"OPTICAL CHARACTERISATION OF MEDICINAL PLANTS HAVING ANTI- HYPERGLYCAEMIC PROPERTIES"

Thesis submitted to Sikkim University for the partial fulfillment of the degree of Master of Philosophy in Physical Sciences (Physics).

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(भारतके संसदके अधिनियमद्वारा स्थापित केंन्द्रीय विश्वविद्यालय)
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DECLARATION

I declare that the thesis entitled **"Optical Characterisation of Medicinal Plants having anti hyperglycaemic properties"** submitted by me for the award of **Master of Philosophy (M. Phil.) Degree in Physical Sciences** of Sikkim University is my original work. The content of this thesis is based on the experiments which I have performed myself. This thesis has not been submitted for any other degree to any other University. The content of this M. Phil. thesis has been subjected to the Anti- Plagiarism Software (Ephorus) and it was found satisfactory.

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Certificate

This is to certify that the thesis entitled **"Optical Characterisation of Medicinal Plants having anti hyperglycaemic properties"** submitted to Sikkim University in partial fulfillment of the requirements for the degree of **Master of Philosophy (Science) in Physical Sciences (Physics)** embodies the result of *bona fide* research work carried out by **Mr. Abijit Bazracharza** under my guidance and supervision. No part of the thesis has been submitted for any other degree, diploma, associate-ship, fellowship.

All the assistance and help received during the course of the investigation have been duly acknowledged by him.

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For my Aja- Aji.........

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Abstract

Medicinal plants have been used for treating various diseases since ages. The uses of these plants in recent years have increased by leaps and bounds. This has resulted in more and more research to be initiated in this field. However, very less research has been done using the fluorescence and absorption spectroscopy. A large number of medicinal plants are found in the Darjeeling and Sikkim Himalayan region. The local communities use these plants for various medicinal and ethno botanical purposes. The thesis presents a study of the fluorescence and the absorption spectroscopy of the medicinal plants used in the cure of diabetes. The plants chosen are the leaves of Chiraito (*Swertia chirayita* Buch. Ham), the seeds of Totola (*Oroxylum indicum* Vent.), and the fruits of Harra (*Terminalia chebula* Retz.) and Barra (*Terminalia berelica* Roxb.). The thin layer chromatography study and the determination of the concentration of trace elements by using Atomic Absorption Spectroscopy in the said plants have also been performed. To check the validity of the plants, the results obtained were also compared with the spectroscopy of the ayurvedic medicines, Maha Sudharsan Churna Vati, Dashmool Kwath, Haritaki Churna and Triphala Churna prescribed for the diabetes

The fluorescence and the absorption study of these medicinal plants revealed the presence of various constituents like flavonoids, terpenes, alkaloids, quercetin, berberine, etc. These constituents have the ability to cure diabetes. Moreover some of these constituents like berberine, quercetin have already been isolated and are prepared synthetically for the treatment of diabetes. As the leaves of Chiraito are boiled and consumed, to check the validity of the method, the temperature and time dependence study was done. The thin layer chromatography also revealed the presence of various components both polar and non-polar.

The atomic absorption study on these plants revealed that they had the presence of all the trace elements tested. As these elements have direct relation with the treatment of diabetes, they help in curing the disease. The comparison of the results with that of the ayurvedic counterparts revealed that these plants were a part of these medicines.

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List of symbols and abbreviations

BC	Before Christ
β	Beta
AYUSH	Ayurveda, Yoga, Unani, siddha and Homeopathy
I	Incident intensity of radiation
I_0	Transmitted intensity
c	Concentration of the solution
l	Path length
κ	Extinction coefficient
T	Transmittance
ϵ	Molar absorption coefficient
A	Absorbance or optical density
λ	Wavelength
K	Potassium
Ca	Calcium
Mn	Manganese
Cu	Copper
Zn	Zinc
Govt.	Government
HCl	Hydrochloric acid
HNO_3	Nitric acid
H_2SO_4	Sulphuric acid
H_2O_2	Hydrogen peroxide
DCM	Dichloromethane
TLC	Thin Layer Chromatography
UV	Ultraviolet
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
Rf	Retention factor
NA	Not available

conc	Concentration
ppm	Parts per million

PART I
INTRODUCTION

Chapter 1
Introduction

1.1. Introduction

Traditionally, medicinal plants are in use for treating various diseases by human beings ever since the dawn of human civilization. Thousands of plant species all around the world are found to have active constituents having direct action on the human body[1]. The history of the medicinal plants and their uses dates back to the pre and early Christian era[2]. These treatments varied over the different traditions and civilizations. These plants apart from being used for curing various diseases have also remained an important aspect in the everyday life; some of them are even used in various religious ceremonies and customs[1]. In the earlier days, the forests provided human civilizations with all the basic necessity like food, water, shelter, medicines etc.[3].

The traditional medicine systems are based on the empirical findings of hundreds and thousands years of their use[4, 5]. The Indian System of Medicine which consists of Ayurveda, Siddha and Unani is considered to be one of the oldest holistic management system in which the majority of the remedies are derived from the plants and their products[6]. More than 80% of the world's population, according to the estimates of the World Health Organisation depends directly or indirectly on the plants for medicine[1, 7]. Also, it has been found that up to 40% of the pharmaceuticals products in the industrialised countries have been derived from the plants[7, 8].

India and China, the two largest countries in South Asia have the richest arrays of registered and relatively well known medicinal plants[9]. Though India has just 2.4% of the total geographical area, yet it accounts for about 8% of the total global diversity. India is known for its rich repository of biological wealth[10]. It has been estimated that there are around 49,000 plants in India of which 17,000 are species of higher plants; also around 4900 plants are endemic and around 7,500 species are used as medicines[11, 12]. The ecosystems of Himalayas, the Khasi and Mizo hills of North Eastern India, the Vindhya and Satpura ranges of the Northern Peninsula and the Western Ghats are the rich treasure houses of bio diversity and accounts for nearly 90% of the country's higher

plants species[12]. The Indian Himalayas alone has around 8000, 44, 600 and 1748 species of angiosperms, gymnosperms, pteridophytes and medicinal plants respectively[13, 14]. Several species of these plants are endemic to the region i.e. they are native to this region[4].

The ministry of Environment and Forests, Government of India estimates that there are around 25,000 effective plant based formulations available in the Indian medicine system[15]. Ancient Indian literatures like the Rig Veda (400-1500 B. C.), Artharva Veda (1500 B. C.) and Ayurveda (1000- 600 B. C.) mention several medicinal plants and their uses[1]. The oldest system of medicine in India which is prevalent even today is the Ayurveda, which alone reports around 2,000 medicinal plants[4]. The Ayurveda flourished greatly in the Northern India. In Southern India, it was taken over by the Siddha. Apart from these, the other systems of medicine prevalent in the country are the Unani and the Tibetan systems[16]. The Charak Samita is one of the oldest written document on herbal therapy that reports of the production of 340 herbal drugs and their indigeneous uses, thus is one of the valuable Indian record for medicinal plants[2, 17]. The rise of Buddhism and the European invasion further enriched the materia medica of the country[2].

In the Darjeeling Himalayas which constitutes the hilly region of Darjeeling and Sikkim, many systems of herbal medicines are practiced which includes the Nepali, Bhutia and the Lepcha system[15, 18]. These systems have been found to be closely associated with the Ayurveda, Folk Medicine, Homeopathy, Siddha, Unani and the Amchi (Tibetan) systems. The spiritual healers as Jhakri, Bijuwa, Dhami, Phedangma, Baidhya (Nepali), Boongthing (Lepchas) and Amji, Pow (Bhutias) are the main guardians of these knowledge of medicinal plants[15, 19, 20]. Around 424 species of medicinal plants found in Sikkim are being presently used in preparing medicines[12]. The largest district of Sikkim, the North Sikkim district, with an area of 4226sq. km. and elevation of 1000-8598m, has the largest number of these medicinal plants[12]. However, though Sikkim is rich in bio-diversity with a large variety of medicinal plants; only a few traditional plants have received scientific or medical scrutiny[21].

With the development of the allopathic method of treatment, the use of herbal medicines gradually declined. The knowledge started becoming obsolete due to lack of recognition by the younger generation because of the shift in attitude and ongoing socio economic changes[22]. However, at present, the demand and consumption for herbal medicine is rising rapidly primarily because of its profound usage in curing various diseases like breast, lung and bowel cancer, heart diseases, rheumatism etc. among others and also because of minimal side effects[23, 24]. The rise has put a heavy strain on the existing bio resources as some species of medicinal plants are being increasingly threatened. The random extraction of medicinal plants has led to overharvesting which could even lead to extinction of the plants.

Diabetes Mellitus, commonly called Diabetes is one of the rapidly growing diseases and is nearing epidemic proportions. Diabetes Mellitus is also known as hyperglycaemia; it is made up of three words: "Hyper" meaning excessive, "glyc" meaning sweet and "emia" meaning of blood [14, 16]. In this disease, the presence of sugar is found to be increased in the blood. There are two main reasons for the occurrence of Diabetes Mellitus [14, 16]:

- Insulin dependent Diabetes Mellitus: Here the body has insufficient or no production of insulin.
- Non-insulin dependent Diabetes Mellitus: In this case, the body does not properly utilize the insulin or is resistant to insulin.

Insulin is the hormone that is produced from the β cells of the pancreas. Its main function is to converts sugar, starches and other foods stuffs into energy[8]. At present the world has around 171 million people suffering from Diabetes, the Indians being in the majority[26]. Many traditional medicinal plants are being used by various communities for the treatment of diabetes; thus they are the hidden wealth of potentially useful natural products to control diabetes[27, 28].

Spectroscopy being one the major analytical tool used in the pharmaceutical industry; they can be used to check and identify the contents to some extent. Today, various spectroscopic methods are available like Raman, FTIR, Absorbance, Fluorescence etc. which can assist in identifying the contents with high accuracy. In a recent work, the use of FTIR Spectroscopy on *Tephrosia tinctoria* and *Atylosia albicans* has shown the

presence of oligosaccharides, phosphates, protein, carbohydrates and carotenoid[28]. In other work, the Fluorescence Spectroscopy analysis was done on Chiraito and has revealed the presence of flavonoid[29].

In this thesis, attempt has been made to understand the constituents of the few medicinal plants by the use of various spectroscopic methods which are being used for curing diabetes by the local masses. The plants chosen are given in Table 1.1[12]:

Table 1.1: Details of the targeted medicinal plants

S. No.	Local Name	Scientific Name	Plant part
1	Chiraito	*Swertia chirayita* Buch. Ham	Leaves
2	Totola	*Oroxylum indicum* Vent.	Seeds
3	Barra	*Terminalia berelica* Roxb.	Fruits
4	Harra	*Terminalia chebula* Retz	Fruits

Further, the obtained spectral results have been compared with their respective ayurvedic counter parts (Maha Sudarshan Churna Vati, Dashmool Kwath, Triphala Churna and Haritaki Churna).

1.2. Objectives:

The main objective behind the study was to identify the local available medicinal plants and find out the constituents present in the plants so as to justify that these plants do help in the curing of Diabetes. The various objectives of study were:

1. **Spectroscopic characterisation of the medicinal plants extract:** The leaves of Chiraito, the seeds of Totola and the fruits of Barra and Harra were chosen for spectroscopic characterisation. The extract of the selected plant parts were prepared in ethanol and distilled water; except that of Chiraito where only the water extract was prepared were spectroscopically characterised. These plant parts have been identified, referred and authenticated by Dr. T. K. Mandal, Research Officer [S-2] in Charge, Ayurveda Regional Research Institute, Department of AYUSH, Gangtok (Appendix 1).

2. **Verification of the traditional practice of consuming medicine:** Chiraito is known to be widely used in curing diabetes and high pressure. Traditionally, the leaves of

Chiraito are boiled in water and the extract is consumed. However, the time of boiling for getting optimal result is not known. For the verification of the same the temperature and time dependent properties of this procedure was also studied.

3. **Comparison of the obtained optical spectra from that of ayurvedic counterparts:** The ayurvedic medicines having the selected medicinal plants as their constituents were selected. The ayurvedic medicines were Maha Sudarsana Churna Vati for Chiraito, Dashmool Kwath for Totola, Haritaki Churna and Triphala Churna for Harra, and Triphala Churna for Barra. These medicines were supplied by Ayurveda Regional Research Institute, Gangtok after due consultation with Dr. T. K. Mandal and Dr. S. K. Debnath, Research Officers of the said institute (Appendix 2). The ethanol and water extract were prepared as that of the plants and their optical characterisation were done. The results were then compared with that obtained from the plants.

1.3. Outline of the thesis

Optical characterisation on the medicinal plants was done and the results were compared with the ayurvedic counterparts. This thesis is structured as follows:

Part I: Introduction

Chapter 1 gives a brief introduction about the medicinal plants and the related works. It also discusses about the objectives and gives a brief outline of the work done.

Chapter 2 discusses some of the basics applications of spectroscopy in the determination of the constituents of the medicinal plants in light of the recent literatures. It also discusses about the chosen medicinal plants and their constituents as have been determined from the phytochemical analysis of the plants. The chapter further discusses about the various trace elements required for the human body and the determination of the trace elements.

Part II: **Materials and methods**

Chapter 3 introduces the materials and methods used in the study. It discusses about the collections of the plant parts and the ayurvedic medicines, the preparation of extracts, the various digestion techniques and the instruments employed.

Part III: Results and Discussions

Chapter 4 discusses the results obtained for Chiraito and it ayurvedic counterpart, Maha Sudharsan Churna Vati. The temperature and post boiling time dependent study on the extract of the leaves has also been discussed.

Chapter 5 discusses the spectroscopic analysis of Harra and its ayurvedic counterparts Haritaki Churna and Triphala Churna. A detailed comparison on the basis of the results obtained has also been done.

Chapter 6 discusses the results obtained from Barra and its ayurvedic counterpart, Triphala Churna where the detailed comparison has been done.

Chapter 7 discusses the optical results as has been obtained from Totola and its ayurvedic counterpart, Dashmool Kwath.

Chapter 8 discusses the results obtained from Atomic Absorption Spectroscopy for all the plants and the ayurvedic counterparts.

Part IV: Conclusion

Chapter 9 gives the summary of the thesis and also discusses about the potential areas for further research.

Part V: Appendix

This section contains the certificates authentication the medicinal plants and their ayurvedic counterparts.

Chapter 2
Literature Review

In this thesis the targeted medicinal plants that are locally used for curing diabetes have been probed by various spectroscopic techniques. For the same the basic workings of the various spectroscopic techniques used in the work have been discussed. The review of the work done so far on the selected plants have also been elaborated

2.1. Spectroscopic techniques

Fluorescence and absorption spectroscopic techniques have been widely used in the fields of biophysics, biochemistry, pharmacology, etc. for determining the constituents and the characteristics of the various medicinal plants[30-33]. Due to their versatility, high accuracy, simplicity, and cost effectiveness, these techniques have become very important analytical instruments in the laboratory[30-33]. Though the components of targeted plants have been studied by various other methods, the same has not been done in large extent using fluorescence and absorption spectroscopy[34, 35].

