_{10}H_{20}O$	156.26	1.39

			monoterpenoids			
5	10.16	Cyclohexanemethanol,4-(1-methylethyl)	Terpene alcohol	$C_{10}H_{20}O$	156.26	0.59
6	10.26	Aceticacid,1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	Terpene ester	$C_{12}H_{20}O_2$	196.29	0.41
7	10.34	2-Octanol, 8,8-dimethoxy-2,6-dimethyl	Terpene alcohol	$C_{12}H_{26}O_3$	218.33	0.85
8	10.39	Dihydro-2(3h)-Furanone	Lactone esters	$C_{12}H_{22}O_2$	198.3	0.35
9	10.66	4-tert-Butylcyclohexyl acetate	Cyclic acetates	$C_{12}H_{22}O_2$	198.3	0.26
10	10.76	6-Octen-1-ol, 3,7-dimethyl-, propanoate	Acyclic monoterpenoids	$C_{13}H_{24}O_2$	212.33	0.21
11	10.8	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	Monoterpenoids	$C_{12}H_{20}O_2$	196.29	7.37
12	10.94	Cyclopentanepropanethiol, thiolacetate	Thiols	$C_5H_{10}S$	102.2	0.95
13	10.99	4-tert-Butylcyclohexyl acetate	Cyclic acetates	$C_{12}H_{22}O_2$	198.3	0.46
14	11.09	Cyclohexanol, 5-methyl-2-(1methylethyl)	Terpene alcohol	$C_{10}H_{20}O$	156.26	0.47
15	11.23	2,4-Diisopropenyl-1-methyl-1vinylcyclohexane	Sesquiterpene	$C_{15}H_{24}$	204.35	0.70
16	11.53	4,7-methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate	Esters	$C_{12}H_{16}O_2$	192.25	0.29
17	11.61	Succinic acid, but-3-yn-2-yl dodec-9-yn-1-yl ester	Esters	$C_{21}H_{34}O_4$	350.49	0.41
18	11.65	Phosphonofluoridic acid, methyl-, octyl ester	Esters	$C_9H_{20}FO_2P$	210.23	0.37
19	11.91	1-Eicosanol	Fatty alcohol	$C_{20}H_{42}O$	298.5	0.66
20	12.02	alpha-Curcumene	Sesquiterpene	$C_{15}H_{22}$	202.33	0.60
21	12.24	Phenol, 3,5-bis(1,1-dimethylethyl)	Phenols	$C_{14}H_{22}O$	206.32	1.29

22	12.37	d-Cadinene	Sesquiterpene	$C_{15}H_{24}$	204.35	1.40
23	12.52	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, (E,E)	Fatty ester	$C_{17}H_{28}O_2$	264.4	0.97
24	12.64	Elemol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	1.46
25	12.72	5,9-Undecadien-2-ol, 6,10-dimethyl	Norsesquiterpene	$C_{13}H_{24}O$	196.33	1.08
26	12.98	Caryophyllene oxide	Terpenes	$C_{15}H_{24}O$	220.35	0.40
27	13.02	Cyclohexane, 1,1,2-trimethyl-3,5-bis(1-methylethenyl)	Menthane monoterpenoids	$C_{14}H_{24}$	192.34	1.10
28	13.09	gamma.-Dodecalactone	Fatty esters	$C_{12}H_{22}O_2$	198.3	4.27
29	13.34	Bornyl isovalerate	Terpenoids	$C_{15}H_{26}O_2$	238.37	0.50
30	13.41	Methyl (3-Oxo-2pentylcyclopentyl)acetate	Aroma compound	$C_{13}H_{22}O_3$	226.31	0.68
31	13.56	4-Isopropylphenyl acetate	Phenyl acetic acid ester	$C_{11}H_{14}O_2$	178.23	1.25
32	13.82	3-Buten-ol, 3-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl) 2-acetate	Alcohol ester	$C_{16}H_{26}O_2$	250.38	0.21
33	13.90	Triacontanoic acid, methyl ester	Fatty acid methyl ester	$C_{31}H_{62}O_2$	466.8	0.67
34	14.23	9-Octadecenoic acid (Z)	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.32
35	14.38	Acetyl cedrene	Terpenoids	$C_{17}H_{26}O$	246.4	0.36
36	14.70	Neophytadiene	Sesquiterpenoids	$C_{20}H_{38}$	278.5	0.74
37	14.88	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	Alkylated tetralin	$C_{18}H_{26}O$	258.4	0.52
38	15.01	Phytol	Diterpene alcohol	$C_{20}H_{40}O$	296.5	0.42
39	15.61	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	2.83

40	15.89	Ambrox	Terpenoid	$C_{16}H_{28}O$	236.39	0.26
41	16.43	9,12-Octadecadienoic acid (Z,Z)	Fatty acid	$C_{18}H_{32}O_2$	280.44	0.10
42	16.47	6-Octadecenoic acid, methyl ester, (Z)	Fatty acid	$C_{19}H_{36}O_2$	296.5	0.61
43	16.62	Methyl stearate	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	0.48
44	18.89	Betulinaldehyde	Triterpenoids	$C_{30}H_{48}O_2$	440.7	0.47
45	19.01	Bis(tridecyl) phthalate	Phthalate ester	$C_{34}H_{58}O_4$	530.8	0.84
46	21.02	Uvaol	Pentacyclic triterpene	$C_{30}H_{50}O$	442.7	3.19
47	22.40	Betulin	Triterpene	$C_{30}H_{50}O_2$	442.72	37.20
48	27.42	Campesterol	Sterol	$C_{28}H_{48}O$	400.7	1.11
49	27.99	Stigmasterol	Phytosterol	$C_{29}H_{48}O$	412.69	1.34
50	29.45	gamma.-Sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	3.47
51	31.57	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	5.62
52	34.65	Methyl commate B	Triterpene	$C_{31}H_{50}O_3$	470.7	3.57

RT= Retention Time, MW= Molecular Weight

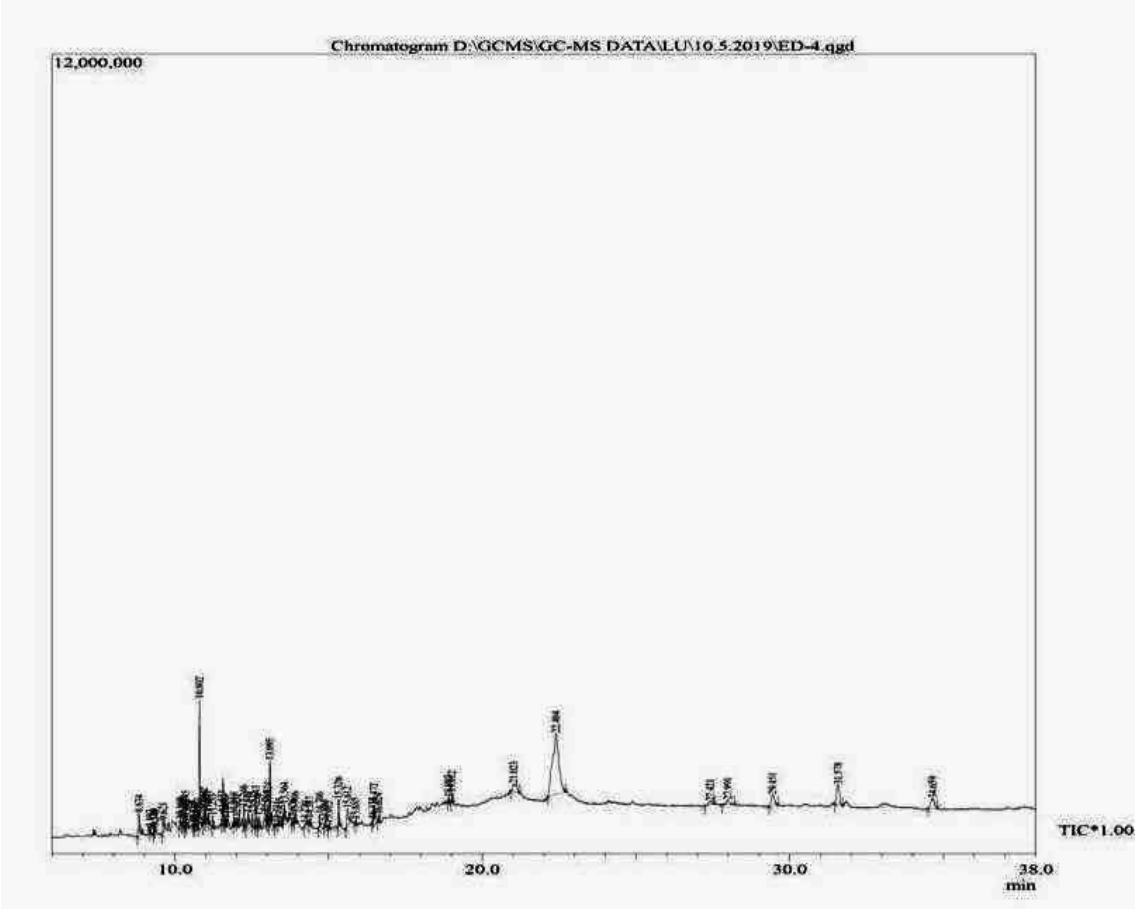

Figure 19: GC-MS chromatogram of *Equisetum diffusum* in methanolic extract

Table 14: Phytocomponents identified in the methanolic extract of *Fraxinus floribunda* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	10.25	endo-1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-ol acetate	Terpene derivative	$C_{10}H_{18}O$	154.25	0.06
2	10.69	Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl	Polycyclic hydrocarbons	$C_{10}H_{16}$	136.23	0.05
3	10.79	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	Monoterpenoids	$C_{12}H_{20}O_2$	196.29	0.73

