STUDIES ON PHYTOCHEMICAL ASPECTS OF SOME MEDICINAL PLANTS USED BY THE NYISHI TRIBE OF ARUNACHAL PRADESH

Thesis submitted to the Nagaland University in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Botany

By

ASHISH KUMAR TRIPATHI

Regd. No. 537/2013

DEPARTMENT OF BOTANY

NAGALAND UNIVERSITY

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Nagaland University

(A Central University Estd. By the Act of Parliament No. 35 of 1989)
Department of Botany, Lumami, Nagaland 798627

CERTIFICATE

This is to certify that the thesis entitled **"Studies on Phytochemical Aspects of Some Medicinal Plants Used by the Nyishi Tribe of Arunachal Pradesh"** submitted to Nagaland University, Lumami in partial fulfillment for the award of the degree of Doctor of Philosophy in Botany is an original research work carried out by **Mr. Ashish Kumar Tripathi**, bearing registration number **537/2013** under our supervision.

Further, it is certified that no part of this thesis has been submitted anywhere for any other research degree to the best of our knowledge.

(Co-Supervisor)
Dr. Rama Shankar,
Scientist-4, Botany
Regional Ayurveda Research
Institute, Jhansi, U.P.
CCRAS, Ministry of AYUSH,
Govt. of India

(Supervisor)
Dr. Limasenla
Assistant Professor,
Department of Botany,
Lumami, Nagaland

(Head)
Department of Botany
Lumami, Nagaland

Nagaland University

(A Central University Estd. By the Act of Parliament No. 35 of 1989)

Lumami 798627, Nagaland, India

April, 2018

DECLARATION

I, Ashish Kumar Tripathi, bearing Ph.D. registration number 537/2013 hereby declare that, the thesis entitled **"Studies on Phytochemical Aspects of Some Medicinal Plants Used by the Nyishi Tribe of Arunachal Pradesh"** being submitted to Nagaland University, Lumami for the degree of Doctor of Philosophy in Botany is the record of original and independent research work carried out by me under the supervision of Dr. Limasenla, Assistant Professor, Department of Botany, Nagaland University, Lumami, and co-supervision of Dr. Rama Shankar, Scientist -4, Regional Ayurveda Research Institute, CCRAS, Ministry of AYUSH, Govt. of India, Jhansi, Uttar Pradesh.

Further, I declare that this thesis has not been previously submitted for the award of any other degree or diploma to any University or institution. My declaration is hereby forwarded by my supervisors and head of the department.

16.04.18

Date: 16.04.18
Place: Lumami

(Ashish Kumar Tripathi)
Regd. No. 537/2013
Department of Botany,
Lumami, Nagaland

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16.04.18

Ashish Kumar Tripathi

CONTENTS

ABBREVIATIONS

µl	Microliter
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
API	Ayurvedic Pharmacopoeia of India,
AYUSH	Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy
bp	Boiling Point
CCRAS	Central Council for Research in Ayurvedic Sciences
cm	Centimeter
Conc.	Concentration
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOAc	Ethyl Acetate
EtOH	Ethanol
FAO	Food and Agriculture Organization
g	Gram
g L^{-1}	Gram per litre
GAE	Gallic Acid Equivalent
h	Hour
M	Molal
MeoH	Methanol
mg	Milligram
ml	Milliliter
mm	Millimeter
N	Normality
nm	Nanometer
PLIM	Pharmacopoeial Laboratory for Indian Medicine
RARI	Regional Ayurveda Research Institute
RE	Rutin Equivalent
ROS	Reactive Oxygen Species
SD	Standard Deviation

SFRI	State Forest Research Institute
Temp.	Temperature
TFC	Total Flavonoid Content
TLC	Thin Layer Chromatography
TPC	Total Phenolic Content
UV-Vis	Ultraviolet - Visible
WHO	World Health Organization

LIST OF TABLES

LIST OF FIGURES

	Plant)
30.	TLC Profiles of *Mesua ferrea* L. (Stem Bark)
31.	TLC Profiles of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark)

Medicinal Plants and Medicine

The Indian indigenous system of medicine is purely based on medicinal plants for the development of drugs. It fulfils the desire of rural population of the country for healthcare management in curing disease. Medicinal plants have medicinal activity based on potentials of the rich source of ingredients in the form of secondary metabolites (alkaloids, glycosides, flavonoids, steroids, protein antioxidant, carbohydrates, crude fibres and essentials oils etc) for drugs. In medicinal plants, phytochemical constituents have been confirmed to have medicinal activity by scientific method and have been approved by the governmental bodies in India such as the Ayurvedic Pharmacopeial Commission or other government agencies.

The World Health Organization (WHO) Expert Group defined Traditional Medicine as the sum of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing (WHO, 1976). Various historical records indicate that claim the primitive use of folklore remedies/plants-based drugs to manage health-related issues in various parts of the worlds (De Pasquale, 1984; Gurib-Fakim, 2006; Balunas & Kingdom, 2005). Importance of medicinal plants was perceived by the human being since ancient time. People started the use of various plant parts in different disease and as a food and medicinal sources.

Folk Medicine

Folk remedies were started by ancient men during the traditional events and festivals and most of these remedies were plants based. In ancient times people tried to find out remedies for diseases based on folk medicine by such medicinal plants. Accordingly, tribal people believed that various diseases and human health were correlated with their culture and traditions. Folklore practices pertaining to these aspects are referred to as the genre of Ethnomedicine. Traditional medicine is diverse in comparison to the modern medicine. In folk communities, any type of knowledge about disease management is transferred from generation to generation. The Government of India has incorporated the traditional medicine in the Drug Acts of 1940, which includes traditional medicaments, demands licensure for practice, and assures the safety and control of drugs, produced in India (WHO, 1976).

Although the natives from their villages and their traditional families have migrated to urban areas, they continued to maintain the use of folk healing practices including traditional medicine (Campbell & Burgess, 2011). The extensive knowledge owned by the tribal people on traditional medicine remains a mystery to the scientists and modern people (Vedavathy, S., 2003).

Medicinal Plants and Nutritional Supplements

Medicinal plants play a vital role in providing the natural nutritional supplement in the form of food as well as medicine. The information and importance about medicinal plants have been accumulated since ancient times and at present; we have many valuable means of ensuring human healthcare management using these methods. Many nutritional

ingredients have been documented in medicinal plants by various workers (Inti, M. & Faoro, F., 2006; Ravi Subbiah, M. T., 2007; Shukla , Y. & Singh, M., 2007).

In remote areas where no modern medical facility is available, peoples depend on sources of medicinal plants and vegetables for food and medicine requirements. People are exploring proteins, minerals, fibres and other ingredients from the sources of medicinal plants and vegetables from the wild for their daily diets and requirements (Mohammed & Sharrif, 2011). Wild sources of medicinal plants have been found to be the basic source of phytomedicine to sustain health and support resistance against diseases (Hyman and Afolayan. 2006).Tribal populations are nutritionally more enduring even during periods of drought (Altieri *et al.*, 1987).

Recently, there has been an increase in awareness for the indigenous system of medicine and health care system which has been recognized as a human right through international organization platform, *i.e*., United Nations Human Rights Commission, Millennium Development Goals (MDGs), the World Health Organization (WHO) and Food and Agriculture Organization (FAO).According to WHO, traditional medicine shows that approximately 80% of the population of the world still depends on their indigenous or traditional systems of medicine, especially in India and other developing countries. Now, it is the core focus of the government, the scientist, the scholar, and research organizations to explore the valuable information related to healthcare systems from folk healers or ethnic groups and bring them to scientific forum after scientific validation. The use of medicinal plants for various purposes is given in figure-1.

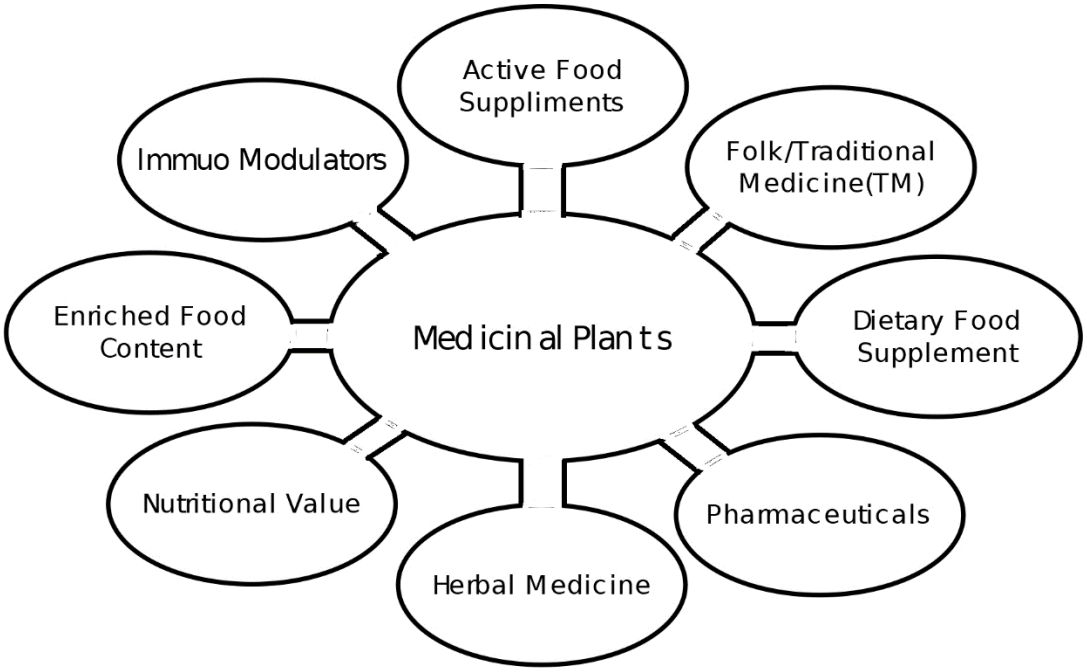


Figure- 1. Role of medicinal plants as food and nutraceuticals.

Antioxidants and Medicinal Plants

Medicinal plants have good natural deposits of antioxidants in the plant parts like fruits, stem, root bark and leaves. Tribal people were using mostly wild sources of medicine including medicinal plants and fruits vegetables and these are the goods source of naturally occurring antioxidants. Antioxidants have the potential to prevent disease and improve the health of human beings.

Most of the oxidative stress resulted by reactive oxygen species (ROS) and increased health-related disorders like cancer, diabetes, inflammatory diseases and other heart-related issues. An antioxidant directly reacts with reactive radicals by stopping the free radicals. Under various types of stress conditions, the human body releases more reactive oxygen species (ROS) and reactive oxygen species are dangerous for health

(Krishnaiah *et al.*, 2011). When our body is producing excess amount of ROS then sudden changes happens to the body through peroxidation and nucleic acid damages. These are responsible for various disorders in our body (Cho & Kleinberger, 2007; Kinnuala & Cropa, 2004; Hyun *et al.*, 2006).

Oxidative stress directly or indirectly affects the human health and creates serious issues in the human body due to the absence of antioxidants, as these increases the reactive free radicals, and initiate signals for degenerative diseases (Shahidi *et al.,* 1992). Cells are protected by enzymatic and non-enzymatic antioxidant systems from free radicals and protective methods were not able to prevent continued stress (Lu J.M., Lin P.H., Yao Q. & Chen C., 2010). Antioxidant is naturally available phytoconstituent found in plants which induced the oxidizing chain reactions by stopping the oxidation process of molecules (Halliwell, B., Gutteridge, J. C., 1995).