Absorption is the phenomenon due to the interaction of radiation with a sample. This results in the transition of the absorber (atoms or molecules) from lower energy level to higher energy level due to absorption of energy i.e. photons (Fig 2.1)[36, 37]. Absorption spectroscopy measures the absorption of radiation as a function of frequency or wavelength. The absorption spectroscopy is performed across the electromagnetic spectrum. The spectra obtained are the characteristics of the molecular structure and they can be also used for the identification of the atomic and the molecular species[38]. The concentration of the sample can also be determined using the absorption spectra with the help of Beer- Lambert Law given by:

$$I/I_o = \exp[\kappa c l]$$

where, I is the incident intensity of radiation, I_o is the transmitted intensity, c the concentration of the solution, l the path length and κ is a constant which depends on the

spectroscopic transition under investigation also called extinction coefficient[39,40,41]. The above equation can also be written as

$$I/I_o = 10^{-\epsilon cl} = T$$

where, T is the transmittance which gives the ratio I/I_0; ϵ is the molar absorption coefficient[39, 40, 41]. Inverting the above equation and taking logarithms, we get

$$I_o/I = 10^{\epsilon cl}$$

$$\text{or, } \log[I_o/I] = \epsilon cl = A$$

where, A is the absorbance or optical density. The absorption coefficient depends on the material and the wavelength of the absorbed light[39, 40, 41]. The absorption coefficient is related to the extinction coefficient by the relation:

$$\epsilon = \frac{4\pi\kappa}{\lambda}$$

where, λ gives the wavelength of incident light[42]

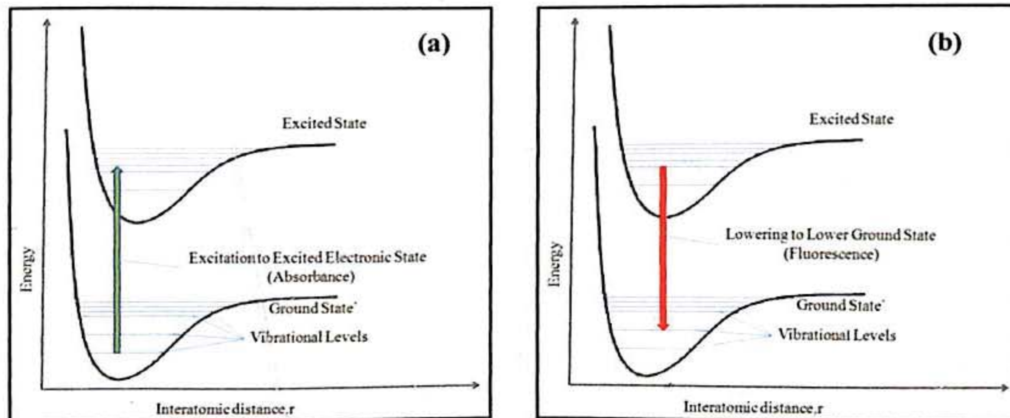

Fig. 2.1: Transition during (a) Absorption, and (b) Fluorescence

After the absorber is in higher vibrational state, the excess energy is lost due to the intermolecular collision and reaches the lower vibrational state. From here, they emit radiation of a different wavelength which is called fluorescence spectrum and reverts back to the ground state (Fig 2.2.)[39, 40, 41].The spectra obtained from the sample gives

both the qualitative and quantitative information of the sample[42]. The emitted radiation normally is of lower frequency than that of initial absorption[39, 40, 43]. The main advantage of fluorescence spectroscopy over the radioactivity and absorption spectroscopy is that it can separate the compounds on the basis of their emission or excitation spectrum[44,45].

Atomic Absorption Spectroscopy is another analytical tool that is widely used for the analysis of various trace elements[46, 47]. The functioning of this technique is based on absorption spectroscopy. Here, the sample is converted to ground state free atoms in vapour state[48]. A lamp of a particular element emits light from the excited elemental atoms; this light passes through the excited atoms in the sample and the radiation is absorbed by the atoms of the same element[48]. The more the amount of the elements, more is the absorption since the absorption is proportional to the number of atoms. The amount of radiation absorbed is compared with the calibration curve constructed by running several known concentration of the element and the concentration of the element in that particular sample is calculated[48].

2.2. Phytochemical analysis on targeted plants

The phytochemical analysis which is basically done by using specific tests like Dragendirff's test for detection of alkaloid, Shinoda test for detection of flavone etc.[49] have been performed on the targeted medicinal plants and the review of the findings are as follows:

2.2.1. Totola

The pods, seeds, stem and root barks of Totola have been found to contain many flavones, weak acids and traces of alkaloids[50]. The leaves contain baicalein-6-glucuronide, baicalein-7-glucuronide, scutellarein, scutellarein-7-glucuronide, alo-emodin and stem bark contains oroxylin-A, baicalein, scutellarein, coumaric acid[51-53].Root bark has been found to contain oroxylin-A and ellagic acid[54]; the wood contains prunetin and ß-sitosterol[55]; the seeds have been found to contain oroxindin, baicalein-6-glucoside, tetuin, glucoside and fixed oils[56-59].

2.2.2. Chiraito

The major constituents of Chiraito are xanthones, triterpenoids, alkaloids, and secoiroids[60]. The bitterness, anthelminthic, hypoglycemic and antipyretic properties are attributed to amarogentin, swerchirin, swertiamarin and other active principles of the plant[61]. It has been found out that decussatin, bellidifolin, isobellidifolin, swertianin, amarogentin, swertianolin and mangiferin are the major phenolic compounds responsible for the free radical-scavenging activity of Chiraito[62]. The active components have been found as swertiamarin and mangiferin flavonoids, iridoid glycosides, dimeric xanthones, and triterpenoids[63, 64]. Apart from these, the plants also contains amaroswerin, b-amyrin, chiratanin, chiratin, chiratogenin, decussating, 1,8-dihydroxy-3,7-dimethoxyxanthone, enicoflavine, gentianine, gentiocrucin, gentiopicrin, 1-hydroxy-3,5,8-trimethoxyxanthone, lupeol, mangiferin, oleic acid, ophelic acid, palmitic acid, resin, stearic acid etc.[65].

2.2.3. Harra

The fruit of Harra has the presence of constituents such as alkaloids, carbohydrates, tannins, and phenolic compounds, flavonoids, saponins, proteins, amino acids, and steroids. The medicinal properties of Harra are attributed to the presence of anthraquinone in the young fruit and tannins in the ripe ones[66-68]. The major tannins found in the fruits are chebulinic acid, chebulagic acid, corilagin along with minor tannins like chebulic acid, galic acid, ellagic acid, terchebin, beta glucogalin, 3-6-digalloylglucose, 1,3,6-tri-O-galloyl-beta-glucose, amino acids, sugar, palmitic acids, etc.[66]. The plant is known to be rich in polyphenolic compounds and the fruits are rich in phytochemicals[69]. It has been found to be antidiabetic, antimutagenic, antifungal, antibacterial and antiviral[70-74].

2.2.4. Barra

The phytochemical analysis done on the fruit of Barra has the presence of alkaloid, steroid, saponins, anthraquinone glycoside, flavonoids, polysaccharides and tannin[49, 75].

2.3. Trace elements

The trace elements plays a vital role in producing the bioactive chemicals in medicinal plants and thus are responsible for their medicinal properties[76].Various research studies have observed the direct association of the trace elements with diabetes[77].The enhancement of insulin action by the trace elements includes the activation of insulin receptor sites which serves as cofactors for enzyme systems that are involved in glucose metabolism, increasing sensitivity etc.[78, 79, 80]. During hyperglycaemia, increased protein glycosylation and varied alterations in the status of the trace elements have been observed[81]. The elements like K, Ca, Mn, Cu and Zn are responsible for the secretion of insulin from the beta cells of the pancreas[82]. Optimum potassium and calcium levels in the body are a must for proper insulin secretion and as such play a vital role in lowering the blood glucose[82]. The role of optimizing carbohydrate metabolism and potentiating insulin is played by chromium[82].

Zinc has been found to have an important metabolic role in production and managing the levels of insulin[82].It promotes cell reproduction, growth of the body tissues and their repair[83]. For an average adult, the total daily requirement of zinc is about 15 milligrams[83]. It is also required for insulin synthesis and storage, the insulin is secreted as zinc crystals and as such maintains the structural integrity of insulin[84].

The estimated daily intake of manganese is 18 mg/day[83, 85]. The deficiency of manganese leads to glucose intolerance[76]. The deficiency produces defective cells in the pancreas and a smaller number of pancreas islet cells which contain fewer β cells that manufacture insulin[86, 87]. Iron's main job is to carry oxygen in the haemoglobin of red blood cells[83]. The total daily requirement for iron is about 18 milligrams[82]. It generates reactive oxygen species and contributes to diabetic neuropathy[88]. Copper is essential for the formation of haemoglobin and is needed to carry oxygen in red blood cells[88]. The daily requirement of copper is about 2.0 milligrams for the average adult[83]. The copper ion serves as an important catalytic cofactor for biological

functions in the body[89]. Sodium takes part in activation of enzymes and in secondary active transport[83].

2.4. Polyherbal formulations

Various medicinal plants are being widely used to prepare the polyherbal formulations; they are available in the form of vati, churna, arka, quath etc. Some important formulations that are available in the market for treating diabetes are madhuhari powder, dianex, madhumeha churna, glucocare, madhunasini vati, diaveda capsule, diamed etc.[8]. However, as these formulations doesnot contain pure compounds extracted from the plants, the Dept. of AYUSH does not give validity to these formulations[90]. They have their own ayurvedic formulation certified by the Govt. of India and is prescribed in the list of Indian Pharmacopeia[90].

PART II
MATERIALS AND METHODS

Chapter 3
Materials and methods

3.1. Plant parts

The plant parts chosen for the study were the leaves of Chiraito, the fruits of Harra and Barra and the seeds of Totola.

The dried leaves of Chiraito were collected from 6th mile, Rongli Rongliot from the district of Darjeeling, West Bengal, India. The dried leaves are boiled and taken by the villagers to cure various diseases. Fig. 3.1(a) shows the dries leaves of Chiraito.

The fruits of Totola were collected from the herbal medicinal practitioner from Ranipool, Gangtok, East Sikkim. Fig. 3.1(b) shows the seeds of Totola.

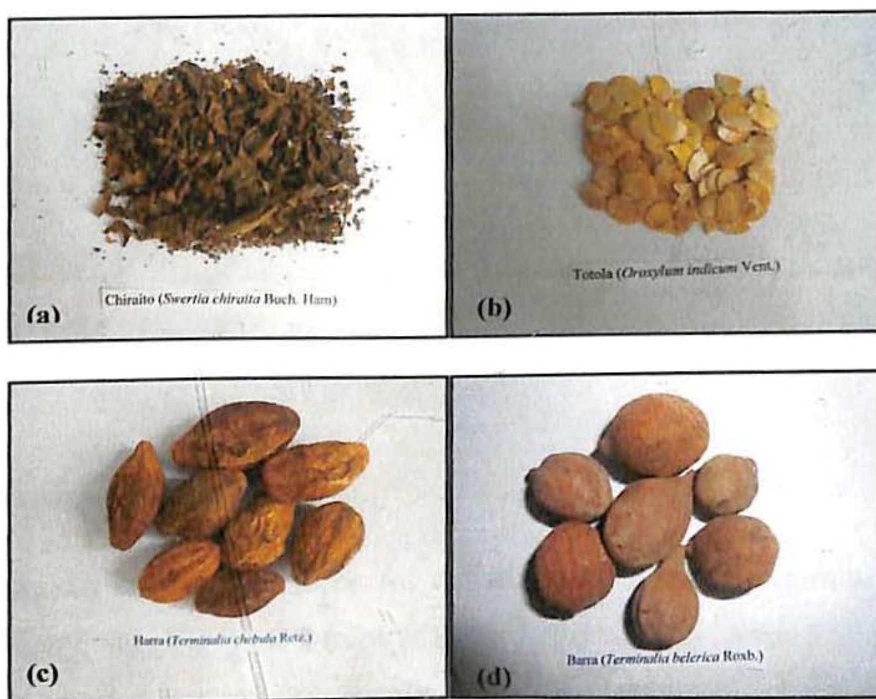

Fig. 3.1: (a) Leaves of Chiraito, (b) Seeds of Totola, (c) Fruits of Harra, and (d) Fruits of Barra

The fruits of Harra and Barra were collected from the herbal medicinal practitioner from Kurseong, Darjeeling, West Bengal. Fig. 3.1 (c) and 3.1 (d) shows the fruits of Harra and Barra respectively.

3.2. Ayurvedic Medicines

To compare the results obtained with the ayurvedic counterparts, four ayurvedic medicines having the presence of the medicinal plant as one of its component were chosen. The chosen medicines were Mahasudarshan Churna Vati, Dashmool Kwath, Haritaki Churna and Triphala Churna. Fig. 3.2 (a) to 3.2 (d) shows the said medicines.

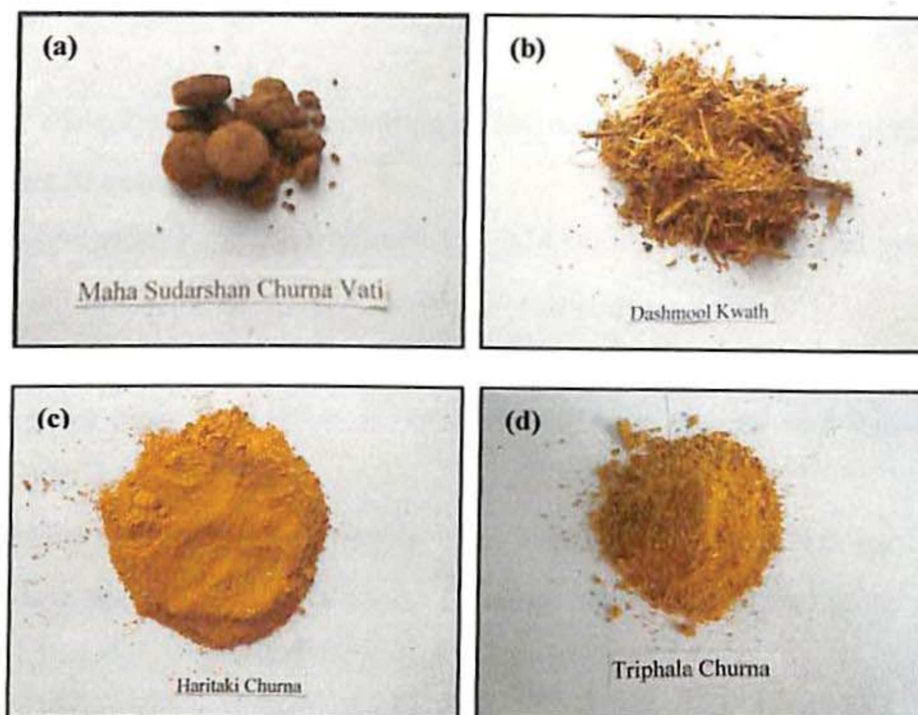

Fig. 3.2: (a) Maha Sudarsana Churna Vati, (b) Dashmool Kwath, (c) Haritaki Churna, and (d) Triphala Churna

3.3. Chemicals

All the chemicals used in the study for the digestion of the samples and Thin Layer Chromatography were purchased from Merck Chemical Ltd., India. The standards used for Atomic absorption spectroscopy were supplied by Perkin Elmer Limited, U. S. A. The deionized water for Atomic Absorption was collected from RCIBSD, Gangtok where it was prepared using Millipore deionization unit (Millipore, Bedford, MA, USA).

3.4. Preparation of the extracts for Fluorescence and Absorption Spectroscopy

The preparation of the extract was done by the process of maceration which was done in the following way [91- 95]:

1. The plant parts were collected from various places as it has been mentioned above.
2. After the collection of the plant parts, the required parts were segregated from the unwanted ones.
3. The segregated parts were then thoroughly cleaned with water so as to remove the soil and other dust particles that might be present in them.
4. The cleaned parts were sundried to remove the excess water.
5. The plant parts were kept soaked overnight in ethanol and distilled water in separate labelled containers.
6. The plant parts were then crushed with the help of mortar and pestle.
7. As some portion of the plant parts however remained in solid form, the same was filtered so that only the extract was obtained.
8. The filtered extracts were stored in labelled glass jars in refrigerator and were used for the purpose of experiment.

However, for Chiraito the study was performed only in water extract and after Step 6, the following process was followed.

7. Some amount of sample was extracted from the solution at room temperature (18°C).
8. The remaining sample was heated in a heating mantle (Model No. W-350, Zenith Glassware and Instrument Corporation, Kolkata, India), from 50 °C with an increment of 10°C till it reached the boiling point.
9. In each step, the extract was collected.
10. Post boiling, the solution was collected at an interval of every 2 minutes.
11. The extracts were filtered and stored in labelled jars in refrigerator and used for experiment.

For the thin layer chromatography experiments, the water extract was completely evaporated using a rotary evaporator (Make: BUCHI, Switzerland) and a few drops of methanol were then added to get the extract for TLC analysis. The TLC plates were viewed under the UV Fluorescence Analysis Cabinet (Make: Zenith Glassware and Instrument Corporation, Kolkata, India).