4	10.93	9-Tetradecynoic acid, methyl ester	Fatty acid esters	$C_{15}H_{26}O_2$	238.37	0.07
5	11.23	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1methylethenyl)	Sesquiterpene	$C_{15}H_{24}$	204.35	0.15
6	11.52	4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate	Esters	$C_{12}H_{16}O_2$	192.25	0.02
7	11.56	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl8- methylene-, [1r-(1R*,4E,9S*)]	Sesquiterpene	$C_{15}H_{24}$	204.35	0.18
8	11.63	6-[2-Butenyl]-1,5,5-trimethyl-1cyclohexene	Sesquiterpenoids	$C_{13}H_{22}$	178.31	0.16
9	11.70	(E)-.beta.-Farnesene	Sesquiterpenoids	$C_{15}H_{24}$	204.35	8.79
10	11.82	1-Naphthol, 2-methyl	Napthols	$C_{11}H_{10}O$	158.2	0.09
11	11.89	(1R,3S,4S)-1,3-Dimethyl-3-(4-methylpent-3-en-1-yl)-2oxab	Monoterpenoids	$C_{15}H_{24}O$	220.35	0.52
12	12.08	Germacrene D	Sesquiterpene	$C_{15}H_{24}$	204.35	1.26
13	12.23	Phenol, 2,5-bis(1,1-dimethylethyl)	Phenols	$C_{14}H_{22}O$	206.32	0.79
14	12.36	d-Cadinene	Sesquiterpene	$C_{15}H_{24}$	204.35	0.41
15	12.49	alpha-Bisabolene	Sesquiterpene	$C_{15}H_{24}$	204.35	0.08
16	12.62	Elemol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	0.83
17	12.80	Bornyl bromide	Acyclic compounds	$C_{10}H_{17}Br$	217.15	0.13
18	12.91	Espatulenol	Sesquiterpene	$C_{15}H_{24}O$	220.35	0.69
19	12.97	Picolinic acid	Pyridinemonocarboxylic acid	$C_6H_5NO_2$	123.11	0.02
20	13.01	Spathulenol	Sesquiterpenoids	$C_{15}H_{24}O$	220.35	0.11
21	13.34	Viridiflorol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	0.40

22	13.40	tau-Cadinol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	0.74
23	13.49	Bisabolol	Sesquiterpene alcohol	$C_{15}H_{26}O$	222.37	8.37
24	13.63	4,8-Dimethyl-3,8-nonadien-2-one	Sesquiterpenoids	$C_{11}H_{18}O$	166.26	0.21
25	13.70	alpha.-Bisabolol	Sesquiterpene alcohol			42.02
26	13.91	Neryl acetone	Monoterpene	$C_{13}H_{22}O$	194.31	1.00
27	14.15	Chamazulene	Sesquiterpenoids	$C_{14}H_{16}$	184.28	0.76
28	14.23	2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl	Terpenes	$C_{10}H_{18}O_2$	170.25	15.72
29	14.38	1-Nonadecene	Alkene	$C_{19}H_{38}$	266.5	0.06
30	14.42	2,5-Octadecadiynoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{30}O_2$	290.44	0.20
31	14.68	(1R,3S,4S)-1,3-Dimethyl-3-(4-methylpent-3-en-1-yl)-2oxabicyclo[2.2.2] oct-5-ene	Cyclic alkene	$C_{15}H_{24}O$	220.35	0.24
32	15.16	(Z)-2-(Hexa-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non-3-ene	Spiro-ether	$C_{13}H_{12}O_2$	200.23	10.63
33	15.67	(E)-2-(Hepta-2,4-diyn-1-ylidene)-1,6-dioxaspiro[4.4]non3-e	Spiro ether	$C_{14}H_{14}O_2$	214.26	0.65
34	16.45	9,12-Octadecadienoic acid (Z,Z)-	Fatty acid	$C_{18}H_{32}O_2$	280.44	0.03
35	16.49	6-Octadecenoic acid, methyl ester, (Z)	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.23
36	16.63	Octadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	0.10
37	31.46	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	1.09
38	38.19	4-Tert butylcalix[4] arene	Ethers	$C_{44}H_{56}O_4$	648.9	0.89

RT= Retention Time, MW= Molecular Weight

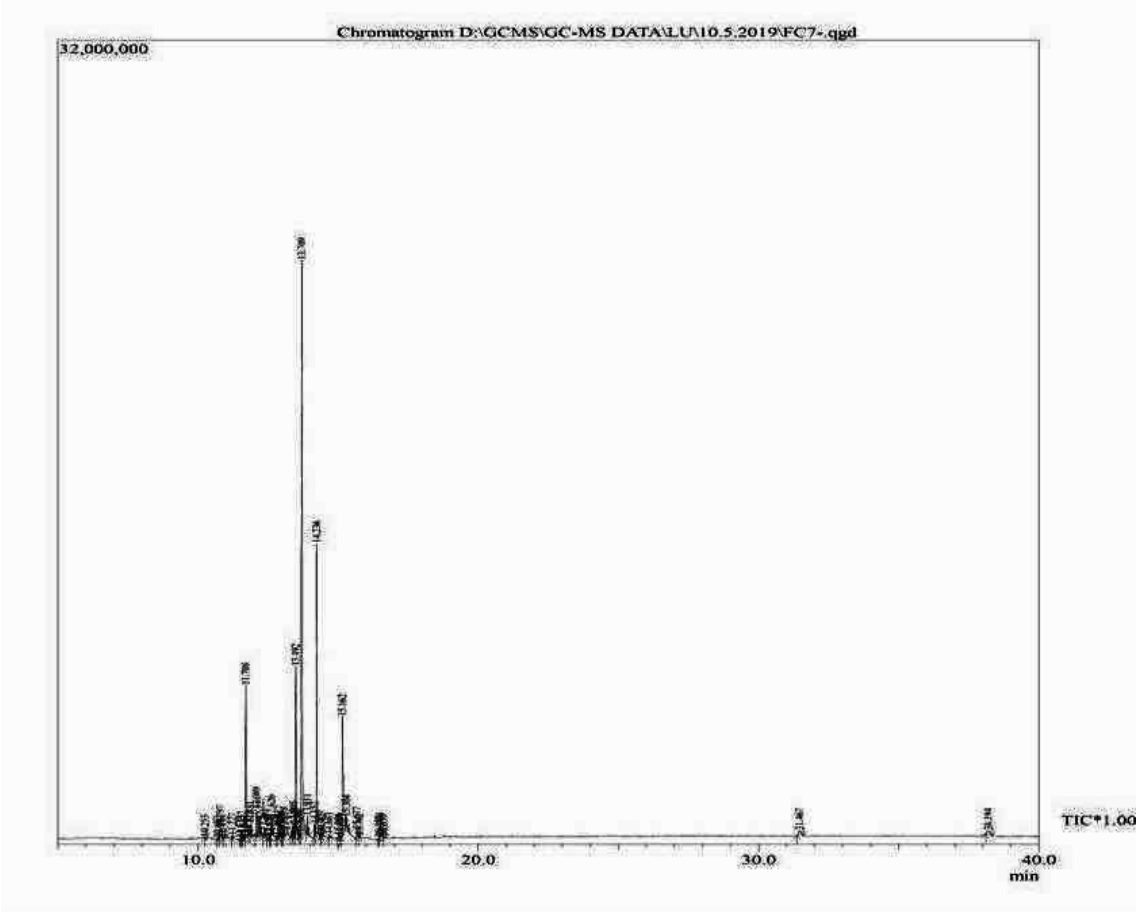

Figure 20: GC-MS chromatogram of *Fraxinus floribunda* in methanolic extract

Table 15: Phytocomponents identified in the methanolic extract of *Litsea cubeba* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	9.82	Benzene, 1,3-bis(1,1-dimethylethyl)	Phenylpropanes	$C_{14}H_{22}$	190.32	0.47
2	10.04	Dodecane, 2,6,11-trimethyl	Alkane	$C_{15}H_{32}$	212.41	0.33
3	10.15	2,9-Dimethyl-5-decyne	Acyclic compound	$C_{12}H_{22}$	166.3	0.28

4	10.26	Cyclohexanemethanol, 2-(2-propenyl)-, trans	Terpene	$C_{10}H_{18}O$	154.25	0.23
5	10.81	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4	Terpineol	$C_{12}H_{20}O_2$	196.29	6.53
6	11.23	2,4-Diisopropenyl-1-Methyl-1vinylcyclohexane	Terpene	$C_{15}H_{24}$	204.18	1.68
7	11.56	Isocaryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.35	2.00
8	11.89	Humulene	Sesquiterpene	$C_{15}H_{24}$	204.35	0.21
9	12.009	Cadinol	Terpene	$C_{15}H_{26}O$	222.37	0.55
10	12.10	Germacrene D	Sesquiterpene	$C_{15}H_{24}$	204.35	2.63
11	12.25	Phenol, 2,5-bis(1,1-dimethylethyl)	Phenols	$C_{14}H_{22}O$	206.32	1.24
12	12.37	d-Cadinene	Sesquiterpene	$C_{15}H_{24}$	204.35	11.06
13	12.50	3,7(11)-Eudesmadiene	Volatile terpene	$C_{15}H_{24}$	204.35	0.41
14	12.64	Elemol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	25.16
15	12.91	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7dienol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	1.47
16	12.98	(3-Amino-1-hydroxy-1-phosphono-propyl)phosphonic acid	Bisphosphonate	$C_3H_{11}NO_7P_2$	235.07	0.31
17	13.34	tau.-Cadinol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	5.74
18	13.59	alpha-Cadinol	Terpene	$C_{15}H_{26}O$	222.37	9.03
19	13.95	Eicosanoic acid, methyl ester	Fatty acid methyl ester	$C_{21}H_{42}O_2$	326.6	1.97
20	14.69	Coumarin	Coumarins and derivatives	$C_9H_6O_2$	146.14	0.07
21	15.35	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	4.17
22	16.49	9-Octadecenoic Acid (Z)-, Methyl Ester	Fatty acid methyl	$C_{19}H_{36}O_2$	296.5	0.63

			ester			
23	16.52	9-Octadecenoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.31
24	16.64	Octadecanoic acid	Fatty acid	$C_{19}H_{38}O_2$	298.5	0.61
25	19.02	1,2-Benzenedicarboxylic acid	aromatic dicarboxylic acid	$C_8H_6O_4$	166.13	0.95
26	29.46	gamma-Sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	3.44
27	31.56	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	16.25