Medicinal plants used by the tribal communities are nutritionally very important for tribal and rural population and also help in the management of various diseases (Ayyanar & Ignacimuthu, 2013).Therefore, it is important to analyse the plants used by tribal people or folk healers for their antioxidant properties.

Pharmacognostical Approach

Medicinal plants are widely used in the form of medicine, food materials and also used in pharmaceutical industries by ethnic group or folk healers globally. But the question is whether, the plant sample is genuine or not. Pharmacognostic study is one of the parameters to check the genuineness or purity of raw drugs (Babu *et al.,* 2010). Pharmacognosy study is focused on structural and phytochemical description and identification of the plants and also secondary metabolite pathways leading to the

identification of bioactive compounds. For standardization of raw drug, the basic key is the correct identification of the plants to examine their quality, safety, and delicacy.

Pharmacognostical study deals with medicinal plant identification, authentication and different parameters pertain to examination of crude drug for drug discovery (Balunus & Kinghorn, 2005). Quality assessment is one of the tools to check their authentication and quality of the raw drug by pharmacognosy studies (Shah *et al.*, 2013). Pharmacognostic study gives the basic tools for the examination of raw drug which assist in drug discovery (Uthaykumari & Sumathy, 2011). Pharmacognosy termed as *"Science of biogenic or naturally derived pharmaceuticals or poisons. It is pertinent to characterized crude drugs pharmacognostically"* (Phillipson, 2007).

Pharmacognosy is the ancient and also modern scientific tool to evaluate medicinal plants (De Pasquale, 1984).Since ancient times medicinal plants were used as medicine, starting with the raw drugs like powder composition, powder extract, decoction and various modes of administration as recorded in Ayurvedic and other systems of medicine (Balunus & Kinghorn, 2005). Pharmacognosy study includes macroscopic, microscopic, powder microscopy physiochemical features, and study of the anatomical description of the plants and their parts.

Phytochemistry

Naturally occurring bioactive chemical compounds found in plants are termed as phytochemicals. It helps in the healthcare system for prevention of the diseases and acts as nutritional supplements in the form of macronutrients and micronutrients (Hasler, C.M. & Blumberg J.B., 1999).Medicinal plants have a basic source of the bioactive compound,

either in the form of traditional medicine or as a pure active chemical ingredient (Farnsworth *et al*., 1985).

The content of sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, etc., under Primary constituents. Contentslike alkaloids, terpenes, flavonoids, lignin, plant steroids, curcumins, saponins, phenolics, flavonoids and glucosides in medicinal plants are called secondary constituents (Hahn, N.I., 1998). Flavonoids are referred to as polyphenolic compounds that are available everywhere in nature. The flavonoids are available in naturally occurring medicinal plants, vegetables, fruits, tea, coffee and fruit drinks. Presently about 4,000 flavonoid compounds have been reported by scientific research (Pridham, J.B., 1960).

Recently, for the standardization of herbal drugs, many techniques are being developed. A technique like spectroscopic techniques, mass spectrometry and nuclear magnetic resonance (NMR) and various chromatographic methods are used for isolation and identification of the chemical compound in plant sources (Cordell, 1995; Phillipson, 1995 & Kinghorn 2001). Chromatographic method is one of the tools used for the isolation of natural chemical products. Primary metabolites are different from Secondary metabolites as described by Kossel in 1891 (Bourguad *et al.*, 2001). Primary and secondary metabolites are categorized on the basis of their biosynthetic pathways and molecular size (Kossel, 1891 & Bourguad *et al.*, 2001). Primary and secondary metabolites are widely used in pharmaceuticals industries for cosmetics, medicine and nutritional supplements.

Role of ethnic or traditional potentials becomes more popularized due to their pharmaceuticals importance (Fabricant & Farnsworth, 2001; & Kunwar *et al.*, 2013). Traditional medicines playa very significant role in the management of healthcare and

prevention of the diseases. Present work has investigated on the identity and potentials of medicinal plants used by Nyishi tribe with respect to their properties like medicinal properties, chemical composition, nutraceuticals status.

Relevance of the Study

The Papum Pare is one of the popular districts of Arunachal Pradesh and is known for its rich traditional knowledge and cultural heritage. Due to the shortage of modern medical systems, the villagers in the remote areas of the district are dependent on the local traditional practitioners. A good number of traditional healers are doing their practices in different remote places of the district. The district is also famous for wild edible plants and locally cultivated vegetables, fruits etc., which gives a very low yield due to lack of knowledge in modern agro-techniques and their trade for socio-economic developments. In addition to this, natural calamities force the inhabitants to use wild edible fruit and vegetables for their day to day consumption, which is also a rich source of carbohydrates, proteins, fats, vitamins, and minerals etc.

The healing practices in Arunachal Pradesh are very common since the ancient times by local tribal communities based on their indigenous knowledge with the help of locally available wild sources of medicinal plants. Some of the healers have good knowledge of healing for the management of chronic diseases. The healing practice of folk medicinal plants for medicine is as old as the mountains in the remote rural areas in the traditional food systems and practices among the Nyishi tribe of Papum pare district of Arunachal Pradesh. The issues viz., hypertension, diabetes, eye disease, cancer and various other ailments were unrest health issues in ancient days. Therefore, it is expected that the medicinal plant sources have good indicators and contributions to those rural remote

population who totally depend on wild fruits and medicinal plants. They are directly or indirectly taking biologically active compounds in addition to nutritional supplements.

There are several medicinal plants used by the natives of Arunachal Pradesh and are reported in the well-established system of medicinal plants of Ayurveda etc., like *Mesua ferrea* L., *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., *Acmella paniculata* (Wall. ex DC.) R.K.Jansen, *Elaeocarpus ganitrus* Roxb. ex G.Don, *Tinospora cordifolia* (Willd.) Miers, *Rauvolfia serpentina* (L.) Benth. ex Kurz, *Oroxylum indicum* (L.) Kurz, *Embelia ribes* Burm.f. and *Leucas aspera* (Willd.) Link etc.

The list of folk medicines has been reported by different researchers from different parts of the district of Arunachal Pradesh, but the detail Phytochemical study of folk medicines has not yet been done by any workers in Papum Pare district of Arunachal Pradesh so far. The studies of medicinal plants will also help in establishing the feature for correct identification of plants used by local healers. The literature available on the folk medicine used by Nyishi Tribe has revealed that Phytochemical studies have not yet been standardized for medicinal plants used in traditional healing system by Nyishi tribe.

Necessity of the Study

Documentation and Phytochemical analysis of medicinal plants obtained from healers during the survey may provide a future research references which could be potential for drug formulation from natural sources against various ailments. It could validate the healing information obtained from the healers based on their healing knowledge. Study of antioxidant composition in medicinal plants may lead to good supplements as an anti-aging agent for the old people and management of human healthcare. The nutritional composition of the selected medicinal plant will be revealed, and hence positive or negative outcomes

will provide encouragement in knowing more about the nutritional status of tribal medicine.

Identification and examination of the local traditional healer's duties and practice method via survey and interview could become the baseline research work for future researchers. This work could provide organized ethnic information and reference related to Nyishi tribe of Arunachal Pradesh.

Objectives of the Study

The objectives of this investigation are as follows:

1. Exploration and selection of prominent medicinal plants used by Nyshi tribe of Arunachal Pradesh.
2. Powder microscopy study of selected prominent medicinal plants
3. Powder study of plants samples fallowed by different photochemical reaction.
4. Physiochemical study of selected plant species.
5. Neutraceutical analysis of selected plant species (Carbohydrates, fat, protein, crude fibre etc.)
6. Antioxidant screening of the selected plants species.
7. Phytochemical analysis of the selected plants species

Review of literature

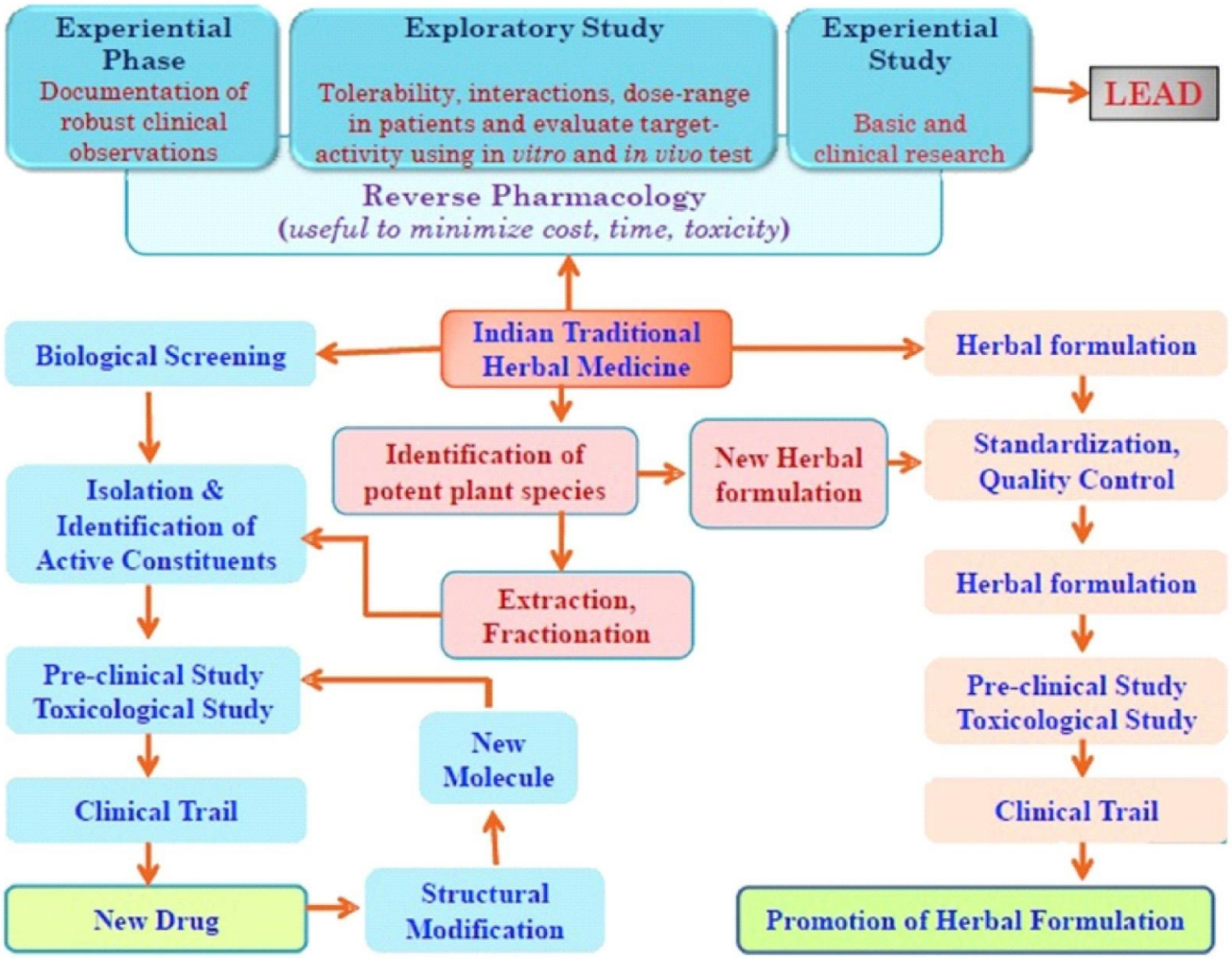
Tilbert & Kaptchuk, (2008) reported that folk or traditional medicines include wild source of medicinal plants, without chemical modification was used by local folk healers in the management of various kind of diseases. As per WHO (1976), it is defined that the folk medicine as "*The sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to preserve health, as well as to prevent, diagnose, improve or treat physical and mental illnesses*". In China, Liya Hong *et al.,* (2015) reported 368 medicinal plant species and their uses in 95 human disorders. These plants were used by the Maonan tribal people as their folk medicine. According to WHO (1976), 82% of the total population are using folk medicines for their basic healthcare needs in the developing country like Asia and Africa. According to Moa *et al.* (2015), folk healing practices is based on plant sources, and in the developing countries uses of medicinal plants has been adapted in their daily routine for medicine and food. Evidences based on ancient scriptures, the *Atharvaveda* (Gupta *et al.,* 2014), the emphasis on ancient literatures for folk medicine can be seen**.** Pharmacognostical characterization study focusing the transparent image for understanding the logic and mystery of *materia-medica* to molecular pharmaceutical approaches has been done by various workers (Balunas and Kinghom, 2005; De Pasquale, 1984 & Gurib-Fakim, 2006). The home-based therapies mostly consist of regular kitchen ingredients and these ingredients are generally used as over the counter (OTC) drugs (Heinrich, M., 2005; Schmidt, B., 2008; Calapai, G., 2008; Ribnicky *et al.,* 2008).