3.5. Sample digestion procedures for atomic absorption spectroscopy

3.5.1. Digestion of the plant parts:

Three methods of digestion were employed primarily because there was no standard procedure for digesting plant parts; the digestion methods employed were the following:

a. Aqua regia extraction[96]

1.5 g of the sample was taken in a 250 ml Borosil beaker and 14 ml of 3:1 mixture of 37% HCl: 70% HNO_3 was added. It was then left for 16 hours at room temperature. The mixture was then digested in a hot plate for 2 hours at 130 °C. The suspension obtained was then filtered with ashless Whatman 41 filter paper and was diluted with 100ml of deionized water.

b. Acid extraction with HNO_3[96]

1g of the sample was taken in a 250 ml Borosil beaker and 50 ml of 70% HNO_3 was added. The solution was then kept at room temperature for 16 hours. The digestion was then done at 140 °C for 2 hours and 30 minutes. The suspension was then filtered using ashless Whatman 41 filter paper and diluted with 50 ml deionized water.

c. Acid extraction with HNO_3, H_2SO_4 and H_2O_2[97]

1g sample was mixed with 5ml 70% HNO_3 and 5ml H_2SO_4. It was then heated in a hot plate up to 100 °C for 5 minutes. Further, 5ml 70% HNO_3, 5ml H_2SO_4 and 2ml of 30% H_2O_2 were added and the solution was gently heated at 60-70 °C. The suspension was then filtered using ashless Whatman 41 filter paper and diluted with 25 ml deionised water.

Three replicates of each of these methods were prepared and the results were analysed by taking their mean.

3.5.2. Digestion of the ayurvedic formulations[98]

2g of the sample was taken in 10ml nitric acid and was heated in hot plate for 15 minutes at 95°C. It was cooled and 5ml nitric acid was added again and was heated in hot plate for 30 minutes at 95°C. This process was repeated again till the volume was reduced to 5ml. The solution was then cooled, 2ml deionised water and 3ml 30% hydrogen peroxide was added to start the peroxide reaction. It was cooled again and 5ml concentrated sulphuric acid and 10ml deionised water was added and heated at constant temperature below boiling point for 15 minutes. The solution was filtered using ashless Whatman 41 filter paper and diluted with 25ml deionised water [98].

3.5. Preparation of the standard solutions for atomic absorption spectroscopy

The individual element working standard solutions [1, 10, 25, 50, 75, 100, 150, 200 mg/l] were prepared by appropriate dilution from the stock element standard [1000mg/l, Perkin Elmer, USA]. The calibration curves for each element were linear and the correlation coefficients ranged from 0.995 to 0.999.

3.6. Experimental techniques

3.6.1. Optical measurements:

The optical measurements were done using the absorption and the fluorescence spectroscopy and the results obtained were compared with that available in the referred literature.

a. Absorption Spectroscopy:

The absorption spectroscopy was performed using a Perkin Elmer made Lambda 750 spectrophotometer (Fig. 3.3) which was equipped with Deuterium and Tungsten lamps

as the light source emitting in the range of 190nm to 380nm and 380nm to 800nm respectively. The incident beam, after passing through the grating monochromator gets directed to the sample in the solvent and the reference solvent. The sample absorbs, transmits and/or reflects the light and the intensity of the emerging light is measured by the detector. The studies were done in the scanning range of 250 nm to 800 nm. The absorption spectrum of the solvent was also measured as a reference which was then subtracted from the corresponding spectra obtained from the plant solutions.

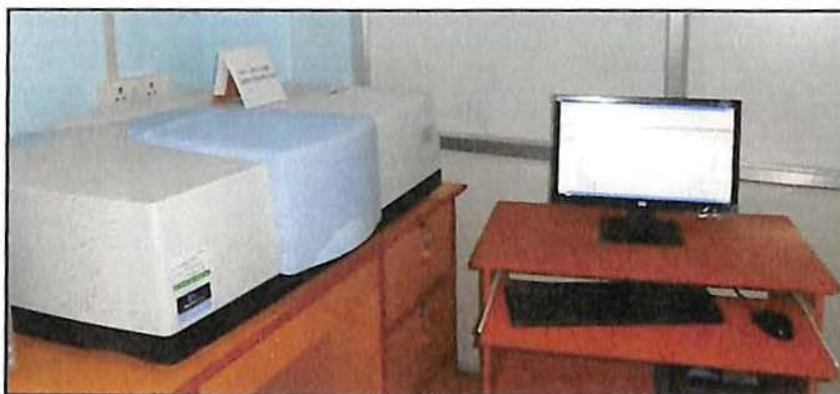

Fig. 3.3: Lambda 750 Spectrometer

b. Fluorescence Spectroscopy

Perkin Elmer LS55 Fluorescence Spectrometer (Fig.3.4) was used for the study of the Fluorescence Spectra of the samples. The excitation was done using the white light at 370nm and the emission was recorded from 400 to 700nm. Here, also the spectrum of solvent as a reference was recorded and was used for background correction.

Fig. 3.4: LS55 Fluorescence Spectrometer

3.6.2. Mass spectroscopic analysis

For the determination of the trace elements, atomic absorption spectroscopy was used. Perkin Elmer made AAnalyst 200 Spectrometer (Fig. 3.5) was used for the determination of the trace elements. Flame atomic absorption technique was employed. For each element, hollow cathode tube was used as radiation source and air acetylene gas was used for all experiments. The flow rate of acetylene was 2.5 l/ min and that of air was 10 l/min.

Fig. 3.5: AAnalyst 200 Spectrometer

3.6.3. Chromatography study

As a backup to the absorption and fluorescence study, thin layer chromatography was performed in the plant samples. The thin layer chromatography analysis was done using trial and error method. The eluent used for Chiraito and its ayurvedic counterpart, Maha Sudarshan Churna Vati was 70% methanol and 30% DCM. For Harra, Barra, Haritaki Churna and Triphala Churna, the eluent used was using ethyl acetate, toluene, methanol and acetic acid in the ratio 75:20:4.5:0.5; while that for Totola and Dashmool Kwath the eluent used was 50% methanol and 50% DCM.

The TLC plates were viewed using the UV Fluorescence Analysis Cabinet (Fig. 3.6). The plates were viewed under daylight, short wavelength and long wavelength conditions. In short wavelength, the wavelength used was 254nm and in long wavelength it was 366nm.

Fig 3.6: UV Fluorescence Analysis Cabinet

PART III
RESULTS AND DISCUSSIONS

Chapter 4

Chiraito: Temperature dependent fluorescence and absorption spectroscopy

4.1. Fluorescence Spectroscopy analysis

Fig. 4.1(a) and (b) shows the fluorescence spectra of Chiraito boiled at 98 °C for 4 minutes and that of Maha Sudarshan Churna Vati. The spectra were obtained using the excitation wavelength of 370nm within the scanning range of 400- 700 nm. Fig. 4.2 shows the Chiraito in water extract prepared at different temperature and post boiling time. Fig. 4.3 shows the Maha Sudarshan Churna Vati extract in water.

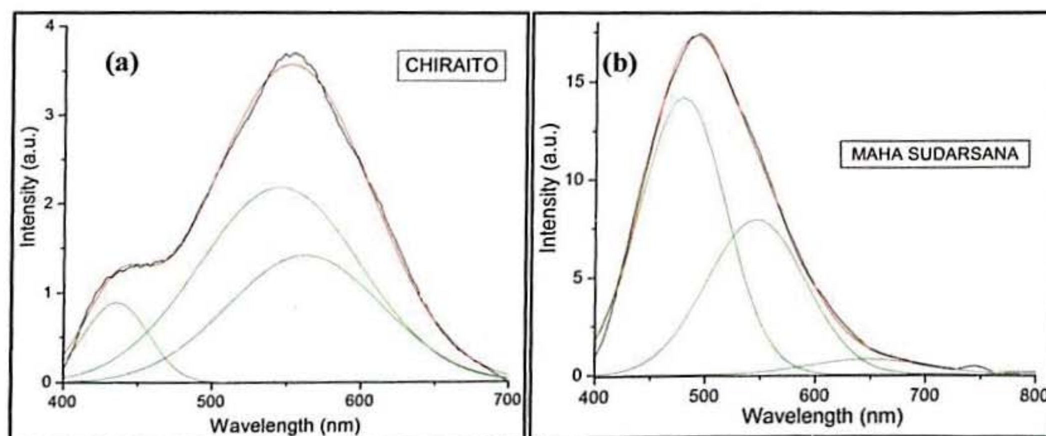

Figure 4.1: Fluorescence spectrum of (a) Chiraito, and (b) Maha Sudarsana Churna Vati [Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

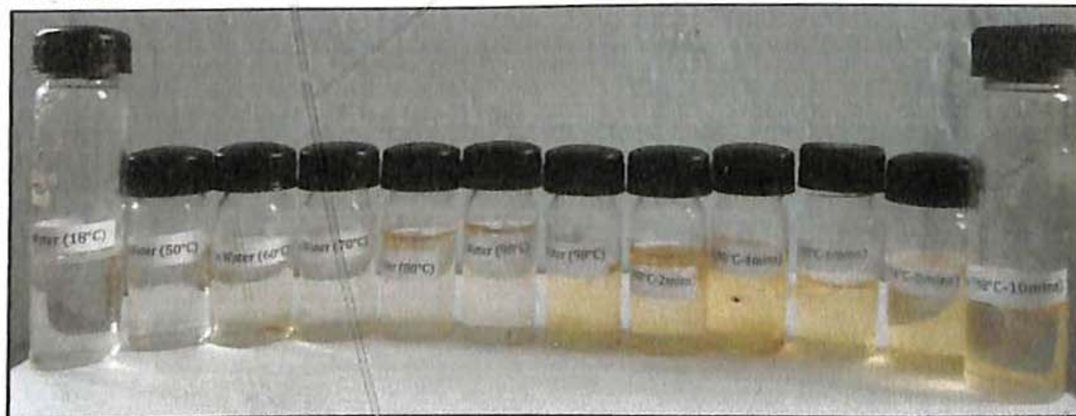

Fig. 4.2: Chiraito extracts obtained at different temperature and time of boiling [The extracts were taken at room temperature(18 °C), and from 50 °C at every interval of 10 °C till it reached the boiling point (98 °C); post the extracts were collected at interval of 2 minutes till 10 minutes post boiling.]

Fig. 4.3: Mahasudarshan Churna Vati extract in Distilled Water

The result of the spectra is summarised in Table 4.1. On the basis of the results obtained, the following can be concluded:

1. The peak at 436 nm with a width of 8±1 nm present in Chiraito is due to NADPH. This peak was however not detected in Maha Sudarsana Churna Vati.

2. The peak 546 nm is present in both Chiraito and its ayurvedic counterpart, Maha Sudarsana Churna Vati with a width of 6±1 nm and 89±4 nm respectively. This peak is attributed to the flavonoid (delphinidin glycoside).

3. The peak detected in Chiraito at 562 nm having a width of 7±1 nm is due to β carotene. This peak was not observed in Maha Sudarsana Churna Vati.

4. The 479 nm peak in Maha Sudarsana Churna Vati with a width of 77±1 nm is due to the presnce of flavonoid, terpenes and alkaloids.

5.The peak at 648nm detected in the ayurvedic counterpart with a width of 105±11 nm has been attributed to phycocyanin.

Table 4.1: Peak details for fluorescence spectra of Chiraito and Maha Sudarsana Churna Vati

Chiraito		**Maha Sudarsana Churna Vati**		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
436±1	8±1			NADPH	99
546±1	6±1	546±2	89±4	Flavonoid (Delphinidin glycoside)	100, 101
562±1	7±1			β carotene	102
		479±1	77±1	Flavonoids, terpenes and alkaloids	101
		648±13	105±11	Phycocyanin	103

As it has been mentioned earlier, the study was performed by boiling the sample and studying its spectral properties at interval of 10 °C until it reached the boiling point at 98°C. Once the boiling started, the spectral properties were studied at interval of every 2 minutes for the time period of 10 minutes. The peak height was found to be maximum for 4 minutes after which it decreases. The spectrum obtained at different temperature is shown in Fig. 4.4(a) and for different time interval in Fig. 4.4(b).

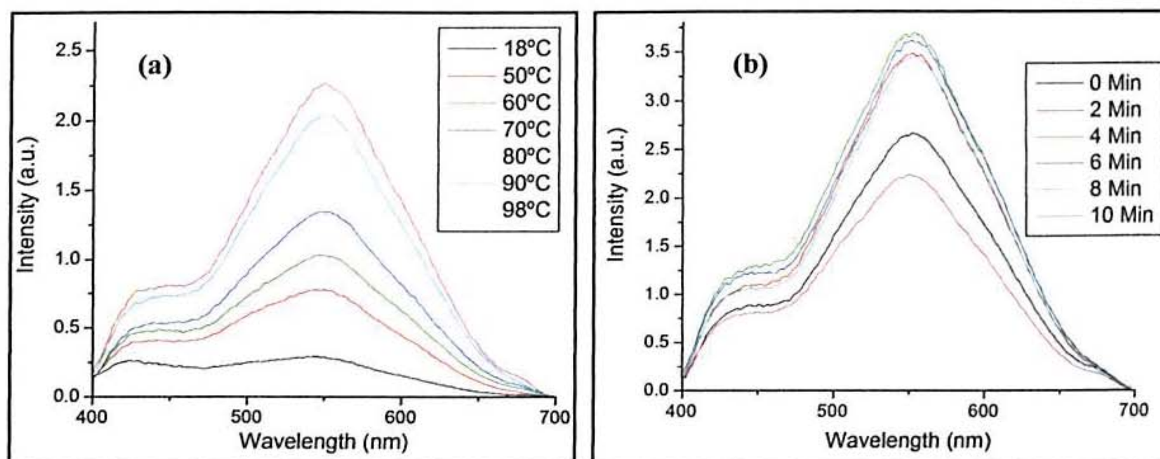

Figure 4.4: Fluorescence spectra of Chiraito (a) corresponding to different heating temperature, and (b) at 98°C boiled at intervals of 2 minutes

For each spectrum obtained at different temperature the area was extracted. Similarly for time dependent the area was extracted. The area plotted against the different temperature and different time interval is shown in Fig. 4.5(a) and (b). From the plotted dependency the following observation have been made:

1- The area of the 436nm peak corresponding to NADPH was found out to be almost same in all the spectra indicating that the concentration of NADPH remains unchanged.
2- The 562nm peak attributed to β carotene shows linear increase reaching the maximum for sample boiled for 4 minutes at 98°C and then it decreases.
3- For flavonoid corresponding to the peak at 546nm, the area was found to increase linearly till 70°C; however as it approaches 80°C, the slope changes. The sudden change in the area for flavonoid between 70- 80°C can be explained by the dissociation of the components. Thus one or more components must be absorbed by the sample and only the remaining part fluorese in the remaining spectra.

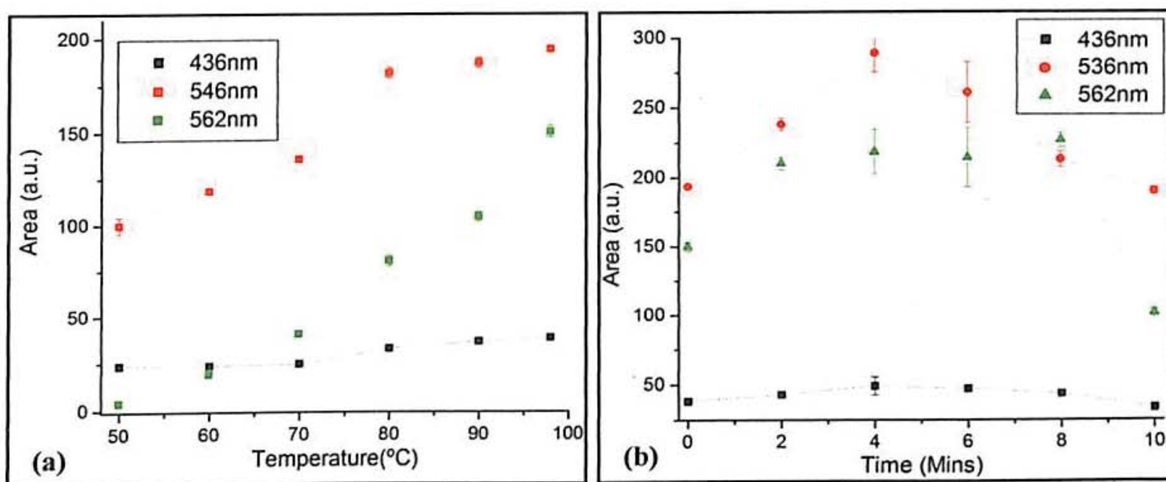

Fig. 4.5: Plot of Temperature versus Area of Chiraito peaks (a) corresponding to different heating temperature, and (b) boiled at 98°C in intervals of 2 minutes. [The dotted lines are for indication only.]