RT= Retention Time, MW= Molecular Weight

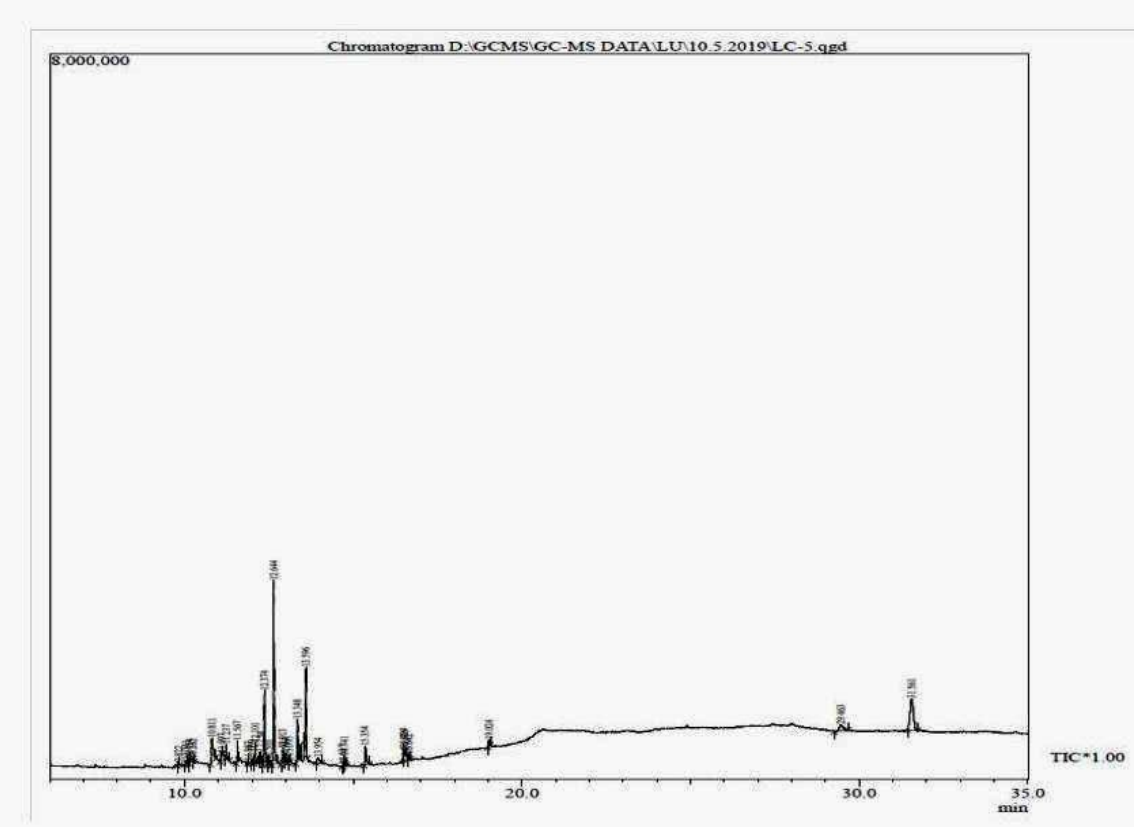

Figure 21: GC-MS chromatogram of *Litsea cubeba* in methanolic extract

Table 16: Phytocomponents identified in the methanolic extract of *Litsea glutinosa* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	10.04	Nonane, 5-(2-methylpropyl)	Heterocyclic compound	$C_{13}H_{28}$	184.36	0.74
2	10.81	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4	Terpineol	$C_{12}H_{20}O_2$	196.29	6.04
3	11.23	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1methylethenyl)	Terpene	$C_{15}H_{24}$	204.35	0.81
4	12.02	alpha-Curcumene	Sesquiterpene	$C_{15}H_{22}$	202.33	6.01
5	12.13	Sesquithujene	Sesquiterpene	$C_{15}H_{24}$	204.35	1.25
6	12.24	Phenol, 3,5-bis(1,1-dimethylethyl)	Phenols	$C_{14}H_{22}O$	206.32	8.76
7	12.64	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4trimethyl	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	1.50
8	12.92	Diethyl Phthalate	Phthalate ester	$C_{12}H_{14}O_4$	222.24	1.96
9	13.095	3,6-Dimethyl-8-(tetrahydro-2h-pyran-2-one	Ether	$C_7H_{12}O_2$	128.17	1.20
10	13.48	Mesitylene	Trimethylbenzene	C_9H_{12}	120.19	4.28
11	13.70	Octadecane	Alkane	$C_{18}H_{38}$	254.5	1.58
12	13.903	Octadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	2.88
13	14.38	Vertofix	Sesquiterpenes	$C_{17}H_{26}O$	246.4	1.55
14	14.70	Neophytadiene	Sesquiterpenoids	$C_{20}H_{38}$	278.5	1.01

15	15.32	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	9.17
16	15.61	n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256.43	6.95
17	16.47	9-Octadecenoic acid, methyl ester, (E)	Fatty acid	$C_{16}H_{36}O_2$	296.5	2.34
18	16.62	*2,6*-Di-tert-butyl-*4-(*dimethylaminomethyl)*phenol*	Phenols	$C_{17}H_{29}NO$	263.4	5.22
19	18.36	Methyl stearate	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	2.34
20	19.01	Bis(2-ethylhexyl) phthalate	Phthalate ester	$C_{24}H_{38}O_4$	390.6	2.34
21	27.98	Stigmasta-7,22-dien-3-ol, (3beta.,5.alpha.,22E,24R)	Steroids	$C_{29}H_{48}O$	412.7	2.76
22	29.45	gamma.-Sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	9.71
23	31.56	Phenol 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	15.04

RT= Retention Time, MW= Molecular Weight

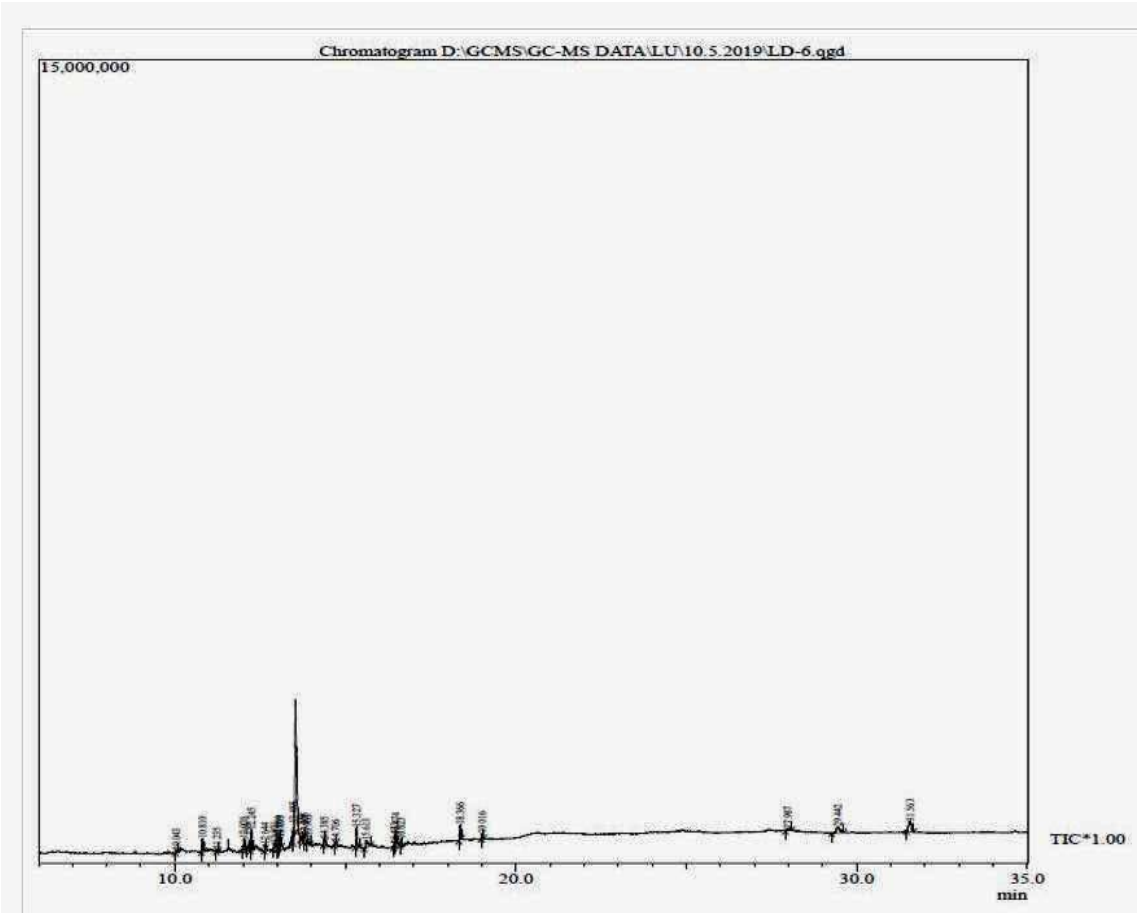

Figure 22: GC-MS chromatogram of *Litsea glutinosa* in methanolic extract

Table 17: Phytocomponents identified in the methanolic extract of *Quercus lamellosa* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	7.34	Cyclobutane, 1,3-diisopropenyl-, trans	Acyclic compound	$C_{10}H_{16}$	136.23	2.45
2	10.12	Cyclohexanemethanol, 2-(2-propenyl)-, trans	Terpene	$C_{10}H_{18}O$	154.25	3.98
3	10.24	Exo-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl acetate	Monoterpene	$C_{12}H_{20}O_2$	196.29	5.49
4	10.85	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	Terpineol	$C_{12}H_{20}O_2$	196.29	15.42

5	11.56	Bicyclo[7.2.0]Undec-4-ene, 4,11,11-Trimethyl-8	Sesquiterpene	$C_{15}H_{24}$	204.35	10.68
6	13.57	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2enyl)-cyclohexene	Terpenoids	$C_{15}H_{26}O$	222.37	1.38
7	14.05	1-Hexanol, 5-methyl-2-(1-methylethyl)		$C_{10}H_{22}O$	158.28	4.24
8	15.43	9-Octadecenoic acid, methyl ester, (E)	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	12.19
9	16.59	Hexadecyl chloroacetate	Esters	$C_{18}H_{35}ClO_2$	318.9	8.69
10	19.03	1,2-Benzenedicarboxylic acid, Diisooctyl ester	Phthalate ester	$C_{24}H_{38}O_4$	390.6	4.25
11	21.13	Squalene	Triterpene	$C_{30}H_{50}$	410.7	3.93
12	31.50	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	16.96