De Pasquale, (1984) had reported knowledge of traditional healing practices by ancient man relating to the problems of disaster, injury, death, etc. Dhami, (2013) studied the scientific skill of man in obtaining the information on the indigenous medicinal plant

used from birth to death processes, in traditional cultures and locally available medicinal plants. Saikat, S. & Chakraborty, R., (2016) has given the systematic exploration and key point that helps in obtaining the knowledge about the plant's world and their medicinal value. The role of traditional medicine and their promotion is illustrated in figure-2.

During the development of civilized society folk information of medicinal plants were conserved in the forms of traditional culture, ethics, festivals, local song etc. In the Indian subcontinent the classical text of Ayurveda like *Nighantu* and *Charaka Samhita* are some of the basic classical (3500 BC) references for the modern study. In ancient Mesopotamia (2600 BC), evidences of drug usage were recorded and defined as "cuneiform" where the importance of oil, specially from *Cedrus* species, *Cupressus empervirens*, *Glycyrrhiza glabra* and *Commiphora* species etc were mentioned (De Pasquale, 1984; Gurib-Fakim, 2006; Dhami, 2013).The oldest and most famous is the "De Material Medica'' written by the Greek physician Dioscorides and is globally accepted as a standard reference book for Ethnomedicine in Europe (De Pasquale, 1984).

Pharmacognosy is an ancient as well as a modern science for drugdevelopment and discovery (De Pasquale, 1984). But the term "Pharmacognosy", has been coined 200 years ago only (Kinghom, 2001). Pharmacognosy is defined as crude drugs originated from plants, animal and mineral origin sources (Wallis, 1985). Pharmacognosy is one of the sciences for medicinal plants and raw drugs to check their authenticity for drug discovery (Balunus & Kinghom, 2005). Systematic and organized knowledge of folk medicine identification and authentication based on pharmacognostic analysis of plant drugs has already been emphasized (Sahoo *et al.,* 2010; Shah *et al.,* 2013).


Experiential Phase
Documentation of robust clinical observations
Exploratory Study
Tolerability, interactions, dose-range in patients and evaluate target-activity using in *vitro* and *in vivo* test
Experiential Study
Basic and clinical research
LEAD
Reverse Pharmacology
(*useful to minimize cost, time, toxicity*)
Indian Traditional Herbal Medicine
Biological Screening
Herbal formulation
Identification of potent plant species
New Herbal formulation
Standardization, Quality Control
Isolation & Identification of Active Constituents
Extraction, Fractionation
Herbal formulation
Pre-clinical Study Toxicological Study
New Molecule
Pre-clinical Study Toxicological Study
Clinical Trail
Clinical Trail
New Drug
Structural Modification
Promotion of Herbal Formulation

Scheele isolated the chemicals in form of acids (oxalic acid, malic acid and tartaric acid) from the plant samples in 18th century (De Pasquale, 1984). Kramer had used microscope for observing powdered samples, screening of starch to avoid duplicity (Cloves, 1894; Elm Bark, 1895). For the determination of Alexandrian and Indian *Sennas*, a method to observe a comparative count of trichomes was developed by Sayre, (1897). Microscopy was used for counting the number of palisade cell under upper epidermal cell also for preliminary screening of the drugs for the different plants species (Wallis, 2011).

African traditional medicines are also a very ancient system of medicine. This system depended on the plants and it is gathered from all healing systems and is named as the "Cradle" (Gurib-Fakim, 2006). In America, folk healer's practices are known as shaman practices. They uselocal medicinal plants which form the basis for the "Pharmacopoeia of the United States" (Gurib-Fakim, 2006). The influence of Chinese medicines has been reflected in South East Asian countries (Gurib-Fakim, 2006). The renowned royal leader Shen Nung also explained about the importance of medicinal plants in his research finding - which may be written in 2500 years B.P as a substitute of their conventional date of 3500 B.P. by the Chinese traditional medicine was organized and written between 100 and 200 BC (Gurib-Fakim, 2006). Chinese Meteria Medica was published in 1977 and it is one of the important reference books for Chinese traditional medicine. It is an information bank of 6,000 drugs of which 4,800 are from medicinal plants sources (Gurib-Fakir, 2006).The Egyptians had recorded their traditional knowledge of medicinal plants in papyrus; the most well-known of these writings is the historic "Ebers papyrus" prepared from *Cyperus aquaticus*. The Eber Papyrus (1500 BC) contains very old information about traditional medicine used before 3000 BC (Gurib-Fakim, 2006). In the 18thcentury Arabs were the first

to establish the personal drug provisions. At that time Persians developed traditional medicine and pharmacopeia work from various sources, for example Canon Medicine – which is also known as "Greco-Roman Medicine" (Cragg & Newmann, 2002). The Mexicans were well known for their hallucination-healing-practices. Reports indicates that they had used *Abrus precatorius* seeds for prompt responses in sexual effectiveness, Camotyl - tuberous medicinal plants for analgesia and tobacco leaves as a cure for headache and diziness (De Pasquale, 1984).

In India, very ancient and famous Grantham "Veda", contains the information about medicinal plants and their uses in healing practices were recorded in Atharvaveda during 2000 to 1000 BC (Shah, 1981). Atharvaveda contains more than 2000 medicinalplants with information regarding their uses and their potential as medicine (Maheshwari, 1987). Cure and prevention of various diseases by Opium, Rauwolfia, Nux vomica, Aconite, Hashish, Datura and mustard are well defined in Artharvaveda (De Pasquale, 1984).

In ancient India, two renowned Indian botanist Charaka and Shushrut, who worked on medicinal plants and healing practices during Vedic period have written valuable the books "Charaka Samitha" and "Shushruta Samhita". They have information about 341 and 395 species medicinal plants respectively (Mukheijee, 1974; Mukheeije, 2001). The Ayurveda, which was a system of medicine practised in the Indian subcontinent, is considered as an Upaveda of Artharbaveda and was written in between 2500 and 500 BC (Mukheeije, 2001).

Ethnographic research has played a long and important role in medicine (Kaplan-Myrth N., 2007; Pope C., 2005). The term ethnography was first recognized in 1922 (Tesch R., 1990) and is foundation of expressive science (Dharamsi, S., 2009). Like, R. C.

(1986) explained that Ethnography is not restricted to study of ethnic observation and practices. They include explanation to a range of social circumstance within various ethics.

Compilation of medicinal plants information and their uses with anthropocentric approach is known as Ethnobotany, a term coined by Power, (1874). The term "Ethnobotany" used in 1895 by J.W. Harsberger to describe the utilization of plants by aborigines. Ethnobotany means cultural and traditional ideas of people about plants (Burchifield, 1972; Jain, 1967 and Ford, 1978). Ethnobotany consists of the study of medicines, food, fibres, dyes, toxic and nontoxic plants, used by people and about 1600 angiosperms are used as medicinal plants Nayar *et al.,* (1989). Chopra, (1958) reported that 1400 species of medicinal plants are available in India and 1500 species of medicinal plants are mentioned in Wealth of India series on raw materials Volume I-XI (1948-1976).

Chopra's Indigenous Drugs of India by Chopra *et al*. (1958), highlighted medicinal plants utilization in the country in a scientific manner. It had a supplement named the Glossary of Indian Medicinal plants (Chopra *et al.,* 1969). S. K Jain who contributed a lot to the Indian ethnobotany with his team founded planned ethnobotanical research (Jain, 1963a, 1963b, 1963c, 1963d, 1964b, 1965a, 1965b, 1976b, 1981a etc). The contribution towards medicinal plants in Northeast India was done by Jain and Borthakur (1980). In his study, "Indian Traditional Medicine in the history of Indian Medicines", Mukheijee, (2001) discussed the government's responsibility and prospects of ethnobotany in detail. Rajasab *et al.,* (2004) also reported 51 plant species out of which 21 species are used by local villagers in Karnataka as indigenous liquor and for medicine purposes. Sinha & Lakra, (2005) have reported more than 130 medicinal plants uses with nutritional value that were used by the tribals of Odisha State. Bhalla & Chand, (2007) worked on medicinal plants used by tribes

of Himachal Pradesh and investigated their medicinal and nutritional values. Binu, (2010) worked on wild medicinal plants used in Kerala and documented 41 medicinal species. Bose *et al.,* (2014) documented 48 medicinal plants for prevention of various ailments used by Paliyar tribes, Tamil Nadu.

In Arunachal Pradesh, the first ethnobotanical survey on "Mishmi teeta", from Mishmi Hills followed by Robinson, (1841) had compared Mishmi teeta from this region to that of outside (Griffith, 1836). Taxonomic introduction and status of forests in Arunachal Pradesh was started in 1987 by Kaul & Haridasan, (1987). Hooker, (1872 – 97) started research on the British India flora within this area without mentioning the ethnomedical and economic important of the plants. Kanjilal *et al.,* (1934-40) reported the higher medicinal plants of NEFA region, which is also included medicinal plants species used by Adi tribes. Some 319 species of angiosperms were explored by Rao & Joseph, (1965). The Botanical Survey of India (BSI), Eastern Zone, had explored the medicinal plants in the Districts of Siang, Kameng, Sibunsiri and Tirap districts of Arunachal Pradesh and reported some species of medicinal plants that were found to have medicinal uses (Rao, 1972). The famous ethnobotanist of Arunachal Pradesh, Bhattacharjee, (1977), explained the flora of Mebo-East Sian, Arunachal Pradesh, and reported some wild medicinal plants in Mebo area. He also described some ethnomedicinal plants used in folk medicine that was similar to *Artocarpus integrifolia*, *Gossypium herbaceum*, *Calamus* species etc. Medicinal plants are used as oral contraceptives in Siang District, Arunachal Pradesh (Joseph, 1977). This research complemented with the works of Thothathri & Pal (1978), who had worked on medicinal plants from Tirap and Sibunsiri Districts. About 500 medicinal plants were explored by (Haridasan & Bhuyan, 2002) and based on medicinal plants used by different tribes of

Arunachal Pradesh, 25 medicinal plants were documented which belonged to the Aka tribes of Arunachal Pradesh with their identification mark and vernacular name for the society (Kar, 2004). Tag & Das, (2004, 2005) have documented 5 medicinal and 11 food based medicinal plants with their ethnomedical value used by the Miri and Nishi Tribe of Arunachal Pradesh. In Arunachal Pradesh, a total 5000 species of angiosperms have been documented and out of these 500 species of plants are used in the folk healing practices (Haridasan K., 2001).