4.2. Absorption spectroscopy analysis

Fig. 4.6 shows the absorption spectrum of Chiraito when it was boiled at 98°C for 4 minutes. The curve fitting gave 2 peaks at 311nm and 365nm with a width of 39±1 nm and 59±1 nm respectively corresponding to Chrysin and Kaempferol (flavonoids)[69, 70]. However, the absorption spectrum of Maha Sudharsan Churna Vati within the scanning range of 250- 800 nm did not yield proper peaks, the same was discarded. The details of the peaks as obtained from the spectrum are shown in Table 4.2.

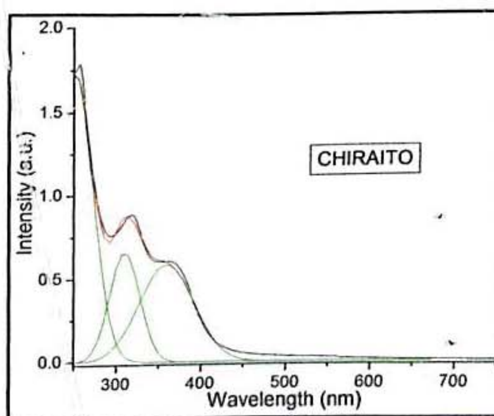

Fig. 4.6: Absorption spectrum of Chiraito[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

Table 4.2: Peak details for absorption spectrum of Chiraito

Position(nm)	Width(nm)	Probable constituent	References
311±1	39±1	Chrysin	104
365±1	59±1	Kaempferol	104, 105

As was performed in case of fluorescence spectra, the temperature and time dependence study was also performed in this case. From Fig. 4.7(a) and (b), we see that similar trends was obtained as in the previous case.

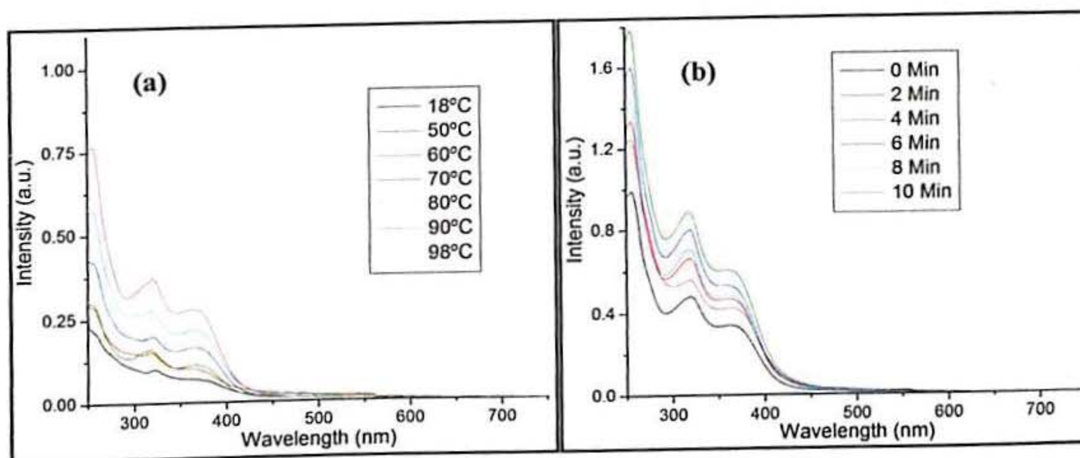

Fig. 4.7: Absorption spectra of Chiraito (a) corresponding to different heating temperature, and (b) at 98°C boiled at intervals of 2 minutes

The plot of area versus temperature and time was plotted as before; it was found that both the flavonoids corresponding to the peaks at 311nm and 365nm showed linear properties both when it was increasing as well as decreasing after reaching the maximum. Fig. 4.8(a) and (b) shows the said plots.

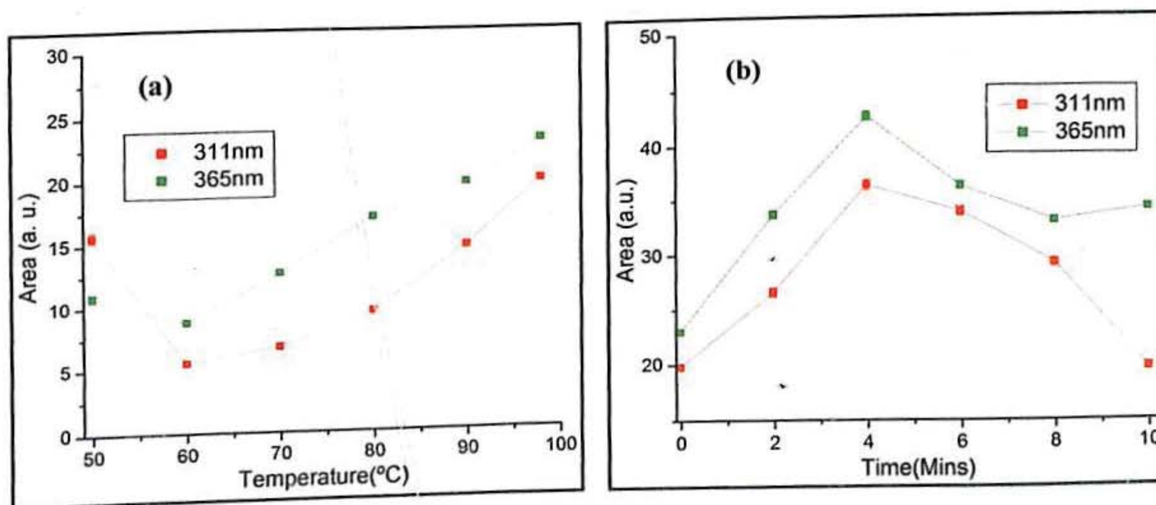

Fig. 4.8: Plot of Temperature versus Area of Chiraito peaks (a) corresponding to different heating temperature, and (b) boiled at 98°C in intervals of 2 minutes. [The dotted lines are for indication only]

4.3. Thin layer chromatography analysis

The thin layer chromatography on Chiraito was performed on following:

1- Sample at 18°C
2- Sample boiled at 98°C for 4 minutes
3- Sample boiled at 98°C for 20 minutes

The eluent used for both Chiraito and its counterpart was 70% methanol and 30% DCM. For the Chiraito sample at 18°C, the obtained Rf values were 0.746 and 0.677, for the sample boiled at 98°C for 4minutes, the Rf values were 0.814, 0.661, 0.739 and 0.898 and for the sample boiled at 98°C for 20 minutes, the calculated Rf values were 0.814, 0.644, 0.746 and 0.898. The results of the Rf values obtained are tabulated when the plates were observed under different conditions viz. daylight, short wavelength and long wavelength in Table 4.3.

Table 4.3: Rf values for Chiraito and Maha Sudarsana Churna Vati when viewed under different conditions

Sample	Viewed under Daylight	Viewed under Short Wavelength	Viewed under Long Wavelength
Chiraito at 18°C	-	4.4/5.9=0.746	4.0/5.9=0.677
Chiraito boiled at 98°C for 4 minutes	4.8/5.9= 0.814	3.9/5.9=0.661 4.8/5.9= 0.814	4.35/5.9=0.737 5.3/5.9= 0.898
Chiraito at 98°C boiled for 20 minutes	4.8/5.9= 0.814 3.8/5.9=0.644	4.8/5.9= 0.814 3.8/5.9=0.644	4.4/5.9= 0.746 5.3/5.9=0.898
Maha Sudarsana Churna Vati	2.5/6.1=0.409 3.3/6.1= 0.541	3.3/6.1= 0.541	6.0/6.1=0.98

The results clearly indicates that only two compounds are present in the sample at 18°C while four compounds have been detected in the sample boiled at 98°C for 4 minutes and 20 minutes. This clearly implies that the two compounds might have dissociated from either of the two initial components while boiling which to some extent gives support to the hypothesis as to the dissociation of the component observed while doing the temperature dependence study in fluorescence spectra. It has also been observed that the relative intensity of the components when viewed under long wavelength is greater for the sample boiled at 98°C for 4 minutes than that boiled at 98°C for 20 minutes. The TLC plates of Chiraito under different viewing conditions are shown by Fig. 4.9.

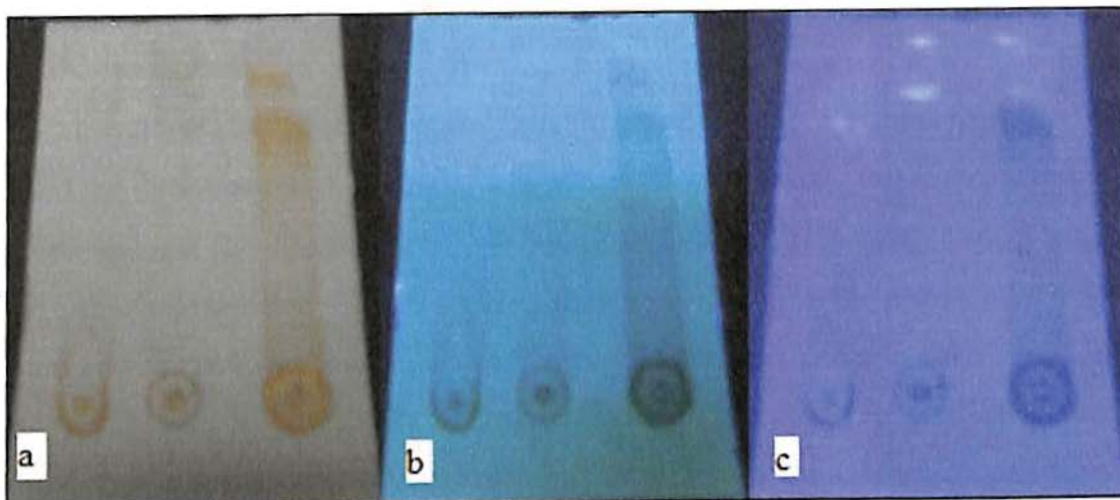

Fig. 4.9: TLC plate of Chiraito when viewed under a. daylight, b. short wavelength and c. long wavelength

On the basis of the obtained values for Maha Sudarsana Churna Vati, 3 constituents with Rf values 0.409, 0.541 and 0.98 may be present in the formulation. The TLC plates of Maha Sudarsana Churna Vati under different viewing conditions are shown by Fig. 4.10. Comparing the Rf values of Chiraito with that of Maha Sudarsana Churna Vati, we find that the Rf values of Chiraito at 0.898 and that of Maha Sudarsana Churna Vati at 0.98 are somewhat similar and may correspond to the same components. As it is evident from the Fig.4.10 that, the spot with Rf value 0.541 shows a large blot indicating that the components have not been separated properly. It is to be mentioned here that Maha Sudarsana Churna Vati contains a total of 12 herbal plants of which Chiraito is one. The other plants contribute some more constituents on their part as such the separation is not proper.

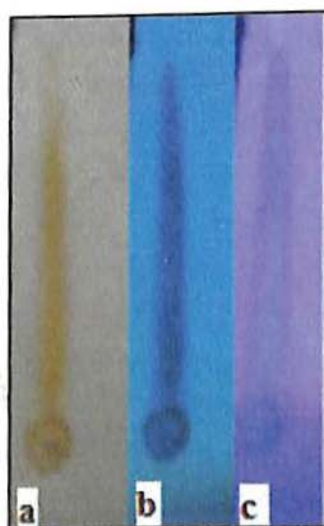

Fig. 4.10: TLC plate of Maha Sudarsana Churna Vati when viewed under a. daylight, b. short wavelength and c. long wavelength

4.4. Conclusion

The temperature and time dependence study done using fluorescence spectroscopy revealed the dissociation of the initial components due to boiling by undergoing structural modification and the formation of the new components. This further reveals that, the newly formed dissociated flavonoids are the ones which have the potential of curing the diseases and as such the boiled extract is more effective. It has also been found out that the concentration of flavonoids was maximum when boiled for a time period of 4 minutes, thus indicating that the optimal result is obtained at the said period of time.

Based on the findings and the reviewed literatures, the leaves of Chiraito were found to have the presence of chrysin, luteolin, kaempferol and flavonoid which helps in curing various diseases. On comparison with its ayurvedic counterpart, Maha Sudarsana Churna Vati, it was found that flavonoid was the main component which helps in curing diabetes. The other components that were detected in Maha Sudarsana Churna Vati were terpenes, alkaloids and phycocyanin.

Chapter 5

Harra

5.1. Fluorescence Spectroscopy analysis

5.1.1. Water extract: Usually medicines are prescribed to be consumed with water. For this reason, the extractions were done in water. Fig. 5.1(a), (b) and (c) shows the water extract for Harra and its ayurvedic counterparts Haritaki Churna and Triphala Churna respectively. The corresponding fluorescence spectra of the water extracts within the scanning range of 400- 700 nm by using the excitation wavelength of 370 nm are shown by Fig. 5.2(a), (b) and (c) respectively. The results obtained have been listed and summarised in Table 5.1.

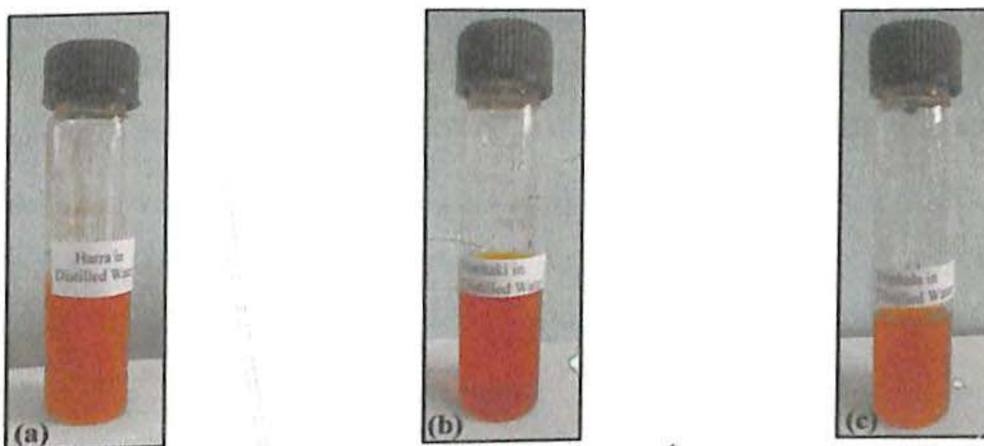

Fig. 5.1: Water extract of (a) Harra, (b) Haritaki Churna and (c) Triphala Churna

Based on Table 5.1, the following observations have been made:

1. The peak at 456nm with a width of 20±1nm in Harra is attributed to NADPH. This peak was detected at 453nm with a width of 28±1nm in Triphala Churna. However this peak was not detected in Haritaki Churna.

2. The fluorescenec in the range between 470-525nm is due to flavonoids, terpenes and alkaloids. The peak at 487nm with a width of 25±1nm in Harra and at 490nm with a

width of 20±1nm in Triphala Churna is due to the said constituents. This peak was however not observed in Haritaki Churna.

3. The peak at 536nm in Harra with a width of 42±1nm is due to quercetin, berberine and other flavonols. This peak was detected at 520nm with a width of 67±2nm and at 541nm with a width of 49±1nm in Haritaki Churna and Triphala Churna respectively.

4. Phycoerythrin has been found to fluoresce in the range 570- 580nm. Thus, the peak at 573nm in Harra with a width of 16±1nm and at 575nm in Triphala Churna with a width of 23±1nm is attributed to phycoerythrin. The said component was however absent in Haritaki Churna.