RT= Retention Time, MW= Molecular Weight

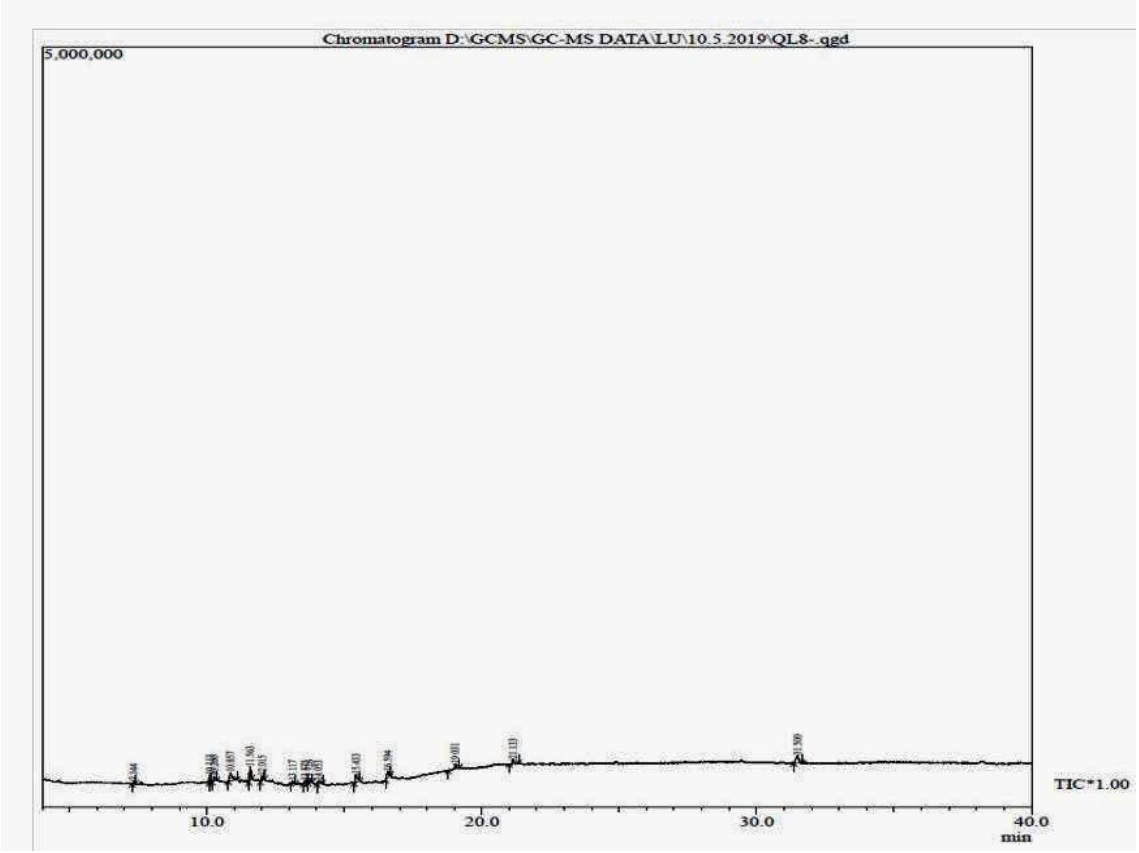

Figure 23: GC-MS chromatogram of *Quercus lamellosa* in methanolic extract

Table 18: Phytocomponents identified in the methanolic extract of *Rheum nobile* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	9.81	Benzene, 1,3-bis(1,1-dimethylethyl)	Phenylpropanes	$C_{14}H_{22}$	190.3245	0.49
2	10.03	3-Ethyl-3-methylheptane	Fatty acyls	$C_{10}H_{22}$	142.28	0.35
3	10.58	Myrtenylacetate	Terpene	$C_{12}H_{18}O_2$	194.27	0.14
4	10.80	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	Monoterpenoids	$C_{12}H_{20}O_2$	196.29	4.17
5	11.08	Cyclohexanol, 5-methyl-2-(1methylethyl)	Terpene alcohol	$C_{10}H_{20}O$	156.26	0.32

6	11.56	beta-Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.36	2.63
7	11.87	Humulene	Sesquiterpene	$C_{15}H_{24}$		0.38
8	11.99	Dodecane, 4,6-dimethyl	Alkane	$C_{14}H_{30}$	198.39	0.19
9	12.17	Caryophylene oxide	Sesquiterpene	$C_{15}H_{24}O$	220.35	0.19
10	12.23	Phenol, 3,5-bis(1,1-dimethylethyl)	Phenols	$C_{14}H_{22}O$	206.32	2.82
11	12.36	10s,11s-Himachala-3(12),4-diene	Terpenoids	$C_{15}H_{24}$	204.35	1.37
12	12.52	Cyclopropane methanol, .alpha.,2-dimethyl-2-(4-methyl-3pentenyl)	Carbocyclic compounds	$C_{12}H_{22}O$	182.3	0.60
13	12.63	Elemol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	1.33
14	12.91	Diethyl Phthalate	Phthalate ester	$C_{12}H_{14}O_4$	222.24	20.60
15	13.08	4-Hexen-3-ol, 2,5-dimethyl-	Carbocyclic compounds	$C_8H_{16}O$	128.21	1.82
16	13.32	Longiborneol	Sesquiterpene	$C_{15}H_{26}O$	222.37	0.90
17	13.41	tau-Cadinol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	0.64
18	13.56	4-Isopropylphenyl acetate	Phenyl acetic acid ester	$C_{11}H_{14}O_2$	178.23	2.28
19	13.69	Dodecane, 4,6-dimethyl	Alkane	$C_{14}H_{30}$	198.39	0.21
20	13.74	Patchouli alcohol	Sesquiterpene alcohol	$C_{15}H_{26}O$	222.36	0.31
21	14.22	E,E-3,13-Octadecadien-1-ol	Ethers	$C_{18}H_{34}O$	266.5	2.66
22	14.29	7-Hexadecyn-1-ol	Terpene	$C_{16}H_{30}O$	238.41	0.83
23	14.37	Acetyl cedrene	Terpenoids	$C_{17}H_{26}O$	246.4	1.49
24	14.43	Naphtho[2,1-b]furan, dodecahydro-3a,6,6,9a-tetramethyl-	Terpenoid	$C_{16}H_{28}O$	236.39	0.16
25	14.88	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8hexahydrocyclopenta(g) Isochromene	Cyclic ether	$C_{18}H_{26}O$	258.4	4.57

26	15.37	Hexadecanoic Acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	2.86
27	15.70	n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256.42	3.57
28	16.07	Ethylene brassylate	Ethylene esters	$C_{15}H_{26}O_4$	270.36	7.03
29	16.49	6-Octadecenoic acid, methyl ester, (Z)	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.24
30	16.53	9-Hexadecenoic acid, methyl ester, (Z)	Fatty acid methyl ester	$C_{17}H_{32}O_2$	268.4	0.15
31	16.64	Octadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	0.52
32	19.01	1,2-Benzenedicarboxylic acid	Aromatic dicarboxylic acid	$C_8H_6O_4$	166.13	0.88
33	31.48	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{36}O_3P$	646.9	10.04
34	38.24	4-Tert butylcalix[4] arene	Ethers	$C_{44}H_{56}O_4$	648.9	15.61

RT= Retention Time, MW= Molecular Weight

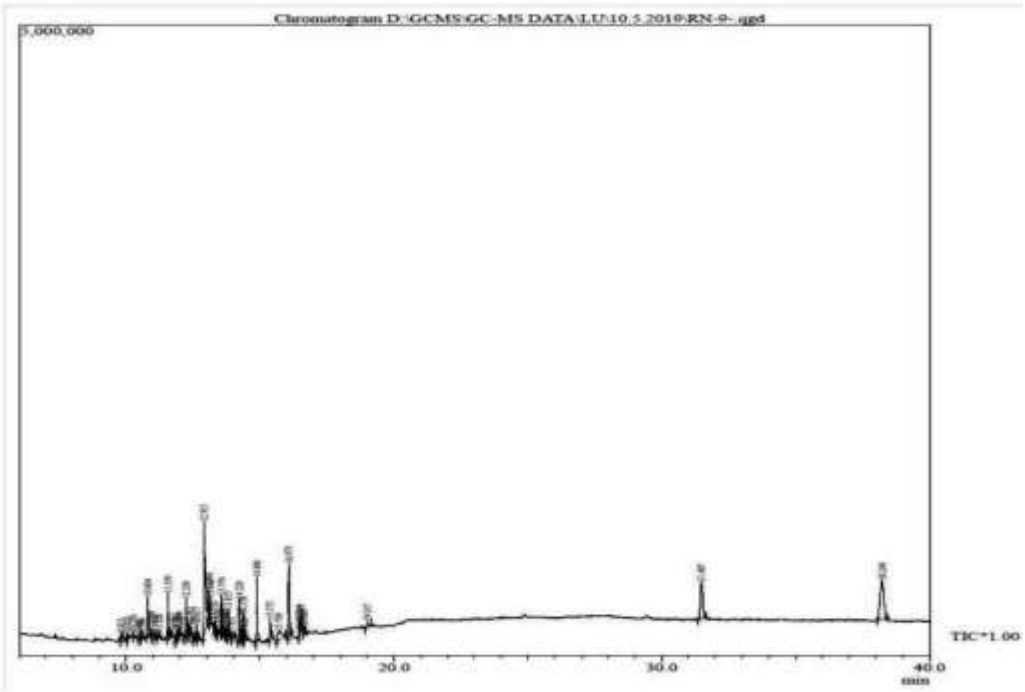

Figure 24: GC-MS chromatogram of *Rheum nobile* in methanolic extract

Table 19: Phytocomponents identified in the methanolic extract of *Stephania glabra* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	8.83	Isopulegol	Terpenes alkenes alcohols	$C_{10}H_{18}O$	154.25	1.34
2	8.94	Cyclohexanol, 5-methyl-2-(1-methylethenyl)	Dipentene terpene hydrocarbon byproducts	$C_{11}H_{18}O_2$	182.26	0.31
3	9.79	1,5-Dimethyl-1-vinyl-4-hexenyl 2aminobenzo	Terpenes	$C_{14}H_{24}O_2$	224.34	0.93
4	10.25	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	Terpene ester	$C_{12}H_{20}O_2$	196.29	0.69

5	10.33	2-Octanol, 8,8-dimethoxy-2,6-dimethyl	Terpene alcohol	$C_{12}H_{26}O_3$	218.33	0.82
6	10.57	Myrtenyl acetate	Terpene	$C_{12}H_{18}O_2$	194.27	0.50
7	10.65	4-tert-Butylcyclohexyl acetate	Acetate	$C_{12}H_{22}O_2$	198.3	0.74
8	10.79	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	Monoterpenoids	$C_{12}H_{20}O_2$	196.29	14.37
9	10.99	4-tert-Butylcyclohexyl acetate	Acetate	$C_{12}H_{22}O_2$	198.3	1.83
10	11.08	Cyclohexanol, 5-methyl-2-(1methylethyl)	Terpene alcohol	$C_{10}H_{20}O$	156.26	1.08
11	11.12	alpha-Copaene	Sesquiterpenoids	$C_{15}H_{24}$	204.35	0.35
12	11.23	2,4-Diisopropenyl-1-methyl-1vinylcyclohexane	Sesquiterpene	$C_{15}H_{24}$	204.35	1.24
13	11.55	Caryophyllene	Sesquiterpene	$C_{15}H_{24}$	204.36	6.43
14	11.60	Hexane, 1-chloro-5-methyl	Ether	$C_7H_{15}Cl$	134.65	0.57
15	11.71	Cycloheptasiloxane, tetradecamethyl	Organosiloxane	$C_{14}H_{42}O_7Si_7$	519.07	0.65
16	11.87	Humulene	Sesquiterpene	$C_{15}H_{24}$	204.35	0.53
17	12.01	alpha-Curcumene	Sesquiterpene	$C_{15}H_{22}$	202.33	1.77
18	12.09	Germacrene D	Sesquiterpernes	$C_{15}H_{24}$	204.35	0.54
19	12.12	alpha-Terpinene	Monoterpenoids	$C_{10}H_{16}$	136.23	0.25
20	12.17	alpha-Humulene	Terpene	$C_{15}H_{24}$	204.5	0.43
21	12.36	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7dimethyl	Sesquiterpene	$C_{15}H_{24}$	204.35	2.38
22	12.51	Cyclopropane methanol, .alpha.,2-dimethyl-2-(4-methyl-3pentenyl)	Carbocyclic compounds	$C_{12}H_{22}O$	182.3	2.10
23	12.59	Benzenemethanol, .alpha.-(trichloromethyl)-, acetate	Benzyloxycarbonyls	$C_{10}H_9Cl_3O_2$	267.5	0.14
24	12.63	Elemol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	2.37