Kala, (2005) worked on Apatani tribe of Arunachal Pradesh and reported 158 medicinal plants species and their uses for various ailments. Murtem & Das (2005), have reported medicinal plants used by different tribes in various kind of diseases of which 40 medicinal plants are used by Mishing tribe of Arunachal Pradesh (Yonggam, 2005). More than 20 medicinal plants species were documented basing on their aromatic and drugs potential (Rawat & Shankar, 2005). Another worker Singh, (2005) has documented and explained the traditional utilization of more than 50 medicinal and aromatic plants of Arunachal Pradesh.

Murtem, (2005) documented and explored the information related to medicinal plants used for different purposes by different tribes of Arunachal Pradesh. Sumpam *et al.,* (2011) reported 74 medicinal plants from 41 families that were used for the treatment of 25 ailments. Namsa *et al.,* (2011) reported that 50 medicinal plants species from 29 families were used for treatment of 22 men and 4 animal diseases. Murtem, G. & Chaudhry, P. (2016) have also reported 140 medicinal plants used by three tribes of Upper Subansiri district of Arunachal Pradesh out of which 18 plants used for treatment of livestock diseases. Chakraborty *et al.,* (2017) conducted studies on folk healing and

ethnopharmacological aspects of drug formation by mixing plants part of different medicinal plants. Bharali P. *et al.* (2017) had conducted a study on the Adi tribe and Nyishi tribe and reported 52 medicinal plants species belongs to 22 families. Tripathi et *al.*, (2017) conducted a survey study in Nyishi dominated areas and reported 21 medicinal plant species by the Nyishi tribes. Traditional medicines as primary drugs have been used for a long time (Kunwar & Adhikari, 2005; Emeka & Elizabeth, 2009).

Central Council for Research in Ayurvedic Sciences (CCRAS) published "Pharmacognosy of Indigenous Drugs" in three volumes in 1999.This publication contains Indian Pharmacognosy. Information on 80 drugs with scientific, Sanskrit, vernacular names and along with plant parts used. The "Meteria Medica" of India has emphasized on folk healing and traditional system of therapeutically valuable drugs. According to Mukheeije (2001), folk healing system is based on Ayurveda. Abid Mahmood *et al.* (2005) have reported folk medicine also contains modern drugs. Gurib-Fakim, (2006) has commented that Indian Ayurvedic science is the foundation of systematic medicine. Charaka's *Samitha* (1900 BC) has contributed the knowledge of Ayurveda science practices (Mukheeije, 2001).

AYUSH (Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy, published the Ayurvedic Pharmacopoeia of India (API), Part-I, Volume I in 1986 which included 80 monographs of Ayurvedic drugs without Thin Layer Chromatography (TLC), however in Volumes II – VI contains TLC patterns of each drug. These Volumes are guidelines for TLC profiling of drugs. The API, Part-I, Volume I (1986) was reprinted in 2009 under the title of Thin Layer Chromatographic Atlas of Ayurvedic Pharmacopoecial Drug, Part-I, Volume I. All over the world, pharmaceutical herbal

companies and local communities are faced with the problems of adulteration and substitution at the raw material stage (Ahmad M., 2010). The work of Jemilat A. & Ibrahim, (2012) to identify and access the quality study of plant *Crotolaria lachnosema* Stapf. suggested some means of checking adulteration. Singh *et al.,* (2012) worked on pharmacognostic studies on roots of *Berberis umbellata* Wall, ex G. Don. known as Barberry wherein they estimated the amount of berberine content through High Profile Thin Layer Chromatography (HPTLC) densitometry.

Review on Pharmacognostical studies revealed that a good number of papers have been published by different workers. Gopalkrishnan & Shimpi, (2012) have conducted a pharmacognostical studies on stem bark of *Madhuca longifolia* (Koen.) Macbr. var. *latifolia* (Roxb.). Jayanthy *et al.,* (2012) have worked on the standardization of different species of Solanaceae. Baskar *et al.,* (2012) reported pharmacognosy study in *Givotia moluccana* leaves. Kaur *et al.,* (2012) have studied on *Lannea coromandelica* Houtt. John *et al.,* (2013) studies on medicinal plants used as Ayurvedic drugs Ativisha and musta to developed pharmacognostical standard of *Aconitum heterophyllum* Wall, ex Royle, (Ativisha) and to compare with its substitutes, *Cyperus rotundus* L. (Musta), *C. scariosus* R. Br. Zaman & Pathak (2013) studied on Pharmacognostical and phytochemistry of leaf and stem bark of *Annona reticulata* L. Isaac Kingsley Amponsah *et al.,* (2014) studied on pharmacognostic authentication and quality study of *Hilleria latifolia*. Umesh *et al.* (2017) reported Arjuna plants as usually non-controversial medicine.

DPPH (2, 2-diphenyl-2- picrylhydrazyl hydrate) technique for screening antioxidant potential was developed by Blois (1958). The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radical scavenging technique was

developed by Rice-Evans and Miller, (1994) and then modified by Re *et al.*,(1999). These two methods help in the screening of total phenolic and flavonoid concentration of the sample. Singleton and Rossi (1965) have designed Folin-Ceocalteu technique to access phenols. Antioxidant stability was first mentioned in the Chinese medical treatise by Su Wen, written 2500 years ago (Ni, 1995). Flavonoids are a major set of polyphenolic antioxidants found in lots of fruits and vegetables (Rice-Evans *et al.,* 1996).

Phenolics or polyphenolics are responsible for antioxidant capacity in fruits, vegetables and in medicinal plants. Some antioxidant like ascorbic acid, alpha-tocopherol and flavonoid cannot be synthesized in vivo (Cao, 2000). Antioxidants induce the prevention of free radical-induced tissue damage by stopping the production of radicals (Young & Woodside, 2001). Most of the green vegetable and medicinal plants are good sources of naturally occurring antioxidants and intake of green vegetables and medicinal plants directly or indirectly may provide antioxidant in a body (Pereira *et al.*, 2011). Green vegetable and medicinal plants include the high amounts of polyphenols and flavonoids (Heimler *et al.*, 2009). An antioxidant can be generally defined as any compound that stop the oxidative damage to a target molecule (Yamagishi *et al.*, 2011) The main work of an antioxidant is the ability to search the free radicals due to their redox hydrogen donors and singlet oxygen quenchers (Wu *et al.*, 2011 & Anokwuru *et al.*, 2011). The free radicals can be scavenged by the medicinal plants and synthetic (butylated hydroxyl toluene, butylated hydroxyl anisol and tetra butyl hydro quinone) antioxidants (Mbaebe, B.O., 2012). But synthetic antioxidants are slowly declining in their usage as the natural antioxidants have gained accepted due nonexistence of side effects (Meenakshi *et al.* 2011). Attanayake *et al.*, (2016) reported that Sri Lankan medicinal plants have been very useful for management

of various ailments including diabetes mellitus, liver diseases and arthritis in Ayurvedic drugs. Medicinal plants are known as good source of nutritional supplements in form of vegetables or food, and medicinal plants also helps in removing deficiency of foods as well as medicine (McBumey *et al.,* 2004).

Lack of nutritional supplements in food is linked with various health disorders (Kumari *et al.,* 2004). Most of the wild medicinal plants are highly valuable and nutritious (Hadjichambis *et al.,* 2008).Wild variety of medicinal plants can act as medicine and good nutritional supplement for the human being (Abitogun, 2010).

India having one of the largest population of tribal community, folk healers have an important role in maintaining health among the tribal communities (Mohan and Kalidass, 2010). The continually growing populations in the developing nations face an ever-rising difference between humans and available nutrients. Researches focused on specific medicinal plants will reduce this gap between population and nutrients supply (Addis *et al.,* 2013). Plants play a significant role in nutritional supplements, source of income and food security to the country (Deshmukh & Rathod, 2013). A major population of developing nations still depends on folk medicine obtained from medicinal plant sources (Stockwell, C., 1988). At present, the trend to analyse the plants possessing anti-microbial activity for different ailments is emerging (Kaushik R.D., 2003). Plants have an unlimited capacity to synthesize aromatic substances and secondary metabolites. Most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963).The majority are secondary metabolites, of which at approximately 12,000 have been isolated, a number estimated to be less than 10% of the total metabolites (Schultes, R.E. 1978). Drugs from medicinal plants are easily found, cheap, harmless and efficient with no side effects.

Although a growing number of people consume medicinal plants, these plants should be examined to know more about their properties, their safety and efficiency (Arunkumar *et al.*, 2009).

Phytochemistry has play an important role in the discovery and inquiry of new drug through phytotochemical analysis (Balunas & Kinghom, 2005). Searching processes for drugs from potential medicinal plants lead to the characterization and isolation of cocaine, codeine, digitoxin and quinine (Newman et *al.,* 2000). Nitisonone obtained from *Callistemon citrinus* Stapf. (Myrtaceae) used to catabolise toxins accumulated in kidneys and liver. Tiotropium isolated from *Atropa belladona* L. helps in prevention of chronic obstructive pulmonary disease (Balanus & Kinghom, 2005). Phytomedicines are obtained from plants parts such asbarks, leaves, flowers, roots, fruits, seeds (Criagg, G.M., 2001). Information about the chemicals present in plants is essentials for drugs development in the form of phytoingredients (Mojab F., 2003 & Parekh J., 2008. The presence of bio active compounds like tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids have been worked out by Edoga, H.O., (2005) and Mann, J., (1978). Fabricant & Farnsworth, (2001) have identified 122 chemical compounds of definite structure obtained from 94 plant species used as traditional medicines.

Balunas & Kinghom, (2005) in their conventional work under National Cooperative Drug Discovery Group have isolated different bioactive compounds. Gurib-Fakim, (2006) have discussed about medicinal plants in term of photochemistry, pharmacognosy and pharmacognosy along with various analytical techniques used in phytochemistry in the context of cardiovascular, HIV, CNS, malarial, cancer, respiratory

and diabetes. Secondary metabolites which contain alkaloids, flavonoids, terpenoids, steroids, anthraquinones and volatile oils etc., are limited (Buchanan *et al.*, 2000).

Thus, the survey of literature has revealed that considerable work has been done to bring the benefits of ethnobotanical studies since the ancient times. This has continued to the modern day with more specialised scientific methods. However, for secluded areas such studies are far from complete and wait proper documentation and exploration.