5. Anthocyanins and azulenes fluoresce in the broad range of 600 – 630nm. Taking into consideration the broadness of the width of the peaks obtained in Harra, Haritaki Churna and Triphala Churna, the peaks at 597 nm having a width of 27±1nm and at 618 nm with a width of 60±7nm in Harra is due to the said components. These peaks were detected in Haritaki Churna at 590nm with a width of 49±2nm and at 610nm with a width of 45±1nm respectively. In Tripahala only one peak was observed at 599nm with a width of 26±1nm.

6. The peaks detected in Haritaki Churna and Triphala Churna at 632nm with a width of 23±2nm and at 633nm with a width of 46±2 nm is attributed to phycoerythrocyanin. However, considering the broadness of these peaks the other components as azulenes and anthocyanins may also be present.

7. The 686nm peak detected in Harra with a width of 53±2nm is due to phycobiliprotein. However, the broadness of the peak also indicates that allophycocyanin may have given the peak. This peak was detected at 680nm with a width of 45±1nm and 679nm with a width of 29±1nm in Haritaki Churna and Triphala Churna respectively.

8. The peaks detected at 736nm, 764nm and 787 nm with a width of 20±1nm, 12±1nm and 55±3nm respectively in Haritaki Churna and at 724nm and 796nm with a width of 59±1nm and 64±2nm could not be identified.

9. The peak at 829nm having a width 20±1nm in Haritaki Churna and at 827nm with a width of 12±1nm has been attributed to chromosome baseplate complex. This peak was not detected in Harra.

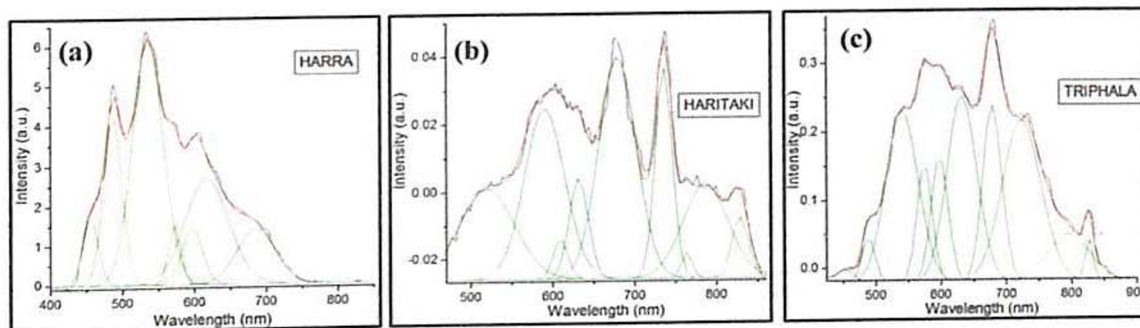

Fig. 5.2: Fluorescence spectrum in water extract of (a) Harra, (b) Haritaki Churna and (c) Triphala Churna[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data].

Table 5.1: Peak details for florescence spectra of Harra, Haritaki Churna and Triphala Churna in water extract.

Harra		**Haritaki Churna**		**Triphala Churna**		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
456±1	20±1			453±1	28±1	NADPH	103, 107
487±1	25±1			490±1	20±1	Flavonoids, terpenes, alkaloids	103
536±1	42±1	520±1	67±2	541±1	49±1	Quercetin, Berberine and other flavonols	108, 109
573±1	16±1			575±1	23±1	Phycoerythrin	104
597±1	27±2	590±1	49±2	599±1	26±1	Anthocyanins, azulenes	103
618±4	60±7	610±1	17±2			-do-	103
		632±1	23±2	633±1	46±2	Phycoerythrocyanin, azulenes, anthocyanins	103, 104
686±2	53±2	680±1	45±1	679±1	29±1	Allophycocyanin, Phycobiliproteins	104, 105
		736±1	20±1	724±1	59±1	NA	
		764±1	12±1			NA	
		787±1	55±3	796±1	64±2	NA	
		829±1	20±1	827±1	12±1	Chromosome baseplate complex	110

5.1.2. Ethanol extract: For an exhaustive study, the ethanol extract were prepared with the perception that some more constituents can be detected. The results have been tabulated in Table 5.2. Fig. 5.3 (a), (b) and (c) shows the ethanol extract for Harra, Haritaki Churna and Triphala Churna respectively. Fig. 5.4 (a), (b) and (c) shows the fluorescence spectra of Harra, Haritaki Churna and Triphala Churna in ethanol extract.

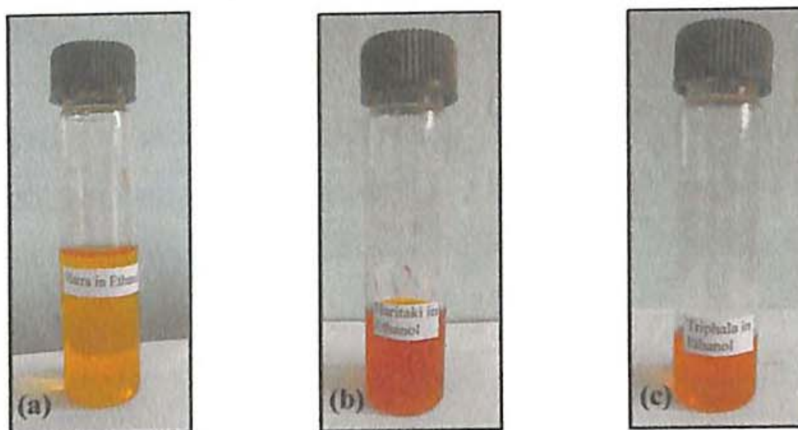

Fig. 5.3: Ethanol extract of (a) Harra, (b) Haritaki Churna and (c) Triphala Churna

Table 5.2: Peak details for fluorescence spectra of Harra, Haritaki Churna and Triphala Churna in ethanol extract.

Harra		Haritaki Churna		Triphala Churna		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
		490±1	19±1	496±1	44±2	Alkaloids,flavonoids, terpenes	103
548±1	15±1	533±1	44±1			Quercetin,berberine, flavonoids	108, 109
577±1	22±1	579±1	44±2	589±1	125±3	Phycoerythrin	104
602±1	20±1					Azulenes, anthocyanins	103
631±1	34±1	630±2	56±3			Azulenes, anthocyanins, phycoerythocyanin	103, 104
680±1	33±1	680±1	25±1	682±1	29±1	Phycobiliproteins, allophycocyanin	104, 105
703±1	7±1					NA	
716±1	9±1			718±1	19±1	NA	
722±2	30±2	722±1	47±1			NA	
		771±1	20±1	773±1	71±1	NA	
				737±1	17±1	NA	

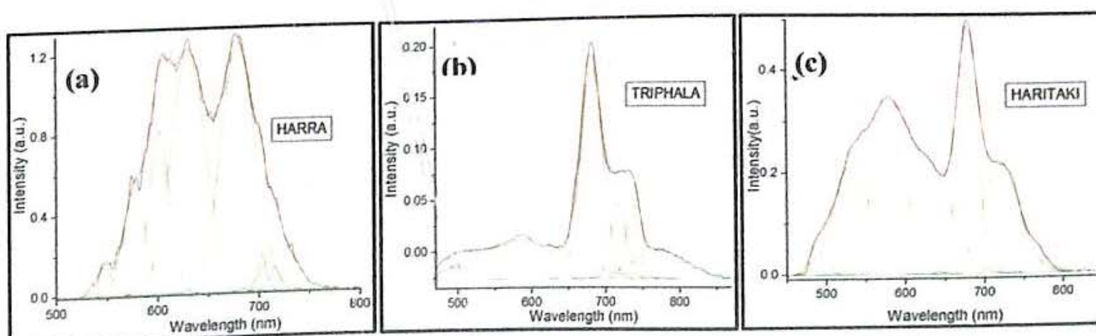

Fig. 5.4: Fluorescence spectrum in ethanol extract of (a)Harra, (b) Haritaki Churna and (c) Triphala Churna[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

On the basis of the obtained spectra and Table 5.2, the following observations can be made:

1. The peak related to alkaloids, terpenes and flavonoids were detected in Haritaki Churna and Triphala Churna at 490nm with a width of 19±1 nm and at 496nm with a width of 44±2 nm respectively. This peak was however not observed in Harra.

2. The peak at 548nm with a width of 15±1 nm in Harra was attributed to Quercetin, Berberine and flavonols. This peak was observed at 533nm with a width of 44±1 nm in Haritaki Churna but was absent in Triphala Churna.

3. The peak at 577 nm in Harra is attributed to phycoerythrin and has a width of 22±1nm. This peak was seen at 579 nm and 589nm with a width of 44±2nm and 125±3nm in Haritaki Churna and Triphala Churna respectively.

4. The peak at 602nm in Harra with a width of 20±1nm is because of azulenes and anthocyanins. The peak was not detected in Triphala Churna and Haritaki Churna.

5. The peak detected in Harra at 631 nm with a width of 34±1nm and in Haritaki Churna at 630nm with a width of 56±3 nm is due to the presence of azulenes and anthocyanins. However, the broadness of the peak indicates that phycoerythocyanin might also be present. This peak was not observed in Triphala Churna

6.Phcobiliproteins and allophycocyanin are responsible for the peak at 680nm in Harra, 680nm in Haritaki Churna and 682nm in Triphala Churna. The peaks have the corresponding width of 33±1nm, 25±1nm and 29±1nm respectively.

7. The peaks at 703nm, 716nm and 722nm in Harra, 722nm and 771nm in Haritaki Churna, and 718nm, 737nm and 773nm in Triphala Churna could not be identified.

5.2. Absorption spectroscopy analysis

5.2.1. Water extract

Absorption spectra in water extract did not yield credible peak in the scanning range of 250-800nm and as such it was discarded from the thesis.

5.2.2. Ethanol extract

Fig. 5.5(a), (b) and (c) shows the absorption spectra of Harra, Haritaki Churna and Triphala Churna in ethanol extract. Table 5.3 summarises the results obtained from the

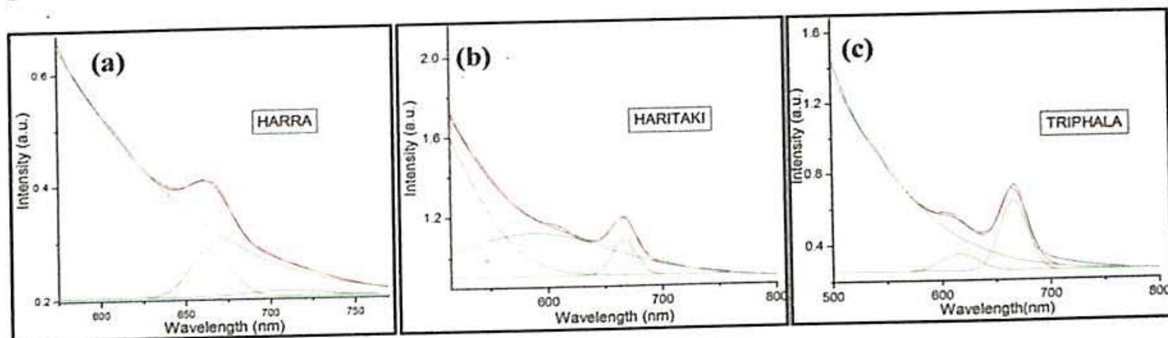

Fig. 5.5: Absorption spectrum in ethanol extract of (a)Harra, (b)Haritaki Churna and (c) Triphala Churna[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

Table 5.3: Peak details for Absorption Spectra of Harra, Haritaki Churna and Triphala Churna in ethanol extract.

Harra		Haritaki Churna		Triphala Churna		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
665±1	24±1	667±1	20±1	666±1	25±1	Allophycocyanins	111
		602±1	134±3	610±1	33±1	Phycobilliprotein	111
709±1	43±2					NA	

On the basis of the Table 5.3 and the references referred, the following observations can be made:

1. The peak at 665nm in Harra with a width of 24±1 nm is because of allophycocyanin. The peak has was observed at 667nm in Haritaki Churna and 666nm in Triphala Churna with a width of 20±1nm and 25±1nm respectively.

2. Phycobilliprotein is responsible for the peak occuring at 602nm in Haritaki Churna having a width of 134±3 nm. This peak was detected at 610nm in Triphala Churna with a width of 33±1nm and was not detected in Harra.

3. The peak detected at 709nm with a width of 43±2 could not be identified from the reviewed literatures.

5.2. Thin layer chromatography analysis

The Thin Layer Chromatography was done using ethyl acetate, toluene, methanol and acetic acid in the ratio 75: 20: 4.5: 0.5 as the eluent. The thin layer chromatography of Harra revealed the presence of three spots with Rf values 0.07, 0.6 and 0.654 revealing the presence of 1 non polar and two polar components. However, there is also the presence of other components in the sample as is evident from Fig. 5.6(i). Just above the fluorescing spot having the Rf value 0.07, there is a huge spot which indicates the presence of a number of non-polar components which was not separable. Also below the spot having Rf value of 0.6, we find a huge spot which corresponds to a number of inseparable polar components. Table 5.4 summarises the calculated Rf values.

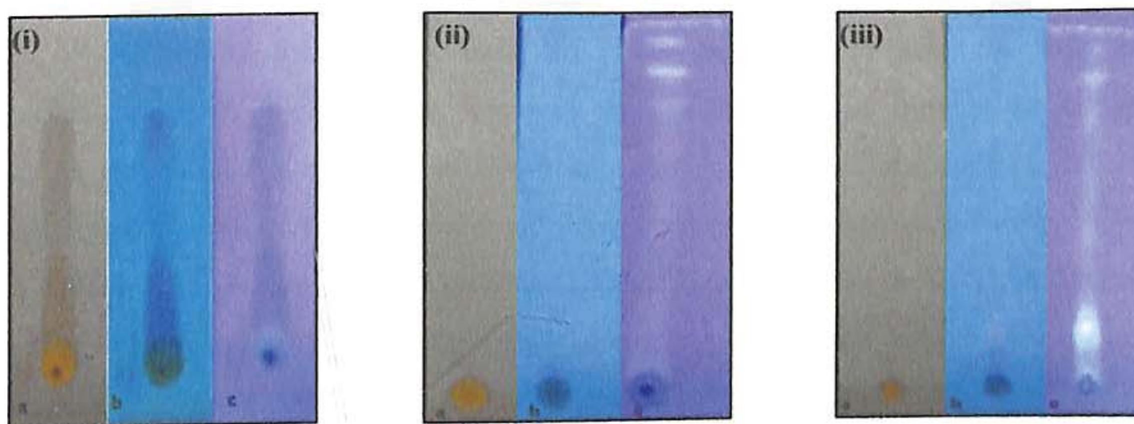

Fig. 5.6: TLC plate of Harra when viewed under (a) daylight, (b) short wavelength and (c) long wavelength for (i) Harra, (ii) Haritaki Churna and (iii) Triphala Churna.

The TLC analysis on Haritaki Churna revealed the presence of 5 probable constituents with Rf values 0.683, 0.8, 0.867, 0.9 and 0.933; the analysis on Triphala Churna had 7 spots with Rf values 0.5, 0.633, 0.8, 0.83, 0.867, 0.9 and 0.933. The Rf values of 0.8, 0.867 and 0.9 which is found in both Triphala Churna and Haritaki Churna clearly indicates that the constituents has been derived from Harra itself. The TLC plates under different viewing conditions are shown in Fig. 5.6(ii) and 5.6(iii); the details of the Rf values are shown in Table 5.4.

Table 5.4: Rf value of Harra, Haritaki Churna and Triphala Churna when viewed under different conditions.

Sample	Viewed under daylight	Viewed under short wavelength	Viewed under long wavelength
Harra	-	3.3/5.5=0.6 3.6/5.5= 0.654	0.4/5.5=0.07
Haritaki Churna	-	4.8/6.0= 0.8 5.2/6.0= 0.867 5.6/6.0= 0.933	4.1/6.0= 0.683 4.8/6.0= 0.8 5.4/6.0= 0.9
Triphala Churna	3.0/6.0=0.5	0.5/6.0= 0.083 3.0/6.0= 0.5 4.8/6.0= 0.8 5.2/6.0= 0.867 5.6/6.0= 0.933	0.5/6.0= 0.083 3.8/6.0= 0.633 4.8/6.0= 0.8 5.4/6.0= 0.9

5.4. Conclusion

Based on the results obtained, we find that Harra was found to have the presence of various components as flavonoids, terpenes, alkaloids and other flavonoids; quercetin, berberine and other flavonols; phycoerythrin; anthocyanins, azulenes, allophycocyanin, phycobiliproteins, etc. On comparison with the ayurvedic counterparts, Haritaki Churna and Triphala Churna, we find the presence of the constituents in them. Thus, Harra has indeed been used in the preparation of these medicines and hence can be used for curing diabetes.