25	12.97	Beta-Caryophyllene oxide	Sesquiterpene	$C_{15}H_{24}O$	220.35	1.81
26	13.08	gamma.-Dodecalactone	Fatty esters	$C_{12}H_{22}O_2$	198.3	7.50
27	13.25	Tetrahydroionyl acetate	Ester	$C_{15}H_{28}O_2$	140.38	0.30
28	13.52	7-epi-alpha-Eudesmol	Sesquiterpenoids	$C_{15}H_{26}O$	222.37	0.65
29	13.90	Cyperotundone	Sesquiterpene	$C_{15}H_{22}O$	218.33	0.61
30	14.29	Oxacycloheptadec-8-en-2-one, (8Z)	Macrocyclic lactones	$C_{16}H_{28}O_2$	252.39	0.25
31	14.37	Acetyl Cedrene	Terpenoids	$C_{17}H_{26}O$	246.4	1.80
32	14.87	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8hexahydrocyclopenta(g) Isochromene	Cyclic ether	$C_{18}H_{26}O$	258.4	1.99
33	14.92	Tonalid	Tetralins	$C_{18}H_{26}O$	258.4	1.02
34	15.26	(E)-Valerenyl isovalerate	Esters	$C_{20}H_{32}O_2$	304.46	0.29
35	15.37	Eicosanoic acid, methyl ester	Fatty acid methyl ester	$C_{21}H_{42}O_2$	326.6	4.20
36	15.87	Naphtho[2,1-b]furan, dodecahydro-3a,6,6,9a-tetramethyl	Terpenoid	$C_{16}H_{28}O$	236.39	0.35
37	16.06	Ethylene brassylate	Ethylene esters	$C_{15}H_{26}O_4$	270.36	0.90
38	16.17	9-Cycloheptadecen-1-one, (Z)	Ketone	$C_{17}H_{30}O$	250.4	0.27
39	16.48	9-Octadecenoic acid, methyl ester, (E)	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.97
40	16.63	Octadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.5	0.47
41	17.65	Heneicosane	Alkane	$C_{21}H_{44}$	296.6	0.24
42	19.01	Bis(2-ethylhexyl) phthalate	Phthalate ester	$C_{24}H_{38}O_4$	390.6	1.17
43	21.96	Hexatriacontane	Alkane	$C_{36}H_{74}$	507	1.90
44	29.40	gamma.-Sitosterol	Phytosterol	$C_{29}H_{50}O$	414.7	1.31

45	31.49	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{36}O_3P$	646.9	4.77

RT= Retention Time, MW= Molecular Weight

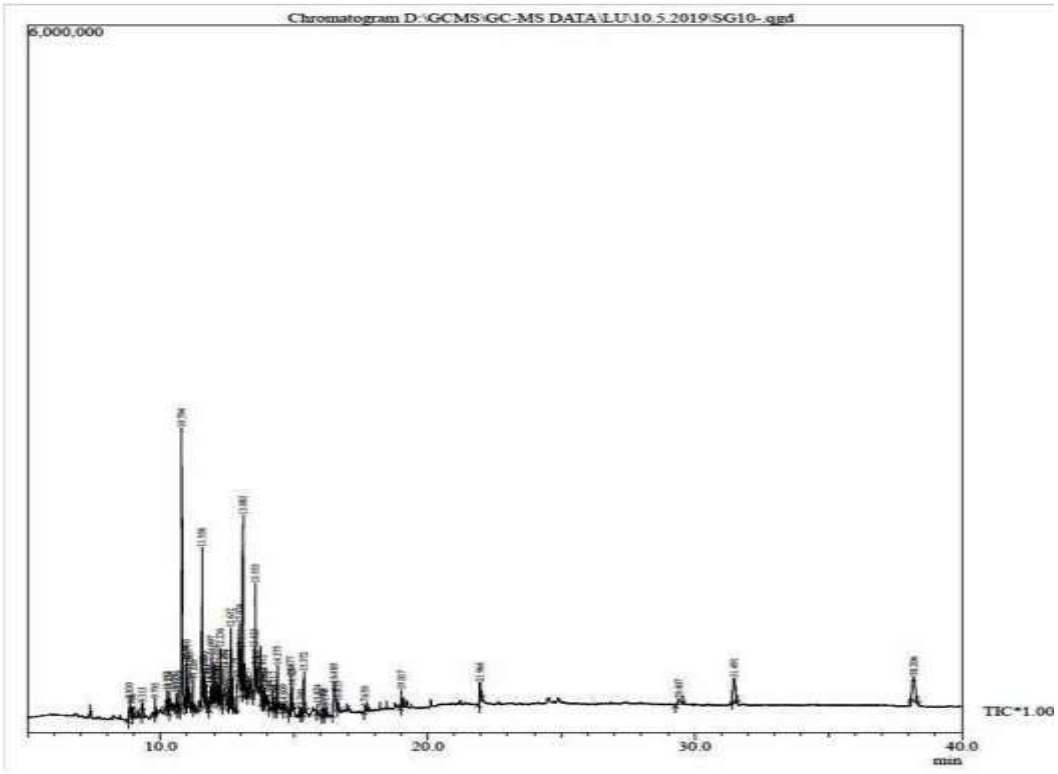

Figure 25: GC-MS chromatogram of *Stephania glabra* in methanolic extract

Table 20: Phytocomponents identified in the methanolic extract of *Viscum nepalense* by GC-MS

Peak No.	**RT (min)**	**Chemical name**	**Natures of the compound**	**Molecular formula**	**MW (g/mol)**	**Peak area (%)**
1	9.81	Benzene, 1,3-bis(1,1-dimethylethyl)	Phenylpropanes	$C_{14}H_{22}$	190.32	0.42
2	10.15	Propanediamide	Chiral diamine	$C_3H_6N_2O_2$	102.09	0.55
3	10.25	Cyclohexanol, 5-methyl-2-	Terpene alcohol	$C_{10}H_{20}O$	156.26	0.36

		(1methylethyl)				
4	10.84	3-Cyclohexene-1-methanol, .alpha.,.alpha., trimethyl-acetate	Monoterpenoids	$C_{12}H_{20}O_2$	196.29	2.93
5	11.23	3-Cyclohexene-1-ethanol, .beta.,4-dimethyl	Terpene alcohols	$C_{10}H_{18}O$	154.24	0.63
6	11.56	Caryophyllene	Terpenes	$C_{15}H_{24}$	204.35	3.06
7	12.25	Phenol, 3,5-bis(1,1-dimethylethyl)	Phenols	$C_{14}H_{22}O$	206.32	4.04
8	12.64	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	Sesquiterpene alcohol	$C_{15}H_{26}O$	222.37	1.19
9	12.98	2,7-Octadiene-1,6-diol, 2,6-dimethyl-(Z)	Terpenoids	$C_{10}H_{18}O_2$	170.25	0.89
10	13.10	2-Octene, 2-methoxy	Steroids	$C_9H_{18}O$	142.24	0.94
11	13.33	2,4,6-Trimethylbiphenyl	Sesquiterpene	$C_{15}H_{16}$	196.29	0.72
12	13.56	4-Isopropylphenyl acetate	Phenyl acetic acid ester	$C_{11}H_{14}O_2$	178.23	1.39
13	13.75	2-Hepten-4-one, 6-hydroxy-2-methyl-6-(4methyl-3-cyclohexen-1-yl)	Terpene	$C_{15}H_{24}O_2$	236.35	0.27
14	14.03	Heptacosanoic acid, methyl ester	Fatty acid methyl ester	$C_{28}H_{56}O_2$	424.7	1.69
15	15.38	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	6.29
16	16.50	Nonanoyl chloride	Acyl chloride	$C_9H_{17}ClO$	176.68	0.54
17	16.53	9-Octadecenoic acid (Z)-, methyl ester	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.73
18	19.02	Bis(2-ethylhexyl) phthalate	Phthalate ester	$C_{24}H_{38}O_4$	390.6	2.17
19	24.87	5-(7a-Isopropenyl-4,5-dimethyl-	Aldehyde	$C_{20}H_{32}O$	288.5	1.41

		octahydroinden-4-Yl) -3-methyl-pent-2-enal				
20	31.49	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Alkylbenzene	$C_{42}H_{63}O_3P$	646.9	34.79
21	38.25	Docosyl docosanoate	Alkyl ester	$C_{44}H_{88}O_2$	649.2	33.03