Brief Introduction of the Study Area

Arunachal Pradesh, the land of down lit- mountainous terrain is situated in extreme North Eastern region of India. It is bounded with China in the North and partly in the East, Myanmar in the East, Bhutan in the West and the state of Assam and partly Nagaland in the South. The state is having an area of 83,743 Sq.km. and is native of more than 25 tribal races and over 100 sub-tribes. Its entire area is extended from valleys, foot hills, Mountainous terrain to snow clad peaks with varying elevation from 100 msl to 7000 msl with several turbulent rivers and rivulets. It also comes under heavy rainfall area with an average rainfall ranging from 250 cm to 350 cm in different regions of the state. Mostly rain fall season is from May to September and it depends on the South- West climate.

The well distributed rainfall, hilly physiographic and altitudinal variation has resulted greater plant diversity in the state. The state is also diverse in culture and has wide traditional beliefs. The inhabitants have enormous knowledge on medicinal plants and depend on forest for their livelihood and food. But this knowledge is going to be extinct due to the modernization of the society. At present there are only a few village elders, who have considerable knowledge on medicinal plants.

Papum Pare, district is positioned between 27°5'10"N longitude and 93°36'48"E latitude and having an elevation of 750 m from msl. The Papum Pare has total forest area of 94.95% of its geographical zone and has the highest area under forests in the State. The district was formed in 1999 when it was seprated from lower subansiri. The district is divided into two sub-divisions: Sagalee and Yupia Capital complex, which are further divided into 10 administrative circles, namely, Mengio, Leporiang, Sagalee, Toru, Kimin,

Doimukh, Balijan, Tarasso, Naharlagun and Itanagar. The district is mainly dominated by Nyishi tribe.

The word 'NYISHI' is originated from two words Nyi and Ishi. Nyi mean human that embodied, from ATU Nyia (son of Abo Tani means a first real man on earth) and Ishi means hill. Therefore, in composite word 'Nyishi' denotes the ancestors of Atu Nyia Tani who stays in the highland. This is the reason why they are called highlanders. The Nyishi tribe is one the dominant ethnic group in Papum Pare and they are linked with Indo Mongoloid communities with respect to their tradition and culture and their language is similar to the Tibeto-Burman people. People of Nyishi tribe have their own specific method of classification, identification and uses of medicinal plants. (Das, A.K., 2003; Tewari *et al*, 1978). Study areas covered during the survey is given in (Table no.1.) and map given in (Figure-3).

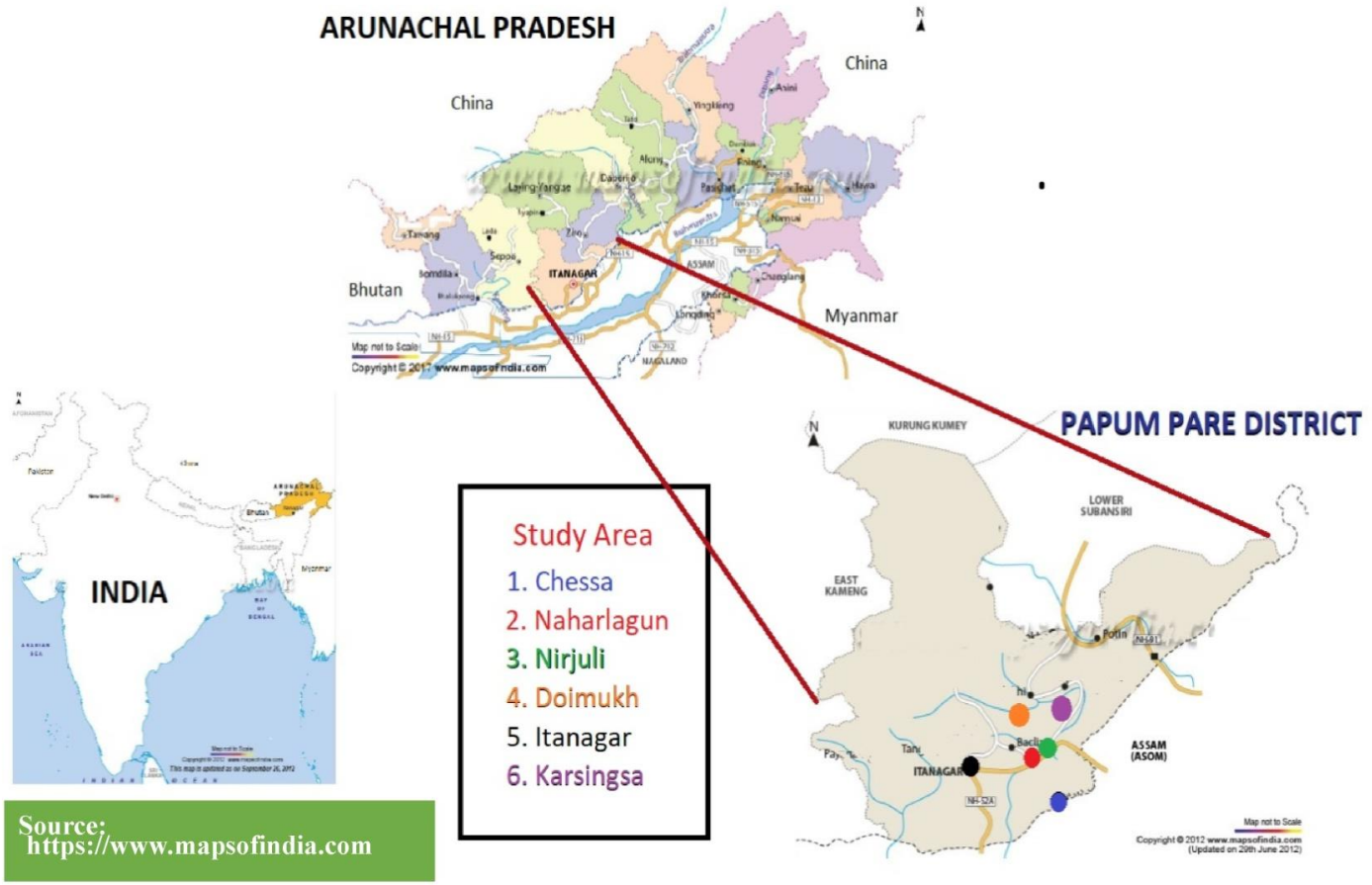
Table-1: Study Area Covered for Exploration of Medicinal Plant during Survey

Name of the Area	District
Chessa forest and village	Papum Pare
Naharlagun	Papum Pare
Nirjuli	Papum Pare
Doimukh	Papum Pare
Itanagar	Papum Pare
Karsingsa	Papum Pare

Exploration and Documentation of Folk Medicinal Plants

In tribal village or remote areas it is customary to obtain permission from the community head or Gaon Burah for any kind of survey in this jurisdiction. Therefore, a specific outline of the research work was prepared for the villagers to explain about the aims and scope of the survey work. Then a data sheet (annexure-1) was prepared which

Map of Study Area



Source: https://www.mapsofindia.com

Figure- 3. Study Area Location Map

includes brief information of the local healers, locality, plants, and mode of administration in respective disease plants parts used etc.

The survey and field visits were conducted in different circles, towns and villages of Nyishi tribe (Map-1) accompanied by some informants of the respective study area and the medicinal plants were collected following the approaches and methodologies given by Martin (1995), Scultes (1962) Jain (1964, 1967, 1987 & 1989). The collected specimen have been identified with the help of local flora and herbarium preserved in different research institute such as Regional Ayurveda Research Institute (RARI), State Forest Research Institute (SFRI), Botanical Survey of India (BSI), Itanagar and CCRAS New Delhi.

The information about the uses of plants was obtained from the folk healer and local medicine practitioners (both man and women), particularly the older person at the age group between 40-80 years. In some cases, younger practitioners of the age group between 20-40 years were also entertained.

Present surveys have collected 38 medicinal plants (Table - 2 and Figs. - 9-13). These were prepared into herbarium sheets following the procedure given by Jain & Rao, (1977) and the voucher specimen were deposited in the Botany Department, Nagaland University.

In the present study, for the evaluation of different parameter *viz.* Phamacognosy (Powder microscopy, florescence study, physiochemical parameters, phytochemical screening), Nutritional analysis of plants sample (Carbohydrate, crude protein, crude fibre, crude fat), Antioxidant (DPPH Free Radical Scavenging, ABTS Free Radical Scavenging Assay, Total Phenolic Content, Total Flavonoid Content) and phytochemical study (TLC of plants sample). Five medicinal plants samples were selected *viz.* *Bauhinia variegata* L.

(Flower), *Leucas aspera* (Willd.) Link (whole plant), *Bixa orellana* L., *Mesua ferrea* L. *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark). The selection of these five plants was based on the information obtained from the folk healers. As per the interview results majority of the healers used these plant parts frequently in different ailments.

Pharmacognostic Study

Methods for Pharmacognostic Study

The present research work includes Powder Microscopy, Ash value, Extractive value following the standard of Ayurvedic Pharmacopoeia of India (Anonymous, API, Vol.III, Part-II, 2010) and Florescent Study of powder sample followed by Gokhale & Kokate, (2011), Kokate *et al*, (2012). Phytochemical Studies have been carried out by the technique described by Raman, (2008); Harbome, (2009), & Harbome, (1999).

Collection and Preparation for Powder Microscopic Evaluation

Plant samples were collected from the study site and micromorphological characters were studied using microscope and Camera Lucida to prepare diagrams. For further study, the samples were oven dried (below 40^0C) or as per requirement of sample type.

Procedures

Whole Plant and Flower

For the evaluation and characterization of the powdered sample of flower and whole plant parts were taken in sufficient amount and treated with Chloral-hydrate solution on a slide and covered it with a cover slip, warm over a low flame for a short time and observed the tissues and cells under Camera Lucida to produced the diagram.

Stem Bark

Little amount powder sample of stem bark were kept on a slide, added 1-2 drops of phloroglucinol and a drop of concentrated hydrochloric acid, covered it with a cover slip, plot out the excess liquid from one side of the slide with filter paper, and then applied 1-2 drops of chloral-hydrate solution from the other side of the slide, lignified elements are stained crimson-red. Observed the tissue and cells under Camera Lucida to produced the diagram.

Fluorescent analysis

For the Fluorescent study of powder samples the method adopted by Shah & Seth, (2010) were employed. When powdered drug is treated with different chemicals, specific colour is observed under UV and visible light for specific drug sample (Tables- 3-7).

Physicochemical parameters

Determination of Total Ash

2 g accurately weighed powdered drug sample was incinerated in a silica dish at a temperature not exceeding 600° C until free from carbon, cool in a desiccator for 30 min and weighed without delay. If carbon free ashes were not obtained then exhaust the charred mass with hot water, and the residue was collected on an ashless filter paper, incinerate the residue on filter paper and evaporate to dryness. Then ignited at a temperature not exceeding 600° C again and the percentage of ash were calculated with reference to the air-dried drug.

Determination of Acid Insoluble Ash

25 ml of dilute hydrochloric acid (HCl) was added drop-wise to the crucible containing total ash. Insoluble matter was collected in ashless filter paper and washed with hot water until the filtrate was neutral. Filter paper containing the insoluble matter was transferred to the crucible, dried on a hot plate and ignited. Allow the residue to cool in a suitable desiccator for 30 minutes and weighed without delay. The content of acid-insoluble ash was calculated with reference to the air-dried drug.

Determination of Alcohol Soluble Extractive

Macerated 5 g sample of coarsely powdered drug, was taken with 100 ml of alcohol (purity 99.9 %) in a closed flask for 24 hours, shaking frequently for 6 hours and allowed to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105° C, to constant weight and weigh the residue. Percentage of obtained alcohol-soluble extractive was calculated with reference to the air-dried drug.