Chapter 6

Barra

6.1. Florescence spectroscopy analysis

6.1.1. Water extract: The fluorescence spectroscopic study on Barra and its ayurvedic counterpart, Triphala Churna was done using the excitation wavelength of 370 nm and the scanning range of 400- 700 nm. Fig. 6.1(a) and (b) shows the Barra and Triphala Churna samples prepared in water extract, the corresponding fluorescence spectra of the water extracts are given by Fig. 6.2 (a) and (b) respectively. Table 6.1 summarises the results obtained.

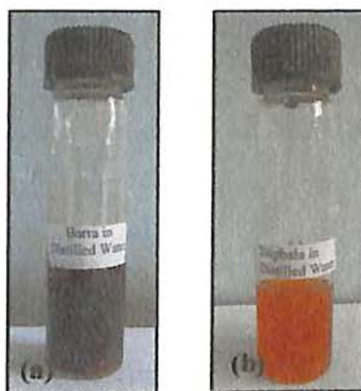

Fig. 6.1: Water extract of (a) Barra and (b) Triphala Churna

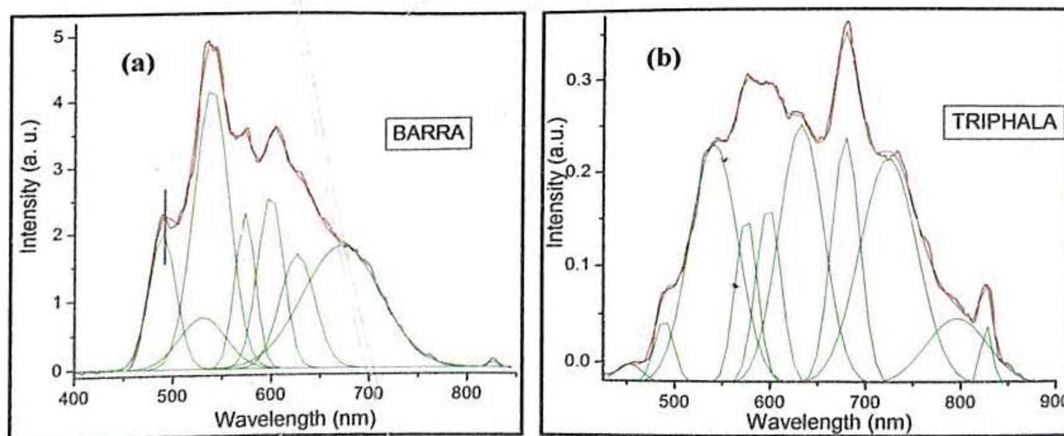

Fig. 6.2: Fluorescence spectrum in water extract of (a) Barra and (b) Triphala Churna[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

Table 6.1: Peak details for fluorescence spectra of Barra and Triphala Churna in water extract

Barra		Triphala Churna		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
		453±1	28±1	NADPH	101,107,108
489±2	27±1	490±1	20±1	Flavonoids, terpenes, alkaloids	103
530±10	46±16			Cinnamic acid, quercetin, berberine	104,109,111
539±3	35±10	541±1	49±1	Quercetin, Berberine and other flavonols	104, 108
573±1	20±1	575±1	23±1	Phycoerythrin	104
599±1	25±1	599±1	26±1	Anthocyanins, azulenes	103
627±1	35±2	633±1	46±2	Phycoerythrin, azulenes, anthocyanins	103, 104
672±1	78±1	679±1	29±1	Allophycocyanin, Phycobiliproteins	104, 105
		724±1	59±1	NA	
		796±1	64±2	NA	
826±1	7±1	827±1	12±1	Chromosome baseplate complex	110

On the basis of the results and reviewed literature, the following can be concluded:

1. The peak at 453nm attributed to NADPH with a width of 28±1 nm was detected in Triphala Churna but was absent in Harra.

2. The peak at 489nm in Harra with a width of 27±1nm was also detected in Triphala Churna at 490nm with a width of 20±1nm. This peak is attributed to flavonoids, terpenes and alkaloids.

3. The peak at 530nm with a width of 46±16nm detected in Harra but absent in Triphala Churna is attributed to cinnamic acid, quercetin and berberine.

4. Quercetin, berberine and other flavonols were responsible for the peak at 539nm detected in Harra with a width of 35±10nm and in Triphala Churna at 541 nm having a width of 49±1nm.

5. The 573nm peak detected in Harra with a width of 20±1nm attributed to phycoerythrin was also detected in Triphala Churna at 575nm with a width of 23±1nm.

6. Anthocyanins and azulenes are responsible for the peaks detected in Harra and Haritaki Churna at 627nm and 633nm with a width of 35±2nm and 46±2nm respectively.

7. The peak detected in Barra at 627 nm with a width of 35±2nm and in Triphala Churna at 633nm with a width of 46±2 nm is due to the presence of azulenes and anthocyanins. Considering the broadness of the peak the presence of phycoerythocyanin can not be ruled out.8. The peak at 672nm in Barra and 682nm in Triphala Churna is due to

phycobiliproteins and allophycocyanin The peaks have the corresponding width of 78±1nm and 29±1nm respectively.

9. The peaks at 724nm and 796nm in Triphala Churna with a width of 59±1nm and 64±2nm could not be identified.

6.1.2. Ethanol extract: As in the case of Barra, the ethanol extract was also prepared for Barra and its counterpart. The results obtained have been tabulated in Table 6.2. Fig. 6.3 (a) and (b) shows the ethanol extract for Barra and Triphala Churna respectively. Fig. 6.4 (a) and (b) shows the corresponding fluorescence spectra.

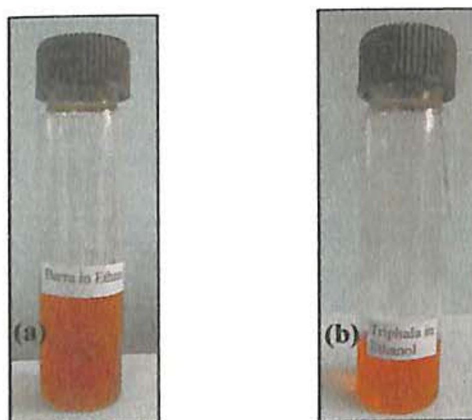

Fig.6.3: Ethanol extract of (a) Barra and (b) Triphala Churna

1. The peak at 496nm detected in Triphala Churna with a width of 44±2nm and attributed to alkaloids, flavonoids and terpenes was not detected in Harra.

2. The phycoerythrin peak at 589nm in Triphala Churna with a width of 125±3nm was also not seen in Harra.

3. Two peaks corresponding to phycobiliproteins and allophycocyanin were detected at 671nm and 682nm in Barra with a width of 14±1nm and 12±1nm respectively. However, only one peak was detected in Triphala Churna at 682nm with a width of 29±1nm.

4. The peaks at 702nm in Harra , and at 718nm, 737nm and 772nm in Triphala Churna could not be identified.

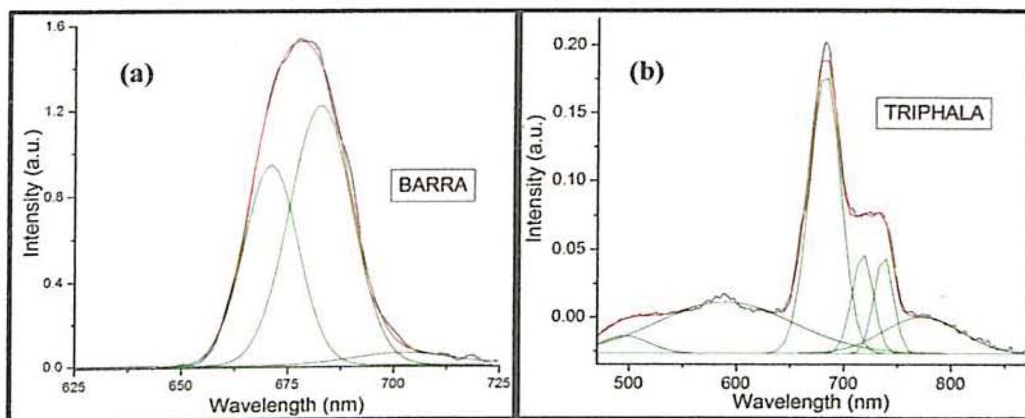

Fig.6.4: Fluorescence spectrum in ethanol extract of (a) Barra, and (b) Triphala Churna[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

Table 6.2: Peak details for fluorescence spectra of Barra and Triphala Churna in water extract

Barra		Triphala Churna		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
682±1	14±1	682±1	29±1	Phycobiliproteins, allophycocyanin	104, 105
671±1	12±1			Phycobiliproteins, allophycocyanin	104, 105
702±4	24±5			NA	
		496±1	44±2	Alkaloids,flavonoids, terpenes	103
		589±1	125±3	Phycoerythrin	104
		718±1	19±1	NA	
		737±1	17±1	NA	
		773±1	71±1	NA	

Triphala Churna is made from three medicinal plants- Harra, Barra and Amla. The presence of all the components present in Barra does indicate that it has indeed been used in the preparation of the ayurvedic formulation Triphala Churna.

6.2. Absorption spectroscopy analysis

6.2.1. Water extract: As has already been mentioned in the earlier chapter, the absorption spectroscopy done using water extract did not yield proper peaks, hence the same was discarded.

6.2.2. Ethanol extract

Fig. 6.5(a) and (b) shows the absorption spectra of Barra and Triphala Churna respectively. The results from the obtained spectra has been tabulated in Table 6.3.

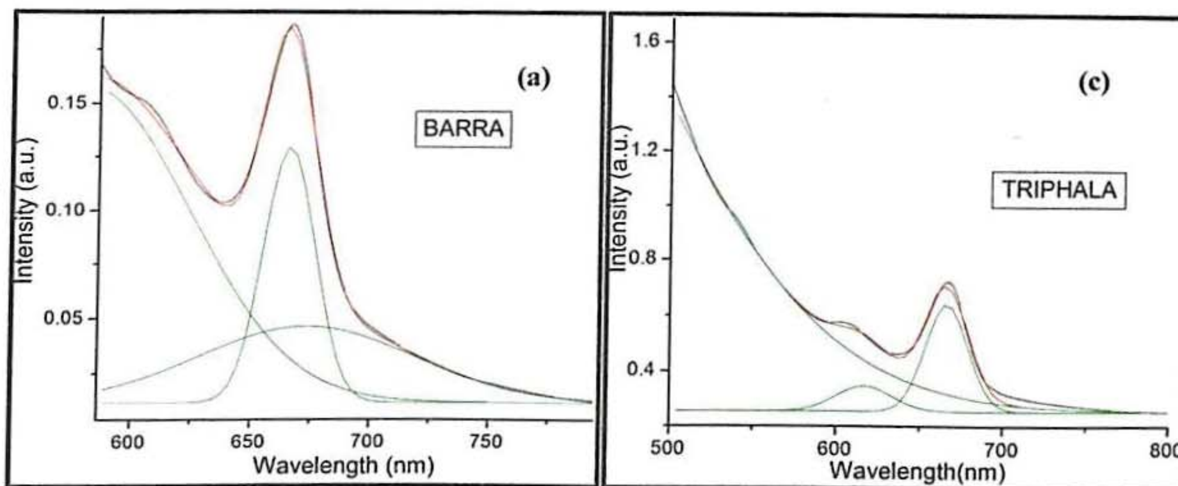

Fig. 6.5: Absorption spectra of ethanol extract of (a) Barra and (b) Triphala Churna[Green line shows the deconvulated peaks. red line is the envelope of deconvulated peaks and black line is the recorded data]

Table 6.3: Peak details for Absorption Spectra of Barra and Triphala Churna in ethanol extract.

Barra		Triphala Churna		Probable constituents	References
Position(nm)	Width (a.u.)	Position(nm)	Width (a.u.)		
603±1	86±4	610±1	33±1	Phycobiliprotein	111
665±1	21±1	666±1	25±1	Allophycocyanins	111

From the spectra obtained from absorption spectroscopy, the peak at 603nm in Barra and at 610nm in Triphala Churna with a width of 86±4 nm and 33±1nm respectively gave the presence of allophycocyanin. The other peak detected at 665nm in Barra with a width of 21±1nm and at 666nm in Triphala Churna with a width of 25±1nm is due to phycobiliproteins. These components were also detected in the fluorescence study of the samples in water extract.

6.3. Thin layer chromatography analysis

For the thin layer chromatography study ethyl acetate, toluene, methanol and acetic acid was taken in the ratio 75: 20: 4.5: 0.5 for preparing the eluent. The study on Barra revealed the presence of two spots with Rf values 0.655 and 0.691 indicating the presence of two polar components. However, there is also the presence of other components in the

sample as is evident from Fig. 6.6(i). In the region just above the sample, there is a huge spot which elongates up to the top. This indicates the presence of a number of non-polar and polar components which could not be separated.

The TLC analysis on Triphala Churna had 7 spots with Rf values 0.5, 0.633, 0.8, 0.83, 0.867, 0.9 and 0.933. The TLC plate under different viewing conditions is shown in the Fig. 6.6(ii); the details of the Rf values are shown in Table 6.4.

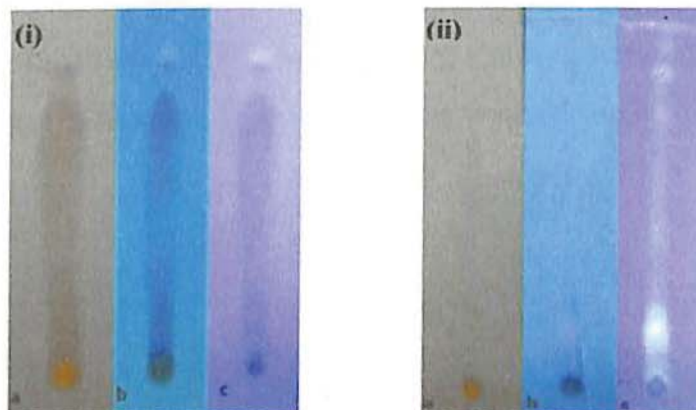

Fig. 6.6: TLC plate when viewed under a. Daylight, b. Short wavelength and c. Long wavelength of (i)Barra and (ii) Triphala Churna

Table 6.4: Rf values for Barra and Triphala Churna when viewed under different conditions

Sample	Viewed under Daylight	Viewed under Short Wavelength	Viewed under Long Wavelength
Barra	3.6/5.5= 0.655	3.6/5.5= 0.655	3.6/5.5= 0.655 3.8/5.5= 0.691
Triphala Churna	3.0/6.0=0.5	0.5/6.0= 0.083 3.0/6.0= 0.5 4.8/6.0= 0.8 5.2/6.0= 0.867 5.6/6.0= 0.933	0.5/6.0= 0.083 3.8/6.0= 0.633 4.8/6.0= 0.8 5.4/6.0= 0.9

6.4. Conclusion

On the basis of the results obtained and the available literature, Barra was found to have the probable presence of flavonoids, terpenes, alkaloids; cinnamic acid, quercetin, berberine and other flavonols; phycoerythrin, anthocyanins, azulenes, allophycocyanin, phycobiliproteins, etc. Apart from cinnamic acid, all the other components were detected in the ayurvedic counterpart, Triphala Churna, thus it indiactes the presence of Barra in the ayurvedic medicine Triphala Churna.

Chapter 7

Totola

7.1. Fluorescence spectroscopy analysis

7.2.1. Water extract: Fig. 7.1(a) and (b) shows the water extract of Totola and Dashmool Kwath respectively. The corresponding fluorescence spectrum is given by the Fig. 7.2 (a) and (b). The results of the spectra have been tabulated in Table 7.1.