RT= Retention Time, MW= Molecular Weight

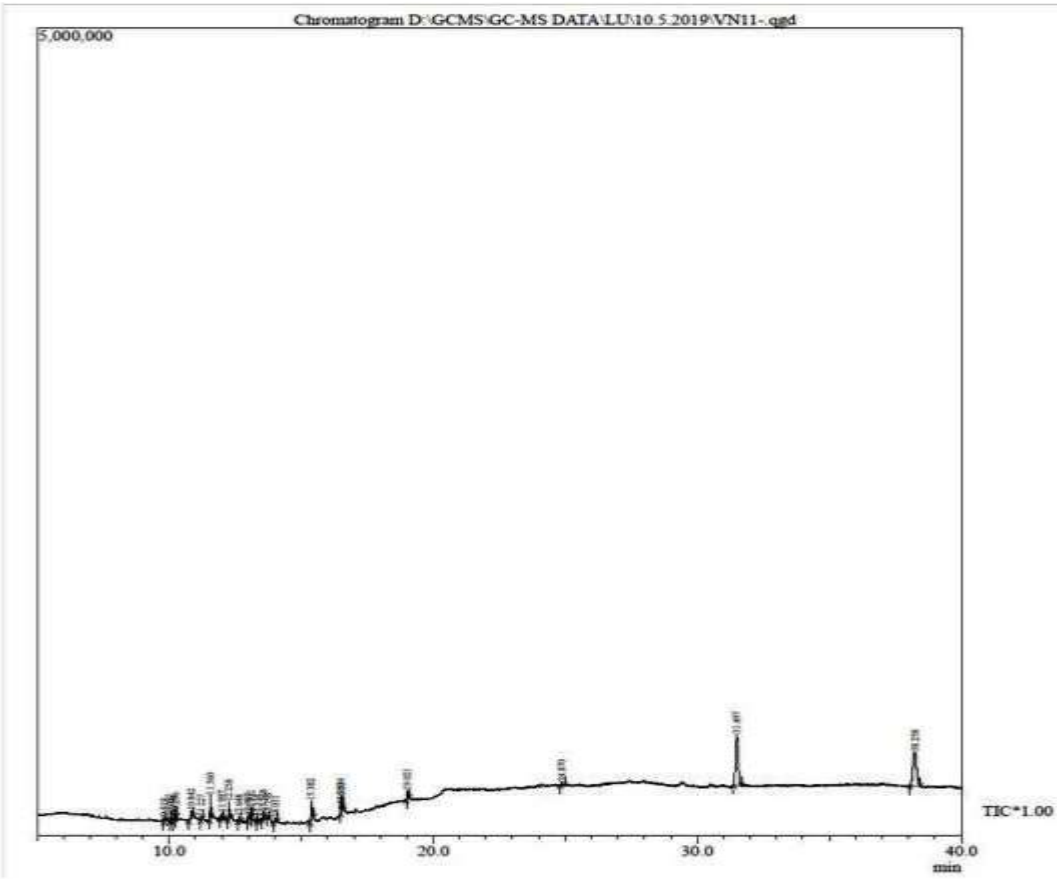

Figure 26: GC-MS chromatogram of *Viscum nepalense* in methanolic extract

Table 21: Some reported biological activities of the identified bioactive compounds

S.no	Bioactive compounds	Biological activity
1.	Limonene	Antitumor (Del Toro-Arreola *et al*., 2005), anti-inflammatory (Srivastava *et al*., 2014).
2.	beta-Sitosterol	Reduction of blood cholesterol (Vissers *et al*., 2000) anti-

		inflammation and anti-diabetic (Malek *et al*., 2009), and anti-peroxidation (Panda *et al*., 2009).
3.	Stigmasterol	Anti-inflammatory effect and cytotoxic against some cancer cell lines (Akihisa *et al*., 2000; Kpoviessi *et al*., 2008).
4.	alpha-bisabolol	Anti-inflammatory (Elsharkawy, 2016).
5.	beta-Caryophyllene	Anaesthetic and anti-inflammatory activities (Ormeño *et al*., 2008), anti-arthritic (Vijayalaxmi *et al*., 2015).
6.	Germacrene	Insecticidal, antimicrobial, and insect pheromones (Bülow & König, 2000; Yang *et al*., 2005).
7.	Chamazulene	Anti-inflammatory and anti rheumatism activity (Ramadan *et al*., 2006), Antioxidant (Sizova, 2012).
8.	Eicosane	Anti-fungal (Nandhini *et al*., 2015), Antitumour (Yu *et al*., 2005).
9.	Squalene	Antioxidant, Antitumor, and Immunostimulant (Kim & Karadeniz, 2012).
10.	Phytol	Antimicrobial, Anti-inflammatory Anticancer, cancer preventive, Diuretic, analgesic, hepatoprotective, anti-androgenic (Tamilselvi *et al*., 2018).
11.	Hexadecanoic acid, methyl ester	Antioxidant, Antiandrogenic, hypocholesterolemic, hemolytic, Alpha reductase inhibitor (Muthusamy *et al*., 2015).
12.	Tetradecanoic acid	Anxiolytic and secure lipid in biomembranes (Muthusamy *et al*., 2015).
13.	9-Octadecanoic acid	Antihypertensive, increase HDL and decrease LDL Cholesterol (Muthusamy *et al*., 2015).
14.	Nonadecanoic acid	Antihypertensive, Increase HDL and decrease LDL

		Cholesterol (Muthusamy *et al*., 2015).
15.	Betulin	Anti-HIV, anti-inflammatory, and anti-cancer (Hordyjewska *et al*., 2019), Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, lipooxygenase-inhibitor, pesticide (Evanjaline & Vr, 2018).
16.	gamma-Sitosterol	Anticancer, anti-inflammatory, and antibacterial activity (Abu-Lafi *et al*., 2019).
17.	Phthalic acid	Anti-inflammatory (Al-Gara *et al*., 2019).
18.	Octadecanoic acid, methyl ester	Antiviral activity (Linton *et al*., 2013).
19.	Coumarin	Anti-inflammatory, antioxidant (Arora *et al*., 2014).
20.	Pamidronic acid	Osteoporosis, bone loss owing to steroid use, and certain cancers with a high propensity to bone (Seifi *et al*., 2017).
21.	alpha-Cadinol	Antimicrobial, antioxidant (Ali *et al*., 2017).
22.	Elemol	Antimicrobial (Romeilah *et al*., 2016).
23.	alpha-Curcumene	Antitumor (Funakoshi *et al*., 1985).
24.	alpha-Copaene	Antioxidant, hepatoprotective, Anticarcinogenic, and anti-inflammatory activities (Junior *et al*., 2007; Vinholes *et al*., 2014).
25.	Cyperotundone	Anti-inflammatory (Gupta *et al*., 1972).
26.	alpha-Terpinene	Anti-inflammatory (Andrade & De Sousa, 2013).
27.	alpha-Humulene	Anti-inflammatory (Chaves *et al*., 2008).
28.	Heneicosane	Antitumor (Poma *et al*., 2019).
29.	Spathulenol	Antiproliferative (Wang *et al*., 2006; Bendaoud *et al*., 2010), anti-inflammatory (Apel *et al*., 2010; Dib *et al*., 2017), and antimicrobial activities (Tan *et al*., 2016).

30.	Betulinaldehyde	Antitumor (Pathak *et al*., 1988).
31.	Methyl commate B	Antioxidant, antimicrobial (Swamy *et al*., 2017).
32.	Campesterol	Anti-inflammatory (Aldini *et al*., 2014).
33.	Uvaol	Anti-inflammatory, antioxidant (Agra *et al*., 2016).
34.	Viridiflorol	Anti-inflammatory, antioxidant, and anti-Mycobacterium tuberculosis (Trevizan *et al*., 2016).
35.	Neophytadiene	Analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compounds (Raman *et al*., 2012).
36.	Isopulegol	Antimicrobial and antioxidant (Mahmud *et al*., 2009).

7.4. DISCUSSION

The GC-MS spectra of selected medicinal plants showed the presence of numerous phytoconstituents like terpenes, steroids, alkanes, alcohols, esters, fatty acids, essential oils, and phenol. The study on these selected plants revealed the presence of a large number of phytocompounds in *Equisetum diffusum* (52), followed by *Stephania glabra* (45), *Betula alnoides* (43), *Fraxinus floribunda* (38), *Rheum nobile* (34), *Litsea cubeba* (27), *Astilbe rivularis* (27), *Bergenia ciliata* (25), *Litsea glutinosa* (23), *Viscum nepalense* (21), and *Quercus lamellosa* (12) and are enumerated in **Table 10-20**. The phytoconstituents were distinguished by comparing GC-MS data with those given in the library and reported in the literature. The selected plant species comprise both similar as well as dissimilar biological active compounds.

Some of the important phytoconstituents present in these selected plants are β-Sitosterol, stigmasterol, α-bisabolol, caryophyllene, chamazulene, eicosane, squalene, phytol, hexadecanoic acid-methyl ester, tetradecanoic acid, 9-octadecanoic acid, nondecanoic acid, betulin, phthalic acid, coumarin, pamidronic acid, α-cadinol,

elemol, α-bisabolene, spathulenol, viridiflorol, etc, which have potent biological activities (**Table 21**). Out of 36 different identified bioactive compounds having reported biological activities, 20 compounds are found to exhibit anti-inflammatory and anti-arthritic properties. Among the phytocompounds present in medicinal plants, phenols, flavonoids, steroids, and terpenes are reported to have anti-inflammatory activity along with anti-arthritic properties (Soetan & Aiyelaagbe, 2009; Patel & Savjani, 2015; Ambriz-Pérez *et al*., 2016; Sarkar *et al*., 2017; Prakash, 2017; Vega *et al*., 2018). In the present plant sample the constituent of phenolic compounds viz. Phenol, 2,5-bis(1,1-dimethylethyl); 3-Cyclohexene-1-Methanol, .alpha.,.alpha.,4; 1-naphthol; Phenol, 3,5-bis(1,1-dimethylethyl) and *2,6*-Di-tert-butyl-*4-(*dimethylamino methyl)*phenol* were also present however, their anti-inflammatory activity was not reported. Nevertheless, other compounds like viridiflorol, uvaol, campesterol, spathulenol, alpha-humulene, alpha-terpinene, cyperotundone, alpha-copaene, pamidronic acid, coumarin, phthalic acid, gamma-sitosterol, betulin, phytol, chamazulene, limonene, alpha-bisabolol, and stigmasterol were present in the plant sample which may directly or indirectly involved in providing anti-inflammatory activity towards rheumatoid arthritis.

7.5. CONCLUSION

Gas Chromatography is a highly developed procedure that cannot be compared with the variant modern analytical tools but can be harmonized by mass spectrophotometer to accomplish GC-MS. It has a wide scope of applications that caters to academic research, quality control as well as industrial purposes. Its short, efficient, and automated system gives fast, reproducible, and effective results that serve a key role

in the uprising of science and technology. This versatile analytical technique could be explored for better prospects in the future.

The biological activities of compounds present in methanolic extract of selected plants support the ethnomedicinal application of these studied plants for treating rheumatoid arthritis. The study uncovered the major bioactive phytoconstituents present in the methanolic extract of the selected plants. Revealing of these bioactive constituents in the plants serves as the basis for assessing the possible medical benefits of plants that are prominent in further biological and pharmacological studies. In addition, the phytocompounds like viridiflorol, uvaol, campesterol, spathulenol, alpha-humulene, alpha-terpinene, cyperotundone, alpha-copaene, pamidronic acid, coumarin, phthalic acid, gamma-sitosterol, betulin, phytol, chamazulene, limonene, alpha-bisabolol, and stigmasterol are stated to contain anti-inflammatory activity.