Determination of Water Soluble Extractive

Macerated 5 g sample of coarsely powdered drug, was taken with 100 ml of chloroform water (2.5 ml chloroform in purified water to produce 1000 ml) in a closed flask for 24 hours, shaking frequently for 6 hours and allowed to stand for 18 hours. Filtered rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105° C, to constant weight and weigh the residue. Percentage of obtained alcohol-soluble extractive was calculated with reference to the air-dried drug.

Phytochemical Screening

Phytochemical screenings was carried out as per the method described by Raman (2008) and Harbome (2009). Medicinal plants samples were taken and soaked in various chemical solvents (Methanol, Acetone, Ethyl acetate, Chloroform, Benzene and Petroleum ether) for three days and filtered and evaporated in water bath. The extracts were treated with various chemical reagents for the phytochemical screening to find out presence or absence of phytochemical present in the samples.

Procedure

Test for Alkaloids

Mayer's test:

Small amounts of plant sample extracts were treated with Mayer's test reagent, observed till the formation of white or cream coloured precipitate.

Wagner's test:

To 5ml. of plant sample extracts few drops of Wagner's reagent were added and wait to observe till the formation of reddish brown precipitate.

Hager's test

To 1ml. of plant sample extracts was treated with 3 ml of Hager s reagent and wait for the formation of prominent yellow precipitate.

Test for flavonoid

NaOH test

To 1ml. of plant sample extracts, few drops of aqueous NaOH and HCL were added along the sides of the test tube and wait for the formation of yellow orange colour.

Sulphuric Acid test

2ml. plant sample extracts was treated with concentrated H_2SO_4 for the formation of

orange colour.

Lead Acetate test

3ml. of plant sample extracts were mixed with 5 drops of lead acetate and wait till the formation of white or cream precipitate.

Test for Glycosides

Plant sample extracts were dissolved (0.lg) in pyridine, added sodium nitropruside reagent and made alkaline with NaOH solution, pink to red colour solution indicates the presence of glycosides.

Test for Phenols

Ferric Chloride test

Plant sample extracts were treated with 5% ferric chloride and wait till the formation of deep blue or dark green colour.

Test for Saponin

Foam test

Plant sample extracts and few drops of distilled water were added and shaken vigorously until a persistent foam forms.

Test for Carbohydrate

Molisch's test

A few drops of Molisch's reagent were added to each of the portion dissolved in distilled water; this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube and kept it for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of two layers was a positive test.

Fehling's test

1 ml of plant sample extract was boiled on water bath with 1 ml each of Fehlingsolutions A and B and wait for red precipitate.

Barfoed s test

To 1 ml of plant sample extract, 1 ml of Barfoed's reagent is added and heated on a boiling water bath for 2 minutes and wait for red precipitate.

Nutritional Analysis of Folk Medicinal Plants

In the present study nutritional analysis of freshly collected plant sample was carried out in five species *viz. Bauhinia variegata* L. (Flower), *Leucas aspera* (Willd.) Link (Whole plant), *Bixa orellana* L., *Mesua ferrea* L., *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark).The samples were first washed with running water followed by rinsing with distilled water and chopping into small pieces. The samples were then placed in an oven at below 40 °C until a constant weight was attained. The dried samples were grounded to powdered form and pass through an 1 mm sieve and were stored in seal in container for analysis.

Estimation of Total Carbohydrates

The estimation of carbohydrate in all the plant material was carried out by Anthrone method (*Yemm, E.W.,* and *Willis, A. J.* 1954). Carbohydrates in the powdered material were first hydrolyzed into simple sugars using dilute HCl. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm (Lamda-25, UV/VIS spectrometer, Perkin Elmer was used). Initially 100mg of the sample was taken into a boiling tube. Then hydrolyzing by keeping it in a boiling water bath for 3 h with 5 mL of

2.5N-HCl and cooled to room temperature. This was neutralized with solid sodium carbonate until the effervescence ceases and the volume was made up to 100mL and centrifuged if necessary. Six concentrations of different volumes of 0, 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard glucose of 10mL taken from stock solution of 100mg glucose in 100mL water and two volumes of 0.5mL & 1mL aliquots from each sample were taken. From the graph of the concentration (x-axis) versus absorbance (y-axis) of glucose concentrations, a straight line characteristics graph was obtained. From this graph we obtained the mg of glucose at the respective absorbance at different concentrations of samples. Taking this amount of glucose corresponding to the test sample, the percentages of total carbohydrate in the plant samples were calculated.

$$\text{Amount of carbohydrate (\%mg)} = \frac{\text{Sugar value from graph (mg)} \times \text{Total Vol. of extract (ml)}}{\text{Aliquot sample used (ml)} \times \text{Weight of sample (mg)}} \times 100$$

Chemicals and Reagent

i) 2.5N –HCl, Anthrone reagent (Dissolve 200mg anthrone in 100 ml of ice cold 95% H_2S0_4 (Prepared fresh before use).

ii) Standard glucose: for stock, dissolve 100mg in 100ml water, for working standard 10 ml of stock solution diluted to 100 ml with distilled water, store in refrigerator after adding few drops of toluene.

Estimation of Crude fibre

In plants sample crude fiber consists largely of cellulose, lignin and mineral matter. The crude fiber content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity.

The estimation of Crude fibre in all the plant samples was carried out by Chopra, S. L. & Kanwar, J. S., (1991). Estimation was done by extraction of 2g powdered material with petroleum ether to remove fat. The dried material was boiled with 200ml of H_2SO_4 for 30 min. Filtered through muslin cloth and washed with boiling water until washings were no longer acidic. The obtained solution was boiled with 200ml of NaOH for 30 min and filtered through muslin cloth and then washed with 25ml of boiling 1.25% H_2SO_4, three 50ml portion of water and 25ml alcohol. The residue was removed and transferred to porcelain dish (preweighted dish W1), the residue was dried for 2 h at 130°C, the dish was cooled in a desiccators and weighed (W2), further ignited for 30 min at 600°C and then cooled in a desiccator and weighed (W3) The crude fiber was than calculated using following formula.

$$\text{Crude fiber \%} = \frac{\text{Loss in weigh on ignition (W2-W1)-(W3-W1)}}{\text{Weight of sample}} \times 100$$

Chemicals:

i) Sulphuric acid solution (0.255 ± 0.005N); 1.25g concentrated sulphuric acid diluted to 100 ml.

ii) Sodium hydroxide solution (0.313 0 ± 0.005N); 1.25g sodium hydroxide in 100ml distilled water. (Titrate to check concentration).

Estimation of Crude Protein

Crude protein was estimated as described by *Kjeldahl* Methods (*Ishwaran, V.,* 1980). A quantity of product containing 100 mg plant sample weighed into a micro digestion flask, then 0.5 g catalyst mixture (i.e. 32 gm of potassium sulphate with 5g of red mercuric oxide) and 2.5 ml concentrated H_2SO_4 was added. This was heated with a small flame until frothing cease and then heated until the solution is clear. It was cool and 8ml distilled water was added into it. The solution was transferred to the distillation apparatus and the flask was rinsed with 3 portion of 2ml distilled water. After that, 15ml sodium hydroxide/sodium sulphide mixture was added and steam distillation was done with 2ml boric acid. 10ml distillate was collected, and an additional 2ml was distilled. The outside of the condenser tube was rinsed and titrated the content of the flask with 0.01N H_2SO_4. Carry out a blank determination. Total Nitrogen (N) was calculated (*Kjeldahl* Methods.) as:

Calculation of Total nitrogen % and Crude protein %:

Weight (in mg) of sample used: W

Volume (in ml) of sulphuric acid used in test: VI

Volume (in ml) of sulphuric acid used in blank: V2

Normality of Sulphuric acid: N

$$\text{Total nitrogen \%} = \frac{1400\ (VI\text{-}V2)\ N}{W}$$

Crude protein % = Total nitrogen × factor (6.25).

Chemical Reagents

i) Sulphuric acid: (a) concentrated: special grade 1.84, nitrogen free, (b) 0.01N, accurately standardized

ii) Catalyst mixture: Grind in a mortar 32g potassium sulphate with 5 gram of red mercuric oxide.

iii) Sodium hydroxide/ sodium sulphide mixture: Dissolve 400g pure sodium hydroxide in about 700ml distilled water, and dilute to 1 liter. Dissolve 40g sodium sulphide in 111 ml distilled water. Mix the two solutions and filter through glass wool.

iv) Mixed indicator. Dissolve 0.lg bromocresol green I 100ml. 96% ethanol (solution A). Dissolve 0.lg methyl red in 100ml 96% ethanol (solution B). Mix 100 ml solution A with 20 ml solution B.

v) Boric acid: Dissolve 40g boric acid in distilled water and dilute to 1 liter. Add 40 ml mixed in

Estimation of Crude Fat

The crude fat content of the plants samples was estimated by extracting with ether petroleum. The petroleum ether dissolves in the fat and hence the fat content was estimated by weighing the plants sample before and after ether extraction; the loss in weight gives the fat content in sample or evaporating the petroleum ether extract to gain the fat and then

weighing. The fat content of the samples were carried out by the method described by Ishwaran (1980). Crude fat content was estimated by extracting 2g of plants samples with petroleum ether (bp 40-60 ^{0}C) in a soxhlet extractor; petroleum ether was then evaporated in vacuum evaporator. Increase in weighed of beaker / decrease in weight of sample gave the crude fat.

Antioxidant Screening

Screening of antioxidant potential of plant samples is based on scavenging activity by 2, 2-Diphenyl-1- picrylhydazyl (DPPH), ABTS radical scavenging, total Phenolic and Flavonoid content.

Chemicals Reagent and Solvents

2,2-Diphenyl-l-picrylhydazyl (DPPH), Gallic acid, ferric chloride, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)- Sigma-Aldrich (Munich, Germany). Merck's Folin-Ceocalteu (Merck (Mumbai, India)) other reagents and solvents used were analytical grade (RANKEM New Delhi, India).

Preparation of Crude Extract

Plants Samples wash in tap water to clean then wash with distilled water. Medicinal plant samples were dried in an oven at below 40 degree Celsius temperature till constant weight was achieved. Dried samples were grinded in laboratory mill and kept in air tight container for farther use. 100g each powder samples were soaked in 500 ml methanol for 48 hours and filtered. The residue of sample was re-extracted twice with 500ml of methanol each. The total filtrate was concentrated by rotatory evaporator at 45^{0}C under reduced pressure.

Determination of Antioxidant using DPPH Free Radical Scavenging

The antioxidant activity in plant sample was determined as per technique described by Aoshima *et al.*, (2004). 100 ml of plant sample extract, 2.9 mL of 2, 2-Diphenyl-l-picrylhydazyl (DPPH) reagent (0.1mM in methanol) was added and vortexed vigorously. The reaction mixture was stored in the dark for 30 minute at room temperature and decolouration of DPPH was measured against a blank at 517 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (Lamda-25, Perkin Elmer, Cambridge, UK). Linear calibration curves were produced with R^2= 0.9994 (Fig. 3) and result was calculated as trolox equivalent per gram dry sample.