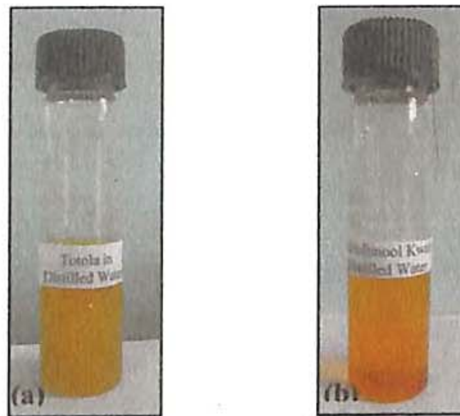

Fig. 7.1: Water extract of (a) Totola and (b) Dashmool Kwath

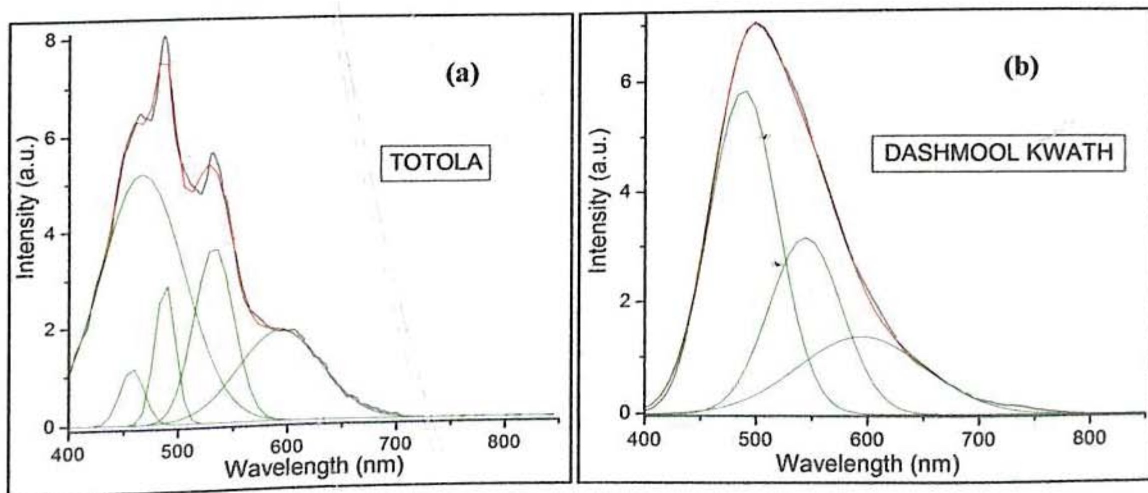

Fig. 7.2: Fluorescence spectrum in water extract of (a) Totola and (b) Dashmool Kwath[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

On the basis of the obtained data, the following can be concluded:

1. The peak at 458 nm in Totola with a width of 21±1 nm was attributed to NADPH. This peak was not detected in Dashmool Kwath.

2. The 467 nm peak in Totola with a width of 75±1 nm is due to β carotene, this peak was also not observed in Dashmool Kwath.

3. The peak due to flavonoids, terpenes and alkaloids were observed in both Totola and Dashmool Kwath at 488nm and 489 nm with the width of 18±1 nm and 61±1 nm respectively.

4. The 532nm peak in Totola with a width of 36±1 nm was due to cinnamic acid, quercetin, berberine and other flavonols. This peak was not observed in Dashmool Kwath.

5. Anthocyanins and azulenes are responsible for the detected peaks at 593nm in Totola and 595nm in Dashmool Kwath. Their respective widths were 75±1 nm and 113±1 nm.

6. The flavins peak detected in Dashmool Kwath at 544 nm with a width of 64±1 nm was not observed in Totola.

Table 7.1: Peak details for Fluorescence Spectra of Totola and Dashmool Kwath in water extract

Totola		Dashmool Kwath		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
458±1	21±1			NADPH	103, 107
467±1	75±1			Beta carotene	112
488±1	18±1	489±1	61±1	Flavonoids, terpenes, alkaloids	103
532±1	36±1			Cinnamic acid, quercetin, berberine and other flavonols	108, 109, 110,111
593±1	75±1	595±3	113±1	Anthocyanins, azulenes	103
		544±1	64±1	Flavonols	113

7.1.2. Ethanol extract: The Fig. 7.3 (a) and (b) shows the ethanol extract of Totola and Dashmool Kwath. The corresponding fluorescence spectra are shown by Fig. 7.4 (a) and (b), the results of which have been tabulated in Table 7.2.

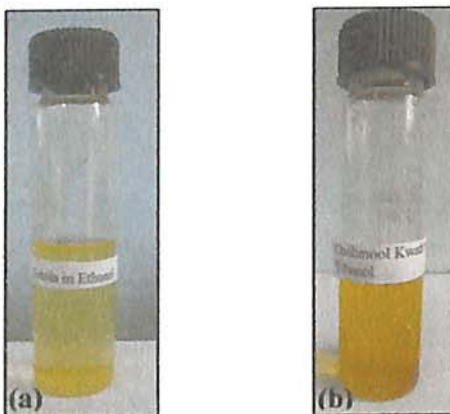

Fig. 7.3: Water extract of (a) Totola, and (b) Dashmool Kwath

On the basis of the results obtained and the reviewed literature, the following can be concluded:

1. The presence of flavonoids, terpenes and alkaloids in Totola were detected due to the presence of peak at 489nm having a width of 9±1 nm.

2. The peak at 507nm with a width of 25±1 nm in Totola is attributed to the presence of carotenoid.

3. The 533nm peak in Totola having a width of 18±1 nm is due to carotenoid, quercetin berberine and other flavonols.

4. The 546nm peak with a width of 9±1 nm is due to the presence of flavonols.

5. β carotene is responsible for the presnce of 557nm peak with a width of 25±3 nm in Totola.

6. The presence of 567nm peak in Totola with a width of 54±20 nm reveals the presence of phycoerythrin.

7. Anthocyanins and azulenes are responsible for the peak at 604nm in Totola. The width of the peak was 5±1 nm.

All these peaks were not detected in Dashmool Kwath.

8. Phycobiliprotein and allophycocyanin are responsible for the peaks 674nm with a width of 14±1 nm and 679nm having width of 18±1 nm in Totola and Dashmool Kwath respectively.

9. The peak at 704nm observed in Dashmool Kwath could not be identified.

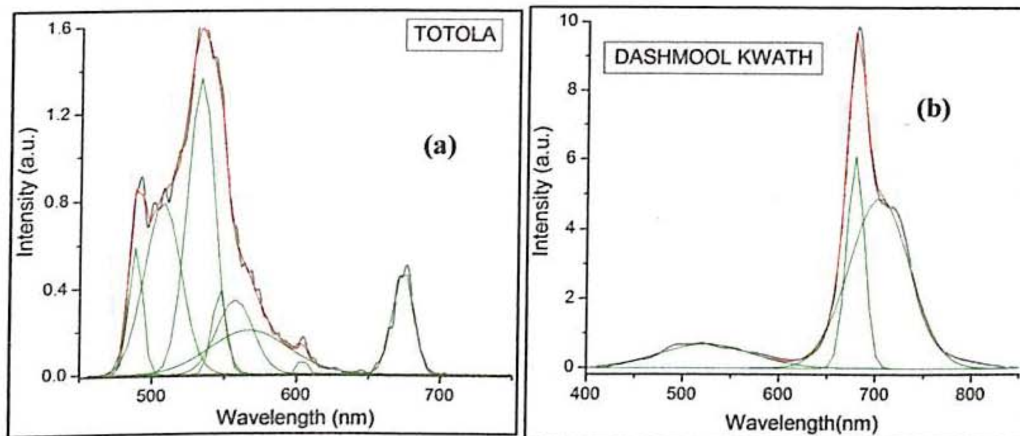

Fig. 7.4: Fluorescence spectrum in ethanol extract of (a) Totola, and (b) Dashmool Kwath[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

Table 7.2: Peak details for fluorescence spectra of Totola and Dashmool Kwath in ethanol extract.

Totola		Dashmool Kwath		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
489±1	9±1			Alkaloids,flavonoids, terpenes	103
507±1	25±1			Carotenoid	112
533±1	18±1			Carotenoid, quercetin, berberine and other flavonols	108, 109, 112
546±1	9±1			Flavins	113
557±1	25±3			Beta carotene	102
567±16	54±20			Phycoerythrin	104
604±1	5±1			Anthocyanins, azulenes	103
674±1	14±1	679±1	18±1	Phycobiliproteins, allophycocyanin	104, 105
		704±1	62±1	NA	

7.2. Absorption spectroscopy analysis

7.2.1. Water extract: As in the case of Harra and Barra, the absorption spectroscopy using water extract did not reveal good peaks in the scanned range, the same was discarded.

7.2.2. Ethanol extract: Fig.s 7.5 (a) and (b) shows the absorption spectra of Totola and Dashmool Kwath in ethanol extract respectively. The details of the peaks obtained from the spectra are summarised in Table 7.3.

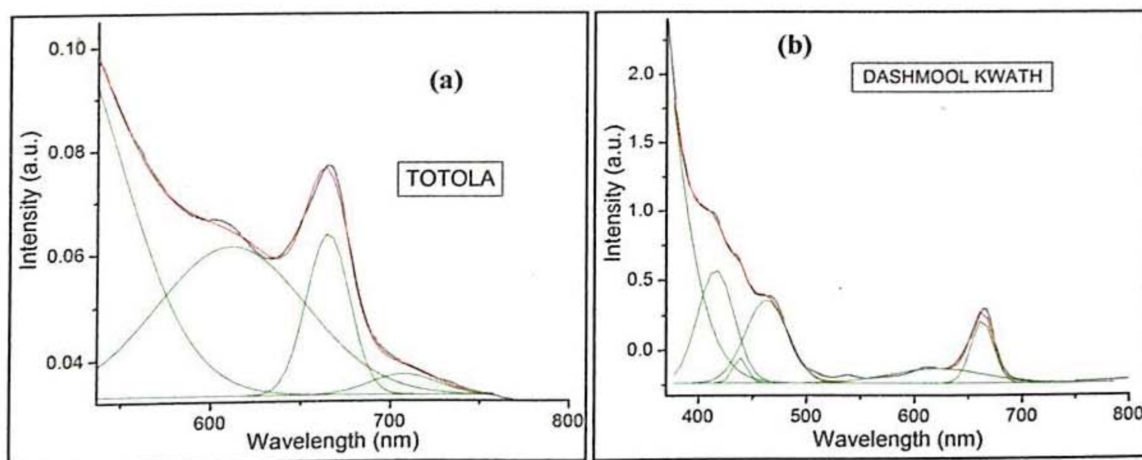

Fig. 7.5: Absorption spectrum in ethanol extract of (a)Totola and (b)Dashmool Kwath[Green line shows the deconvulated peaks, red line is the envelope of deconvulated peaks and black line is the recorded data]

Table 7.3: Peak details for absorption spectra of Totola and Dashmool Kwath in ethanol extract.

Totola		Dashmool Kwath		Probable constituents	References
Position (nm)	Width (a.u.)	Position (nm)	Width (a.u.)		
		416±1	33±1	Carotenoid	102
		439±1	13±1	Diadoxanthin, Peridinin, Carotenoid	102, 105
		463±1	39±1	-do-	102, 105
611±3	83±5	610±1	33±1	Phycobiliprotein	102
665±1	24±1	663±1	20±1	Allophycocyanins	102
707±1	37±2			NA	

On the basis of the obtained curves and available literature, the following were observed:

1. The presence of 416nm peak in Dashmool Kwath having a width of 33±1nm revealed the presence of carotenoid, this peak was however not observed in Totola

2. The two peaks detected in Dashmool Kwath at 439nm and 463nm with a width of 13±1nm and 39±1nm is attributed to the presence of diadoxanthin, peridinin and carotenoid which was not detected in Totola.

3. The presence of allophycocyanin were detected in both by the presence of 611nm peak in Totola with a width of 83±5nm and at 610nm in Dashmool Kwath with a width of 33±1nm .

4. The 665nm peak having a width of 37±2nm in Totola revealed the presence of allophycocyanins.

5. The peak at 707nm in Totola could not be identified.

7.3. Thin layer chromatography

For the thin layer chromatography analysis, methanol and DCM were taken in the ratio 50:50 as the eluent. The study on Totola revealed the presence of three spots with Rf values 0.311, 0.475 and 0.689 revealing the presence of 2 non polar and one polar components. Fig. 7.6 (a) shows the TLC plate under different viewing conditions for Totola. However, the TLC analysis on Dashmool Kwath revealed one spot with Rf value 0.97. The other components were not separated properly as is depicted by a long tail like structure as seen in Fig. 7.6 (b) which thus indicates the presence of innumerable components have similar Rf values. The calculated Rf values of Totola and Dashmool Kwath is shown in Table 7.4.

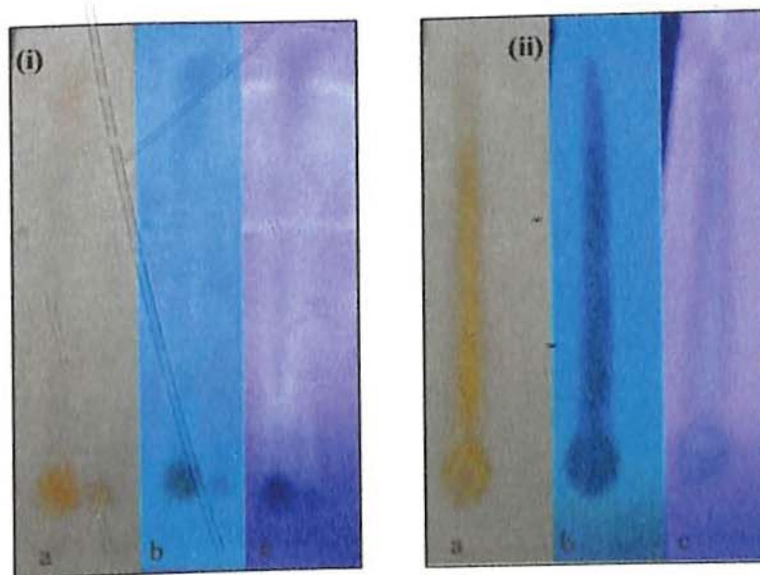

Fig.7.6: TLC plate when viewed under (a) Daylight, (b) Short Wavelength and c. Long Wavelength of (i)Totola, and (ii) Dashmool Kwath

Table 7.4: Rf values for Totola and Dashmool Kwath when viewed under different conditions

Sample	Viewed under daylight	Viewed under short wavelength	Viewed under long wavelength
Totola	1.9/6.1= 0.311 2.9/6.1= 0.475 4.2/6.1= 0.689	1.9/6.1= 0.311 2.9/6.1= 0.475 4.2/6.1= 0.689	2.9/6.1= 0.475 4.2/6.1= 0.689
Dashmool Kwath	-	-	5.9/6.1= 0.97

7.4. Conclusion

On the basis of the results obtained, we find that there is huge difference in the constituents present. In this regard, it is to be mentioned that the ayurvedic medicine, Dashmool Kwath contains the roots of Totola and not the seed. It was chosen for the study because at present there is no ayurvedic medicine made from the seed and also to find out whether the seeds and the roots have similar components or not. Thus, we find that the seeds contains a variety of constituents like flavonoids, terpenes, alkaloids, etc. which has been found to be absent in Dashmool Kwath. Thus, the seeds of Totola are more useful in the treatment of Diabetes.

Chapter 8

Atomic Absorption Analysis

The sample were analysed for zinc, sodium, iron, copper and manganese. The atomic absorption data for various plants obtained by using the three different digestion method and the ayurvedic counterparts are discussed below. It has been found out that the Method 1 was the most effective method for digestion of plant parts.

8.1. Chiraito and Maha Sudarshan Churna Vati

The atomic absorption data for Chiraito for the three different digestion methods and the ayurvedic medicines, Maha Sudarshan Churna Vati are shown in Table 8.1.