Chapter 8

Summary and Conclusion

The practice of complementary and alternative remedies is more prominent at present than ever and it offers exceptional opportunities for the improvement of natural medicine. For the development of the new drug, conventional knowledge of the past and present generation is of immense importance. Investigations of plants for pharmacological applications have contributed much to the identification and isolation of compounds that are responsible for their actions and confer therapeutic values in various ailments. At present, a large number of drugs that are prescribed have been obtained from natural sources. Owing to the adaptation of modern lifestyle, associate diseases like inflammation, rheumatoid arthritis, hypertension, obesity, diabetes, etc are rapidly growing. The use of herbal plants as medicine is growing globally due to the presence of various active phytochemicals with potential therapeutic activities. Medicinal plants are considered as one of the major sources of antioxidants, anti-inflammatory as well as anti-arthritic agents with minimum or negligible side effects. The present study is the first of its kind in documenting traditional knowledge of the indigenous people of the Sikkim Himalayan region for the treatment of rheumatoid arthritis (RA) along with phytochemical analysis, *in-vitro* anti-RA activity, and analysis of bioactive phytoconstituents by GC-MS of the relevant plants.

In this study, a total of 33 ethnomedicinal plants belonging to 24 families and 29 genera were documented. Of these, 22 plants were further selected for phytochemical analysis after extraction in three different solvents: methanol, acetone, and water. The

selection of plants for the analysis was based on the number of recommendations of the informants for a particular plant species during the field study as ascertained by RFC values. Qualitative phytochemical study on these plants exhibited the existence of a wide range of phytoconstituents viz. flavonoids, carbohydrates, terpenoids, phenols, tannins, saponins, anthraquinones, alkaloids, glycosides, and phlobatannins. Quantitative estimation for some major phytoconstituents such as phenol, flavonoid, flavonol, and tannin from the above-selected plants was also performed. Comparative studies of total phenol content showed the methanolic extract of *Litsea cubeba* contained the maximum (71.72±0.022 mgGAE/g dry extract wt.) and the aqueous extract of *Kaempferia rotunda* contained the minimum (9.08±0.021mgGAE/g dry extract wt.). Similarly, the total flavonoid content was expressed as rutin equivalent (RE) per gram dry extract and the content varied among the selected plants of which the methanolic extract of *Zingiber zerumbet* contained maximum (83.5±0.022 dry extract wt.) and aqueous extract of *Litsea cubeba* contained the minimum (3.17±0.01mgRE/g dry extract wt). Regarding the total flavonol content, the methanolic extract of *Equisetum diffusum* contained a maximum 92±0.038mgRE/g dry extract wt) and water extract of *Datura metel* contained the minimum (22.9±0.003mgRE/g dry extract wt). Total tannin contents were expressed as gallic acid equivalent (GAE) per gram dry extract weight and in the selected plant species, tannin content was found to be maximum in methanolic extract of *Curcuma caesia* (72.3±0.026mgGAE/g dry extract wt.) and minimum in aqueous extract of *Pentapanax leschenaultii* (16.29±0.006mgGAE/g dry extract wt.). Regarding the quantification of phytochemical constituents, it is found that the selected medicinal plants were solvent dependent and most of the plants in methanol extract showed high content of phytoconstituents as compared to acetone and aqueous extracts. Therefore,

out of the three extracts, only methanolic extract was considered for antioxidant tests and the results showed that the selected plants contain potential antioxidant activities in three different scavenging assays which were found to increase with increasing concentrations.

Furthermore, based on the RFC value, phytochemical contents, and antioxidant activity, 11 plant species **(***Astilbe rivularis*, *Bergenia ciliata*, *Betula alnoides*, *Equisetum diffusum*, *Fraxinus floribunda*, *Litsea cubeba*, *Litsea glutinosa*, *Quercus lamellosa*, *Rheum nobile*, *Stephania glabra*, and *Viscum nepalense***)** were further selected for the determination of anti-RA activity *in-vitro* which was conducted by the methods of protein denaturation inhibition and HRBC (human red blood cell) membrane stabilization assays. Results of HRBC membrane stabilization assay suggested that all the selected plants acquired significant stabilization towards HRBC membrane in a dose-dependent manner, which increased with increasing concentration and maximum protection was provided by the extracts at 5000µg/ml of *Astilbe rivularis* (85.73±0.345), *Bergenia ciliata* (92.29±0.053), *Betula alnoides* (68.46±0.244), *Equisetum diffusum* (77.79±0.138), *Fraxinus floribunda* (73.96±0.203), *Litsea cubeba* (96.80±0.045), *Litsea glutinosa* (87.41±0.036), *Quercus lamellosa* (80.15±0.032), *Rheum nobile* (73.11±0.062), *Stephania glabra* (84.58±0.259), and *Viscum nepalense* (72.29±0.380) respectively which were found to be comparable to the standard drug diclofenac sodium (77.98±0.082). Unlike other plant samples, the stabilization activity of *Litsea cubeba* was significantly different (p=0.004, $P<0.05$) indicating better efficacy of plant extract than the standard drug. Similarly, the inhibition property of protein denaturation was also exhibited by all the selected plants which may prevent the development of rheumatoid arthritis. At 5000 µg/ml, the inhibition percentages of protein denaturation were *Astilbe rivularis*

(73.98±0.037), *Bergenia ciliata* (70.70±0.053), *Betula alnoides* (79.05±0.037), *Equisetum diffusum* (74.14±0.048), *Fraxinus floribunda* (63.29±0.038), *Litsea cubeba* (81.80±0.078), *Litsea glutinosa* (77.57±0.087), *Quercus lamellosa* (68.59±0.025), *Rheum nobile* (79.98±0.037), *Stephania glabra* (76.90±0.055), and *Viscum nepalense* (78.50±0.086) which were found to be effective and again comparable to the standard drug diclofenac (83.83±0.054). The presented results, therefore, indicated that the selected plants were capable of controlling the development of autoantigens and inhibiting protein denaturation in arthritic conditions. A T-test revealed that the protection activity of the selected plant extracts did not vary significantly from that of the standard drug, indicating that the activity of selected plant species is comparable to the standard drug utilized. The significant *in-vitro* anti-arthritis activity of selected plant extracts is possibly due to the influence of diverse phytochemical constituents like Phenols, terpenoids, tannin, etc. This finding on selected medicinal plants possessing numerous phytoconstituents, antioxidant activity, and *in-vitro* anti-RA activity justified the traditional use of the plants in the treatment of rheumatoid arthritis.

GC-MS analysis on methanolic extracts of 11 selected medicinal plants also showed the presence of numerous phytoconstituents including terpenes, steroids, alkanes, phenols, alcohols, esters, fatty acids, and essential oils. Results revealed the presence of maximum phytocompounds in *Equisetum diffusum* (52), followed by *Stephania glabra* (45), *Betula alnoides* (43), *Fraxinus floribunda* (38), *Rheum nobile* (34), *Litsea cubeba* (27), *Astilbe rivularis* (27), *Bergenia ciliata* (25), *Litsea glutinosa* (23), *Viscum nepalense* (21), and *Quercus lamellosa* (12). Some of the significant phytoconstituents present in the selected plants were viridiflorol, uvaol, campesterol, spathulenol, alpha-humulene, alpha-terpinene, cyperotundone, alpha-copaene,

pamidronic acid, coumarin, phthalic acid, gamma-sitosterol, betulin, phytol, chamazulene, limonene, alpha-bisabolol, and stigmasterol which may directly or indirectly involved in providing anti-inflammatory activity towards rheumatoid arthritis. Out of 36 different bioactive compounds identified with the help of GC-MS, 20 bioactive compounds from the 11 plant samples were found to already have reported anti-inflammatory and anti-arthritic properties. These findings are in consonance with the ethnomedicinal information obtained from the indigenous respondents from the study area about the use of plants for treating rheumatoid arthritis. In addition, this study pioneers the documentation, involving *in-vitro* anti-rheumatoid arthritis activity and phytochemicals constituents of ethnomedicinal plants validating the traditional knowledge of indigenous people of the Sikkim Himalayan region.

Future perspective

On the basis of this study for documenting ethnomedicinal plants used by ethnic people of Sikkim Himalaya for treating rheumatoid arthritis (RA) along with phytochemical analysis, *in-vitro* RA activity, and identification of bioactive phytoconstituents, it was confirmed that the ethnic people of Sikkim Himalaya have a good knowledge of medicinal plants for treating RA. And the findings of selected medicinal plants with the presence of numerous phytoconstituents, antioxidant activity, *in-vitro* anti-RA activity, and bioactive phytoconstituents are in consonance with earlier findings of bioactive components with anti-RA properties and support the ethnomedicinal uses of selected plant species for treating RA by ethnic people of Sikkim Himalayan region. Future studies, therefore, should be done on analysis of anti-RA activity by an *in-vivo* method. Furthermore, separation, characterization, and structural clarification of active compounds and clinical studies on the isolated phytocompounds will provide further light on their remedial value and usage and pave the way for the development of novel medicinal compounds for treating rheumatoid arthritis.

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Annexure

Photo plate 1: Questionnaire Survey at the study site (A)-(D).

Photo plate 2: Plants herbarium deposited in Department of Botany, Sikkim University (A) *Acorus calamus*, (B) Asparagus *racemosus*, (C) *Astilbe rivularis*, (D) *Bergenia ciliata*, (E) *Betula alnoides*, (F) *Costus speciosus*.