The inhibition % was calculated using the formula

$$\text{Inhibition\%} = \frac{\text{A (control)-A (test sample)}}{\text{A (control)}} \text{ X } 100$$

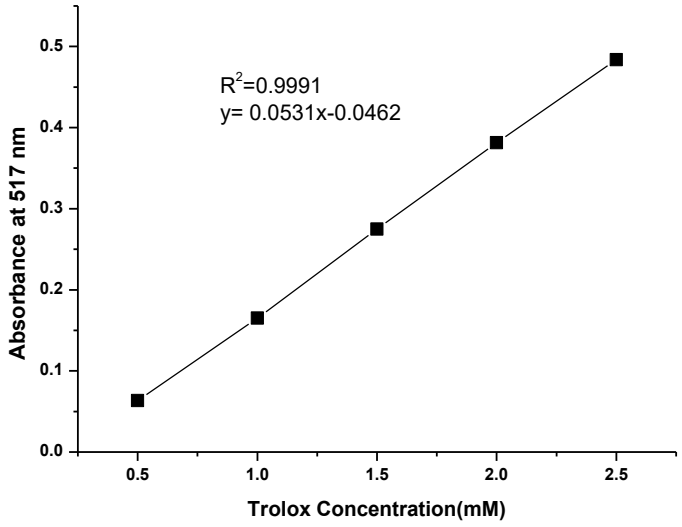


Figure -3, Trolox concentration vs absorbance for DPPH standard curve

ABTS Free Radical Scavenging Assay

The ABTS radical cation scavenging activity in plant sample was performed as per method described by Re *et al*., (1999) with slight modifications. The ABTS solution (**7**mM) was reacted with potassium per sulfate (2.45mM) solution and kept overnight in dark to yield a dark green-colored solution containing ABTS radical cation. Prior to use in the assay, the ABTS radical cation was diluted with 50% methanol for an initial absorbance of about 0.700± 0.02 at 743nm using UV-Vis spectrophotometer (UV-Vis) spectrophotometer (Lamda-25, Perkin Elmer, Cambridge, UK). With the temperature set at 30°C. Free radical scavenging activity was assayed by mixing 100µL of test sample with 2.9ml of an ABTS working standard in a microcuvette. The decrease in absorbance was measured at exactly 1 minute after mixing the solution and then at **1** minute intervals up to 6 minutes when final absorbance was recorded. Linear calibration curves were produced with R^2= 0.9988 (Fig. -4) for evaluation of antioxidant activity in ABTS and result was calculated as trolox

equivalent per gram dry sample.

The inhibition % was calculated using the formula

$$\text{Inhibition\%} = \frac{\text{A (control)-A (test sample)}}{\text{A (control)}} \times 100$$

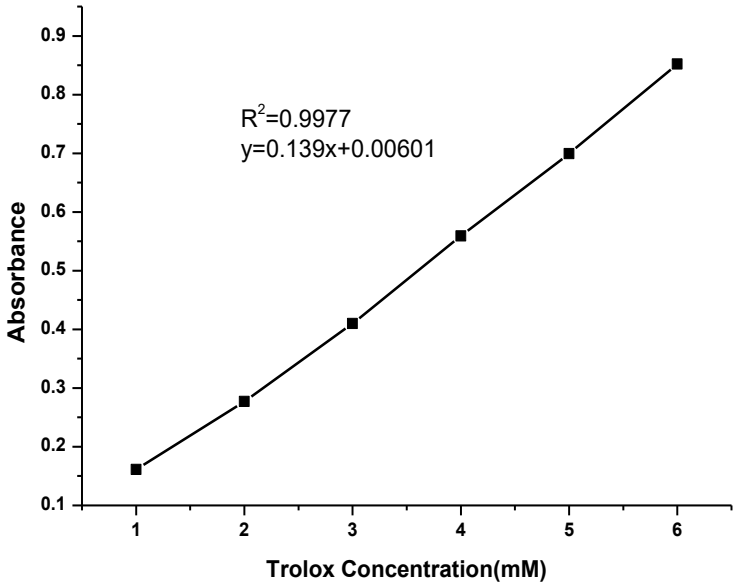


Figure - 4, Trolox concentration vs absorbance for ABTS standard curve

Determination of Total Phenolic Content

Total phenolic content in plant sample was estimated by the Folin-Ciocalteu method followed by Singleton and Rossi (1965). 900µL of distilled water and 1mL of the Folin- Ciocalteu reagent l00µL of filtered extract was added. After 5 minutes, 2mLof saturated sodium carbonate (75g.L-l) and 2 mL water was added. Absorbance of the

resulting blue- colored solution was measured at 765nm using UV-Vis spectrophotometer (UV-Vis) spectrophotometer (Lamda-25, Perkin Elmer, Cambridge, UK). After incubation at 30^0C for 1.5 h with intermittent shaking. Quantification measurement was performed based on a standard calibration curve of 20, 40, 60, 80 and 100mg/100mL of Gallic acid in 80% methanol. Total phenolic content was expressed as Gallic acid equivalent (GAE) in the dry sample. Linear calibration curves were produced with R^2=0.9989 (Fig. 5).

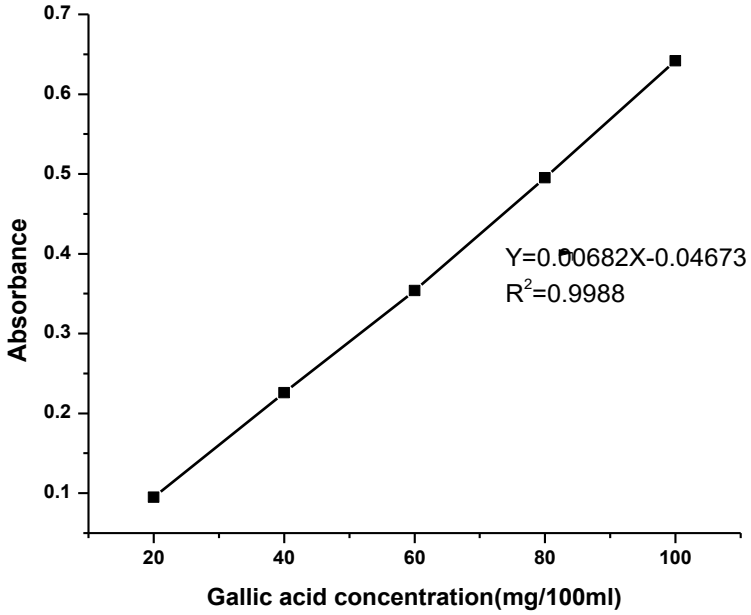


Figure -5, Gallic acid standard curve for TPC

Determination of Total Flavonoid Content

Total flavonoid content in plant sample was estimated by the colorimetric technique of Sahreen and Khan (2010) with slight modification. 50mg of sample was dissolved in 10 ml of 80% aqueous methanol and filtered through Whatman filter paper No.42 (125mm). In a 10mL test tube, 0.3ml of extract, 3.4 mL of 30% methanol, 0.15 mL of 0.5M sodium nitrite, and 0.15 mL of 0.3 M aluminium chloride

hexahydrate were added and mixed after 5 minutes, 1mL of 1M sodium hydroxide was added. The absorbance of the mixture was measured at 510 nm using UV-Vis spectrophotometer (Lamda-25, Perkin Elmer Cambridge, UK) and values were express as rutin equivalent antioxidant capacity. Linear calibration curves were produced with R '=0 9994 (Fig. 6).

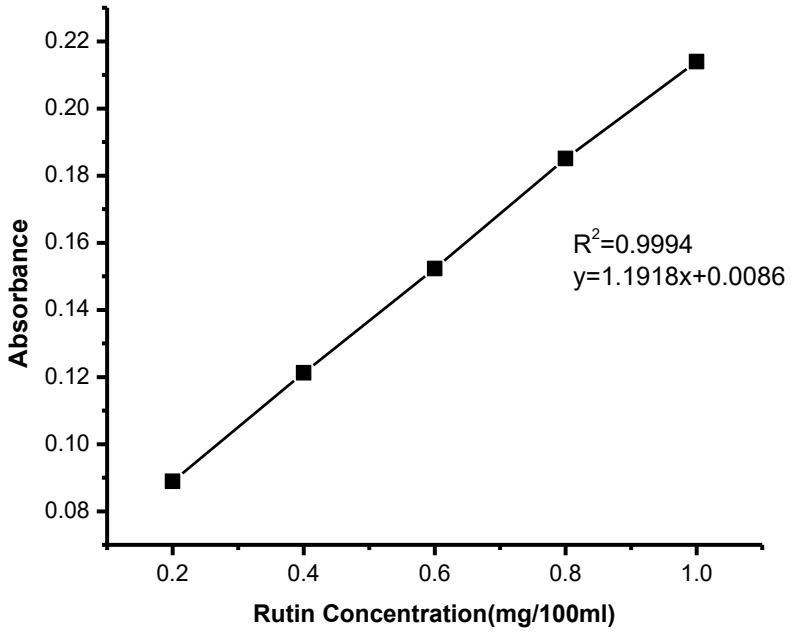


Figure -6, Rutin standard curve for TFC

Statistical Analysis All the estimations were carried out in triplicate and the experimental results obtained were expressed as Mean±SD.

Phytochemistry

Materials and Methods

Phytochemistry (Thin layer chromatograph) study is carried out by the methods described in standard of Ayurvedic Pharmacopoeia of India (Anonymous, API, Vol.II, Part-III, 2010).

Equipments Used

CAMAG HPTLC system (Muttenz, Switzerland) with Win CATS software version 1.4.2. equipped with a semi automatic TLC applicator Linomat IV and Hamilton (Reno, Nevada, USA) Syringe (100 µl), used for application of sample on TLC plate. After applying sample TLC plate was dried for a while and developed in Twin Trough plate development chamber. Developed plate was photo-documented under 254nm, 366nm and white light with the help of CAMAG Reprostar 3.

Materials and Reagents

All chemicals, reagents *viz. n*-hexane (95.0 %), ethyl acetate (purity 99.5 %), methanol (purity 99.7 %), chloroform (purity 99.0-99.4 %), toluene (purity 99.5 %), formic acid (85.0 -90.0 %), ethanol (purity 99.9 %) and glacial acetic acid (purity 99.8-100.5 %) used during the experimentation were of analytical grade and TLC plates were purchased from E. Merck KGaA, 6427 Darmstadt, Germany (Product no. 1.05554.007, Batch no. HX360379). All chemicals (Analytical grade) and TLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

Sample Preparation

All the plant parts were dried under a gentle stream of air in the laboratory till no loss in weight (temperature 30 ± 2° C and relative humidity 50 ± 5% and powdered in an electric grinder. Conventional extraction of all batches of ingredients was performed at room

temperature (28 ± 3° C) with a variety of solvents ranging from non polar to polar ones, *i.e.* *n*-hexane, ethyl acetate, methanol and chloroform. Dried and powdered parts of each batch (10 g each) were extracted three times (3 × 50 ml) for 18 hours and each successive extract was collected in a conical flasks. Samples were sonicated for 5 minute in digital ultrasonicator for good extraction. After 18 hours all samples were filtered by using Whatman's filter paper no. 1 and the solvent was removed under vacuum at 50°C, separately and concentrated up to 10 ml to get the sample solution of 100 mg/ml. 10µl of each sample was applied separately to TLC plate for the development of fingerprints.

R_f Value:

It was measured by recording the distance of each spot from the point of its application and calculate the R_f value by dividing the distance travelled by the spots and by the distance travelled by the front of the mobile phase.