Table 8.1: Atomic absorption data for Chiraito and Maha Sudarsana Churna Vati

	Chiraito				Maha Sudarshan Churna Vati	
Element	Digestion Method	Conc. (ppm)	Standard Deviation	Chosen Conc. (ppm)	Conc. (ppm)	Standard Deviation
Sodium	1 2 3	28.213 34.273 25.657	±2.027 ±2.258 ±2.385	28.213	54.945	±0.134
Zinc	1 2 3	17.585 12.862 11.376	±0.958 ±0.752 ±0.098	17.585	4.729	±0.172
Iron	1 2 3	12.137 6.256 4.976	±0.156 ±0.047 ±0.086	12.137	6.729	±0.053
Manga-nese	1 2 3	22.444 5.021 5.996	±0.331 ±0.374 ±0.039	22.444	26.235	±0.269
Copper	1 2 3	2.276 0.495 0.624	±0.055 ±0.014 ±0.015	2.276	1.196	±0.012

The concentrations of Sodium, Zinc, Iron, Manganese and Copper in Chiraito were found out to be in the range 28.213±2.027, 17.585±0.958, 12.137±0.156, 22.444±0.331 and

2.276±0.055 respectively. The values for these elements in Maha Sudharsan Churna Vati were found out to be 54.945±0.134, 4.729±0.172, 6.729±0.053, 26.235±0.269 and 1.196±0.012 respectively. Apart from copper, the concentration of other elements in Chiraito is relatively high. This makes Chiraito a very effective medicine that helps in curing diabetes as the concentration of almost all the elements is above the average requirement of the human body. The presence of the trace elements makes it efficient for its therapeutic efficiency. On comparing the data with that of Maha Sudarshan Churna Vati, it has been found out that the concentration of Sodium and Manganese has relatively increased, while that of Zinc, Iron and Copper has relatively decreased. This is because apart from Chiraito, there are 11 other herbal medicinal plants that have been used in the preparation of the formulation. The combined effect of all these herbs must have given the above result.

8.2. Harra, Haritaki Churna and Triphala Churna

The atomic absorption data for Harra for the three different digestion methods and the ayurvedic medicines Haritaki Churna and Triphala Churna are shown in Tables 8.2 and 8.3 respectively.

Table 8. 2: Atomic absorption data for Harra

Element	Digestion Method	Concentration (ppm)	Standard Deviation	Chosen Concentration (ppm)
Sodium	1	18.539	±3.117	18.539
	2	32.574	±0.238	
	3	35.889	±0.149	
Zinc	1	4.398	±0.734	4.398
	2	4.548	±0.230	
	3	3.519	±0.789	
Iron	1	4.761	±0.049	4.761
	2	1.972	±0.277	
	3	2.270	±0.051	
Manganese	1	0.096	±0.016	0.096
	2	0.160	±0.028	
	3	0.25	±0.013	
Copper	1	0.280	±0.03	0.280
	2	0.447	±0.460	
	3	0.309	±0.026	

The concentration of Sodium, Zinc, Iron, Manganese and Copper in Harra were found out to be 18.539±3.117, 4.398±0.734, 4.761±0.049, 0.096±0.016 and 0.280±0.03 respectively. The concentrations in Haritaki Churna and Triphala Churna for the same elements were found as 47.149±0.093 and 55.620±0.149; 6.027±0.049 and 7.823±0.058; 61.153±0.059 and 52.789±0.09; 7.223±0.039 and 4.050±0.061; and 0.772±0.016 and 0.511±0.011 respectively.

Table 8.3: Atomic Absorption Data for Haritaki Churna and Triphala Churna

	Haritaki Churna		Triphala Churna	
Element	Concentration [ppm]	Standard Deviation	Concentration [ppm]	Standard Deviation
Sodium	47.149	±0.093	55.620	±0.149
Zinc	6.027	±0.049	7.823	±0.058
Iron	61.153	±0.059	52.789	±0.090
Manganese	7.223	±0.039	4.050	±0.061
Copper	0.772	±0.016	0.511	±0.011

We find that the concentration of other compounds apart from sodium in Harra is relatively low. The presence of sodium, zinc and iron with the above concentration makes it efficient in curing diabetes. However, on comparing with the ayurvedic counterparts, Haritaki Churna and Triphala Churna, the concentration of all the elements have increased with sodium and iron increasing the most. Of the two, the increase is much higher in Haritaki Churna than Triphala Churna. Even though Haritaki Churna has been prepared from Harra itself, the values of elements are higher which might have resulted from the purification and concentration of the e-fruits during the preparation of the medicine. However, the relative decrease in concentration of the elements and increase in

concentration of sodium in Triphala Churna might be because of the presence of Barra and Amla in the medicine.

8.3. Barra and Triphala Churna

The atomic absorption data for Barra for the three different digestion methods and the ayurvedic medicine, Triphala Churna are summarised in Table 8.4.

Table 8.4: Atomic Absorption Data for Barra and Triphala Churna

	Barra				Triphala Churna	
Element	Digestion Method	Conc. (ppm)	Standard Deviation	Chosen Conc. (ppm)	Conc. (ppm)	Standard Deviation
Sodium	1 2 3	0.382 0.235 0.303	±0.032 ±0.019 ±0.045	0.382	55.620	±0.149
Zinc	1 2 3	4.448 3.997 3.602	±0.790 ±0.475 ±0.045	4.448	7.823	±0.058
Iron	1 2 3	39.550 31.806 51.210	±1.314 ±0.264 ±0.139	39.550	52.789	±0.090
Manga-nese	1 2 3	4.763 1.052 2.858	±0.101 ±0.046 ±0.049	4.763	4.050	±0.061
Copper	1 2 3	0.663 0.160 0.526	±0.015 ±0.008 ±0.012	0.663	0.511	±0.011

The concentration of Sodium, Zinc, Iron, Manganese and Copper in Barra were found out to be 0.382±0.032, 4.488±0.790, 39.550±1.314, 4.763±0.101 and 0.663±0.015 respectively. Apart from iron, the concentration of other elements in Barra is relatively low. The presence of zinc, iron and manganese in the given concentration in Barra, makes it more efficient than Harra in treatment of diabetes; this is because of the fact that these three elements along with chromium plays a very important role in the diagnosis.

On comparing the data with that of the ayurvedic counterpart, Triphala Churna, the concentrations for Sodium, Zinc, Iron, Manganese and Copper were found as 55.620±0.149, 7.823±0.058, 52.789±0.09, 4.050±0.061 and 0.511±0.011 respectively.

We find that the concentrations of Sodium, Iron and Zinc has increased but the concentration of Manganese and Copper has decreased. As Triphala Churna is made from Harra, Barra and Amla, the present concentration was the result of these three constituents combined together.

8.4. Totola and Dashmool Kwath

The atomic absorption data for Totola for the three different digestion methods and the ayurvedic medicine Dashmool Kwath are shown in Tables 8.5. The concentration of Sodium, Zinc, Iron, Manganese and Copper in Totola were found out to be 24.981±4.354, 12.210±1.360, 3.053±0.023, 1.708±0.028 and 4.377±0.193 respectively. The concentrations in Triphala Churna for the same elements were found as 36.943±0.091, 6.080±0.154, 59.718±0.053, 10.988±0.055 and 1.236±0.017 respectively.

Table 8.5: Atomic absorption data for Totola and Dashmool Kwath

	Totola				**Dashmool Kwath**	
Element	Digestion Method	Conc. (ppm)	Standard Deviation	Chosen Conc.(ppm)	Conc. (ppm)	Standard Deviation
Sodium	1	24.981	±4.354	24.981	36.943	±0.091
	2	28.436	±3.497			
	3	14.550	±4.352			
Zinc	1	12.210	±1.360	12.210	6.080	±0.154
	2	10.363	±2.150			
	3	8.881	±1.673			
Iron	1	3.053	±0.023	3.053	59.718	±0.053
	2	3.944	±0.041			
	3	1.695	±0.052			
Manga-nese	1	1.708	±0.028	1.708	10.988	±0.055
	2	0.757	±0.024			
	3	0.914	±0.013			
Copper	1	4.377	±0.193	4.377	1.236	±0.017
	2	0.773	±0.007			
	3	2.476	±0.182			

It is seen that apart from sodium and zinc, the concentration of other elements in Totola is relatively low. However all the elements have the concentration almost in the range required for an average adult; thus it is therapeutically efficient. Dashmool Kwath is prepared from the roots of ten different medicinal plants which have yielded the above results. The values of the elements on comparison yielded the increase in the concentration of sodium, iron and manganese and decrease in zinc and copper.

8.5. Conclusion

The Atomic Absorption Spectroscopy data has revealed that all these medicinal plants are therapeutically efficient. Among the four medicinal plants, Chiraito has been found out to be most efficient than the others. All the medicinal plant picked up for the study was found to contain all the elements tested, thus they are efficient in the treatment of diabetes.

PART IV
CONCLUSION

Chapter 9

Conclusion

9.1. Conclusion

The analysis of the various spectra of the samples revealed the presence of various components which have already been proved to be very effective in curing various diseases. Some of these components like berberine, quercetin, cinnamic acid etc. have been isolated and are being used for the treatment of Diabetes. A brief details of the results obtained is done below:

9.1.1. Chiraito

The study revealed the dissociation of the initial components due to boiling by undergoing structural modification and the formation of the new dissociated flavonoids On the basis of the study it can be concluded that the boiled extract is more effective than the raw sample, the optimal boiling condition being 4 minutes post initiation on boiling.

Our findings shows that the presence of flavonoid in Chiraito and also in Maha Sudarshan Churna Vati. Apart from this, Chiraito had the presence of chrysin, β carotene and kaempferol; its ayurvedic counterpart had the presence of terpenes, alkaloids and phycocyanin. The findings reveal that Chiraito has the ability to diagnose diabetes as the presence of flavonoid was detected in it. It has been found out that flavonoid have a number of medicinal use, they are used as anti- oxidant, anthelminthic etc.

9.1.2. Harra

The studies revealed that Harra had the presence of NADPH; flavonoids, terpenes, alkaloids; quercetin, berberine and other flavonols; phycoerythrin; anthocyanins, azulenes, allophycocyanin, phycobiliproteins, etc. As has already been mentioned, these components have a number of medicinal uses. Thus, they do help in the treatment of diabetes. On comparison Haritaki Churna and Triphala Churna, it was found that these constituents were present in both the medicines. This clearly indicates that Harra has been used in preparing the medicines and Harra has the capability to cure diabetes.

9.1.3. Barra

Barra was found to have the presence of flavonoids, terpenes, alkaloids; cinnamic acid, quercetin, berberine and other flavonols, phycoerythrin, anthocyanins, azulenes, allophycocyanin, phycobiliproteins, etc.. Majority of these components were also found in its ayurvedic counterpart, Triphala Churna. This clearly indicates that Barra is used in the formation of the ayurvedic medicines which have been found to be effective against diabetes. This we can conclude that Barra is useful for treating Diabetes.

9.1.4. Totola

From the observed results, Totola was found to have the presence of NADPH, β carotene, flavonoids, terpenes, alkaloids, cinnamic acid, quercetin, berberine and other flavonols,anthocyanins, and azulenes. On comparison with its ayurvedic counterparts, only flavonoids, terpenes, alkaloids; azulenes and anthocyanins were present in the ayurvedic medicine, Dashmool Kwath. The differences is because of the fact that the medicine is prepared from the roots of Totola and not from the seeds. Dashmool Kwath was chosen for comparison as no ayurvedic medicine made from seed was available. The results indicates that the majority of components present in Totola were not present in Dashmool Kwath. Thus, the seeds of Totola are more effective in the treatment of diabetes than the roots.

9.1.5. Atomic Absorption analysis

Based on the atomic absorption results, all the plants had the presence of the trace elements tested. This indicates that they have various medicinal properties as these elements helps in various metabolism in the human body which are also related to diabetes. Thus, all these medicinal plants are therapeutically efficient. Among the four medicinal plants chosen for study, Chiraito had the maximum concentration of all the elements tested; as such it is most efficient.

The results thus obtained provide validity to the medicinal plants as being useful in curing diabetes. On comparing the results obtained with the ayurvedic counterparts, the results revealed the presence of the medicinal plants in them.

9.2. Future Prospects

The results obtained through this study could be coupled up with other methods like HPLC, NMR etc. whereby the active components can be determined with certainity. The determination of the active components and their isolation would be of great benefit both for the local masses and the medical community at large. This would also open doors for studies on other plants; some of which are endemic. The positive results if obtained on the other plants would validate the traditional knowledge of medicinal plants and such would be of great benefit to the society. The medicinal plants could then be patented which would greatly boost the economy of the local masses.

Comparative study can also be done taking the plants of the same species but different genre. Many such medicinal plants are found in this region like for the specie *Swertia* we find the plants as *Swertia Chiraita, Swertia Augustifolia, Swertia punica* among others. This would enable us to understand the medicinal values of the various plants belonging to the same species.

Also, with regard to the Atomic Absorption analysis, the study can be further taken forward by analyzing the concentration of the other elements like chromium, magnesium, etc. The atomic absorption analysis can also be done on the other medicinal plants. The trace element determination can also be done using Inductively Coupled Plasma Mass Spectroscopy and the results compared.

PART V
APPENDIX

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पत्र सं0................ दिनांक......................
F. No. 3/2012/ARRI-GTK/Gen/223 Date: 11-04-2013

To Whom It May Concern

This is to certify and confirm that the following plant parts collected by Mr. Abijit Bazracharza, IVth Semester (M. Phil- Ph. D.), Department of Physical Science, Sikkim University have been referred, identified and authenticated as detailed below:

S. No	Local Name	Scientific Name	Family	Plant parts
1	Chiraito	*Swertia chiraita* Buch. Ham	Gentianaceae	Leaves
2	Totola	*Oroxylum indicum* Vent.	Bignoniaceae	Seed
3	Barra	*Terminalia belerica* Roxb.	Combretaceae	Fruit
4	Harra	*Terminalia chebula* Retz.	Combretaceae	Fruit

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पत्र सं0................
F. No. 3/2012/ARRI-GTK/Gen./338

दिनांक....................
Date: 27/06/13....

This is to certify that the following Medicines are provided to Mr. Abijit Bazracharza, IVth Semester (M. Phil- Ph. D.), Department of Physical Science, Sikkim University, for research purpose to carry out his academic study. The details of these Medicines are as below :

Name of the Medicines:

1. **Maha Sudarshan Churna Vati (Tablet)**
2. **Dashmool Kwath (Crude powder)**
3. **Triphala Churna**
4. **Haritaki (powder)**

Ingredients' (Main)

Maha Sudarshan Churna Vati (Tablet)

	Sanskrit Name	Botanical Name	Part
1.	Haritaki	Terminalia chebula	1
2.	Vibhitaki	Terminalia belerica	1
3.	Amalaki	Emblica officinalis	1
4.	Shunthi	Zingiber offcinale	1
5.	Mariacha	Piper nigrum	1
6.	Pippali	Piper longum	1
7.	Katuka	Picrorrhiza kurroa	1
8.	Ativisha	Aconitum heterphyllum	1
9.	Shalaparni	Desmodium gangeticum	1
10.	Prishniparni	Uraria picta	1
11.	Guduchi	Tinospora cordifolia	1
12.	Kiratatikta	Swertia chiraita	26.5

Dashmool Kwath (Crude powder)

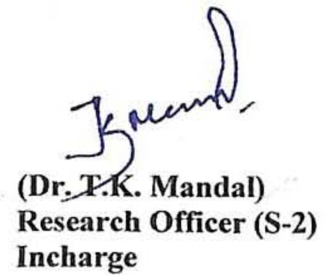
	Sanskrit Name	Botanical Name	Part
1.	Bilwa	Aegle marmelos	1
2.	Shyonak	Oroxylum indicum	1
3.	Gambhari	Gmelina arborea	1
4.	Patala	Stereospermum suaveolens	1
5.	Ganikarika	Premna integrifolia	1
6.	Shalaparni	Desmodium gangeticum	1
7.	Prishniparni	Uraria picta	1
8.	Brihati	Solanum indicum	1
9.	Kantakari	Solonum xanthocarpum	1
10.	Gokshru	Tribulus terrestris	1
11.	Pippali	Piper longum	1

Triphala Churna

	Sanskrit Name	Botanical Name	Part
1.	Haritaki	Terminalia chebula	1
2.	Vibhitaki	Terminalia belerica	1
3.	Amalaki	Emblica officinalis	1

Haritaki (powder)

	Sanskrit Name	Botanical Name
1.	Haritaki	Terminalia chebula



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