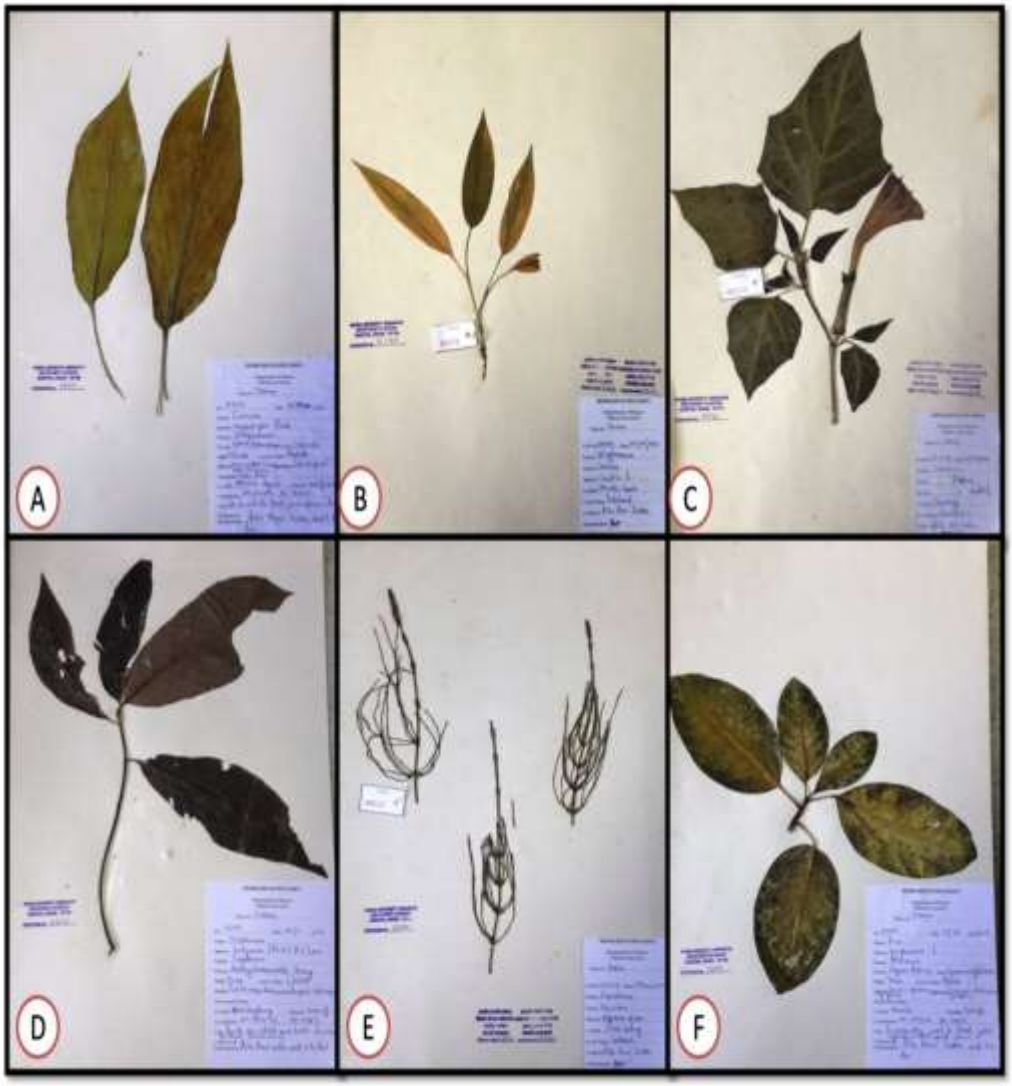

Photo plate 3: Plants herbarium deposited in Department of Botany, Sikkim University (A) *Curcuma angustifolia*, (B) *Curcuma caesia*, (C) *Datura metel*, (D) *Diploknema butyracea*, (E) *Equisetum diffusum*, (F) *Ficus benghalensis*.

Photo plate 4: Plants herbarium deposited in Department of Botany, Sikkim University (A) *Fraxinus floribunda*, (B) *Juglans regia*, (C) *Kaempferia rotunda*, (D) *Litsea cubeba*, (E) *Litsea glutinosa*, (F) *Morus macroura*.

Photo plate 5: Plants herbarium deposited in Department of Botany, Sikkim University (A) *Pentapanax leschenaultii*, (B) *Piper longum*, (C) *Prunus cerasoides*, (D) *Quercus lamellosa*, (E) *Quercus thomsoniana*, (F) *Rheum australe*.

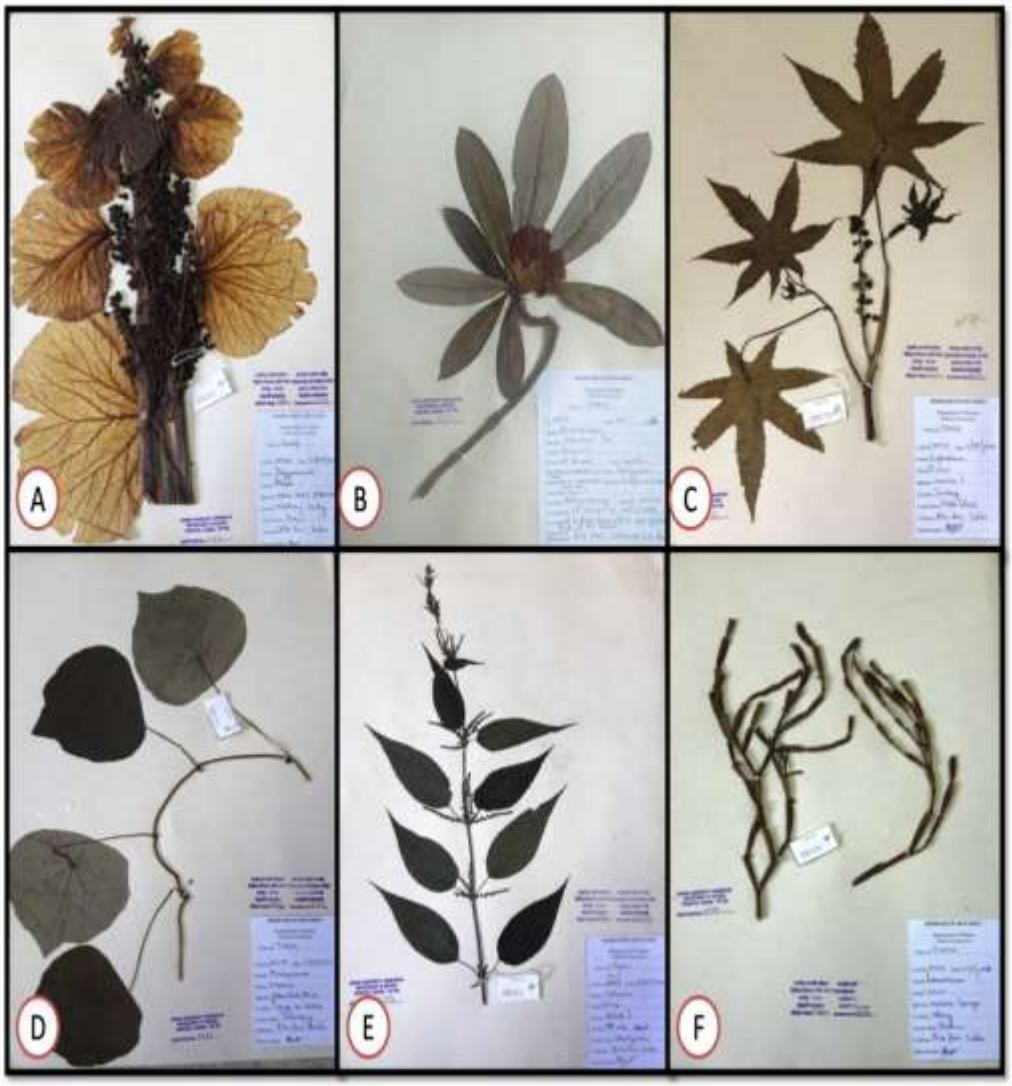

Photo plate 6: Plants herbarium deposited in Department of Botany, Sikkim University (A) *Rheum nobile*, (B) *Rhododendron arboreum*, (C) *Ricinus communis*, (D) *Stephania glabra*, (E) *Urtica dioica*, (F) *Viscum nepalense*.

Photo plate 7: Plants herbarium deposited in Department of Botany, Sikkim University (A) *Vitex negundo*, (B) *Zingiber capitatum*, (C) *Zingiber zerumbet*.

Indian Journal of Natural Products and Resources
Vol. 9(3), September 2018, pp 235-243

Phytochemical screening, physico-chemical analysis and antioxidant activity of some ethnomedicinal plants from Sikkim Himalaya

Aita Rani Subba and Santosh Kumar Rai*

Department of Botany, Sikkim University, 6th Mile, Tadong, Gangtok, 737102, Sikkim, India

Received 08 December 2017; Revised 11 May 2018

The present study was carried out to determine the phytochemical constituents, physicochemical values and antioxidant activity of six traditionally used medicinal plants from Sikkim Himalaya according to the standard pharmacopoeial method. Antioxidant activity was determined in methanolic extracts by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ferrous chelating assay and ferric ion reducing assay for methanol extracts, which showed that methanolic extracts of all six plants particularly at higher concentration exhibited better antioxidant property. The phytochemical investigation revealed the presence of various secondary metabolites such as flavonoids, tannins, phenols, terpenoids, phlobatannins, saponins, glycosides and good content of four major phytochemicals (Phenol, flavonoid, flavonol and tannin), which could account for the high antioxidant activity. The results suggest the potential of selected medicinal plants as a source of new agents for the treatment of diseases related to oxidative stresses.

Keywords: Antioxidant activity, Reactive oxygen species, Secondary metabolites, Sikkim.

IPC code; Int. cl. (2015.01)-A61K36/00, A61P 39/06

Introduction

From the ancient times, people are using medicinal plants for treating their common ailments. Nowadays uses of herbal plants are growing all over the world for the treatment of various diseases, due to their potential antioxidant activities. It is well known that oxidative stress produces the number of reactive oxygen species (ROS)/free radicals, which play an important role in the pathogenesis of various diseases including inflammatory diseases. Oxidative stress is produced when there is an imbalance between the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the amount of cellular antioxidants that can lead to multiple reactions causing damage or death of cells[1]. Due to an imbalance between body's antioxidant mechanisms and oxidative stress production, results in the development of chronic disease as autoimmunity like Rheumatoid Arthritis (RA), cancer, etc.[2-4]. Antioxidants are the compounds of nature which either prevent the production or obstruct any that are produced and prevent the progression of chain reaction produced by various ROS[5]. In the case of active RA, it is found that oxidative stress increased while the level of endogenous antioxidants decreased[6]. Antioxidants constitute an important mechanism to block the action of free radicals which are implicated in the pathogenesis of many diseases and in the ageing process[7,8]. Antioxidants are our first line of defence against free radical damages and play an important role in maintaining optimum health and well-being[9]. Most of the antioxidant compounds are derived from plant sources belonging to various classes of secondary metabolites with a variety of physical and chemical properties[10]. Although our body has effective defence mechanisms that protect itself from the harmful oxidative reaction, the ability slowly reduces with ageing, creating a need for the constant supply of antioxidants in our daily food supplements. This has led to increased interest among the researchers globally to find and evaluate the plants having effective antioxidants in order to treat the diseases related to oxidative stress like RA. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay is one of the widely used methods to test the activity of compounds to act as free radical scavengers and to evaluate antioxidant activity. This test has been the most accepted model for evaluating the free radical scavenging activity of the new drug[11]. Many free radicals can be of ferrous iron (Fe^{2+}) due to its ability to transfer single electrons, starting

*Correspondent author
Email: skrai@cus.ac.in