Thin Layer Chromatography

Test Solution

1g Plant sample was extracted in 15 ml of each solvent *viz. n*-Hexane, ethyl acetate, methanol and chloroform respectively by cold percolation method dipped overnight and sonicated the next day and filtered. Remove the solvent under reduced pressure. Dissolve the residue in 10 ml of each solvent mentioned above.

Solvent System for Plant Samples

After several trials the following solvent ratio was found to be most suitable to devolve TLC plate for different samples.

1. ***Bauhinia variegata* L.**

Solvent Ratio Toluene: Ethyl acetate: formic acid **(**6.0: 4.0: 0.1)

2. ***Bixa orellana* L.**

   Solvent Ratio: Toluene: Ethyl acetate: formic acid (7.0: 2.2: 0.8)

3. ***Leucas aspera* (Willd.) Link**

   Solvent Ratio Toluene: Ethyl acetate: formic acid (9.0: 1.0: 0.2)

4. ***Mesua ferrea* L.**

   Solvent Ratio Toluene: Ethyl acetate: formic acid (7.0: 2.2: 0.8)

5. ***Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.**

   Solvent Ratio Toluene: Ethyl acetate: formic acid (5.0: 4.2: 0.8)

Procedure

10µl of each extract prepared in the solvents *n*-haxane, ethyl acetate, methanol and chloroform was applied on aluminum TLC plates pre-coated with Silica gel 60 F_{254} (E. Merck) of uniform thickness of 0.2 mm as a track T_1, T_2, T_3 and T_4, respectively. Extracts were applied in the form of band. After applying the extract, dry the plate in air and developed in the solvent system in a twin trough chamber up to a distance of 8 cm.

Results and Discussion on Exploration of Folk Medicinal Plants

On the basis of survey tour and folk healer's interaction, through questionnaires in study site a total of 38 medicinal plants were recorded. These medicinal plants are helpful in various kind of disease as per information given by folk healers. The details of information are enlisted in Table-2.

Table – 2. List of folk medicinal plants explored from the study area

S.No.	Botanical Name & Family	Accession No.	Local/Hindi Name	Mode of administration of folk medicinal plants used by Nyishi tribe
1.	*Abrus precatorius* L. (Leguminosae)	3252	Raho	**Part Used:** Seed, Leaf **Used in folk medicine:** Roots, leaves and seeds mixed powder used in treatment of tetanus, tonic, used in nervous disorder. Leaves decoction used to cure fever, cough and cold and lungs ailments. Roots are used to treat jaundice.
2.	*Achyranthes aspera* L. (Amaranthaceae)	3242	Chirchita / Latjira	**Used Part:** Seed, Leaf **Used in folk medicine:** Seed and leaves mixed powder used as anti-inflammatory and also used in treatment of indigestion, cough, asthma, anemia, jaundice and snake bite.
3.	*Acmella paniculata* (Wall. ex DC.) R.K.Jansen (Compositae)	3238	Sonaful/ Kulekhara	**Used Part:** Flower, Whole Plant, Leaf **Used in folk medicine:** Flower, leaf paste used for epilepsy, depression, Allergic asthma, chronic, Headache, Flowers used as toothache, nerve infections, dryness of mouth, Oral problems, toothache, bad breath, Paralysis of tongue or throat, relaxed uvula, Rheumatism, Seminal debility, Speech disorders.
4.	*Adenanthera pavonina* L. (Leguminosae)	3250	Chandan	**Used Part:** Flower, Whole Plant, Leaf **Used in folk medicine:** Decoction of the young

				leaves and stem bark used to treat diarrhoea. Seeds powder is used to treat inflammation and bacterial disease.
5.	*Aloe vera* (L.) Burm.f. (Xanthorrhoeaceae)	3239	Lu Hui/ Gawar Patha/ Ghirita	**Used Part:** Leaf pulp, Rhizome **Used in folk medicine:** Pulp juice used in stomach illness, purgative, pulp and rhizome in menstrual disorders, dried juice in constipation, root in colic disorders.
6.	*Amaranthus spinosus* L. (Amaranthaceae)	3231	Tai	**Used Part:** Seed, Leaf, Whole plant **Used in folk medicine:** leaves decoction used internally in the treatment of internal bleeding, diarrhoea and excessive menstruation. Seed and leaves used in the treatment of snake bites. Whole plant powder and decoction to treat ulcerated mouths, vaginal discharges, nosebleeds and wounds.
7.	*Andrographis paniculata* (Burm.f.) Nees (Acanthaceae)	3261	Chirata/kalmegh	**Used Part:** Whole plant **Used in folk medicine:** Whole plant powder decoction used in treatment of fever, cough, cold and various other ailments in body.
8.	*Asparagus racemosus* Willd. (Asparagaceae)	3251	Satawari	**Used Part:** leaf, Rhizome **Used in folk medicine:** Rhizome powder with milk used in tonic to prevent diarrhoea, dysentery, and general debility, immunity booster, sex related disorder, milk enhancement in women during delivery. Leaf are also used for milk enhancement in women during delivery
9.	*Bauhinia variegata* L. (Leguminosae)	3230	Og-yok / Kachnar	**Used Part:** Stem Bark, Flower

				Used in folk medicine: Stem bark is used in throat disorder Throat disorder. Flower used in tonsillitis, pharyngitis.
10.	*Bixa orellana* L. (Bixaceae)	3229	Sindhura /Latkan	**Used Part:** Seed, Stem Bark, fruit Leaf **Used in folk medicine:** Root bark, fruits mixed powder used as antiperiodic, antipyretic. Leaves in jaundice. Seeds used in cardiac illness, febrifuge, Seed oil used in bronchitis, sore throat, and eye inflammation.
11.	*Cannabis sativa* L. (Cannabaceae)	3260	Bhang	**Used Part:** Leaf, Seed **Used in folk medicine:** leaf paste used locally for severe pain, severe nausea, as brain tonic, anaesthesia. Seed used as locally for chatni in prevent constipation.
12.	*Cassia tora* L. (Leguminosae)	3240	kulb	**Used Part:** Seeds, Leaves, Whole Plant **Used in folk medicine:** Seed and leaves powder used in abdominal disorders, blood disorders, Constipation Inflammations, glandular swellings.
13.	*Clitoria ternatea* L. (Leguminosae)	3244	Aparajita	**Used Part:** leaf, Flower, whole plant **Used in folk medicine:** leaves and flower paste used as swelling and pain, bleeding piles. Decoction of leaves helps in Piles and the paste of whole plant is applied over it. Leaf juice is used as nasal drops in headache. Whole plant powder with oil and dhamasa is used for rheumatoid arthritis.
14.	*Costus speciosus* (J.Koenig) Sm. (Costaceae)	3253	Kebuk/keu	**Used Part :** Rhizome, Flower, leaf **Used in folk medicine:** Rhizome powder used

				bitter astringent tonic, aphrodisiac. Flower and leaf used in fever.
15.	*Curcuma caesia* Roxb. (Zingiberaceae)	3234	Ama Haldi/ Kali Haldi/ Asthma Plant/ Kola-haladhi	**Used Part :** Rhizome **Used in folk medicine:** Decoction of rhizome powder with milk used in rheumatism, asthma, cough, pain, rheumatism.
16.	*Cyperus involucratus* Rottb. (Cyperaceae)	3263	Payobhara/ Boriala	**Used Part :** leaf, whole **Used in folk medicine:** Used in locally by folk people in "Jhad phook" for the treatment of fever and other ailments.
17.	*Eclipta alba* (L.) Hassk. (Compositae)	3265	Kehraj/ Bhringraj	**Used Part :** Flower, Whole Plant **Used in folk medicine:** flower and whole Plant used in hair fall treatment, liver disorders, skin diseases etc.
18.	*Elaeocarpus ganitrus* Roxb. ex G.Don (Elaeocarpaceae)	3247	Rudraksh	**Used Part :** Seed **Used in folk medicine:** Seeds powder used in cardiac disease and hypertension.
19.	*Embelia ribes* Burm.f. (Primulaceae)	3245	Bidang	**Used Part :** Seed **Used in folk medicine:** Seeds used to prevents formation of gas in the gastrointestinal tract and in tapeworms infection.
20.	*Euphorbia hirta* L. (Euphorbiaceae)	3241	Gakhiroti-bon / Asthma plant	**Used Part:** Flower, leaf, whole plant **Used in folk Medicine:** Flowers and leaves used to treatment of bronchitis, fever, cough, asthma, cancer, diarrhoea, dysentery, intestinal, helminthic infestations, wounds, kidney stones. Decoction of whole plant used for skin diseases. Fresh leaves are used as gargle for the treatment of thrush. Root decoction is used for nursing mothers

				deficient in milk and in the treatment of snake bites.
21.	*Holarrhena antidysenterica* (Roth) Wall. ex A.DC. (Apocynaceae)	3254	Girimallik / Dudkhuri/ kutuj	**Used Part :** Stem bark, seed **Used in folk medicine:** Stem bark used in diarrhoea, dysentery, worm infestation, seeds in digestive disorder, colic,
22.	*Leucas aspera* (Willd.) Link (Lamiaceae)	3237	Guma / Eki-sipyak/ Dronpuspi	**Used Part:** Leaf, Whole Plant **Used in folk medicine**: whole plant is used as an insecticidal, anti inflammatory, analgesic and used for wound healing. Also used in snake bite. Whole plant decoction used as nutritional supplement and anti aging
23.	*Mesua ferrea* L. (Calophyllaceae)	3232	Nahor/ Nagkeshar	**Used Part :** Flower, Seed, Stem Bark **Used in folk medicine:** Flower and seed used as impotency. Stem bark is used for injury and bacterial infection. Flower and stem bark used for HIV and sexual transmitted disease.
24.	*Oroxylum indicum* (L.) Kurz (Bignoniaceae)	3258	Sona Patha/ Shyonak	**Used Part:** Seed, Stem Bark, Leaf, **Used in folk medicine:** Paste of bark powder is applied for mouth cancer, scabies and other skin diseases. The seed and leaf is ground with fire-soot and the paste applied to the neck for quick relief of tonsil pain. 10 seed powder in two doses per day used in diabetes, Stem bark decoction used in breast cancer, gastric ulcer.
25.	*Persicaria hydropiperoides* (Michx.) Small (Polygonaceae)	3162	Patchouli	**Used Part:** whole plant, Root **Used in folk medicine:** The whole plant used as

				antiseptic and astringent Abdominal disorders, fevers, etc. Dried root powder used for termination of pregnancy.
26.	*Phlogacanthus thyrsiflorus* Nees (Acanthaceae)	3255	Pilamola / Barakkanta	**Used Part :**Whole Plant, Flowers **Used in folk medicine:** Whole plant powder used in cough and menorrhagia. Useful for curing coughs, colds and asthma .Flowers are antidote to pox, prevents skin diseases like sore etc.
27.	*Plumbago zeylanica* L. (Plumbaginaceae)	3246	Chita/ Chitrak	**Used Part:** Seed, Leaf, Root **Used in folk medicine:** Seed and leaf powder used for weight loss, obesity, cardiac disease. Leaves and seed used in liver disorders, Root used in bone fracture, grains cooling agent, inflammation
28.	*Pongamia pinnata* (L.) Pierre (Leguminosae)	3243	Karos	**Used Part :** Root, Seed **Used in folk medicine**: The root and seed powder used for treating gonorrhoea, cleaning gums, teeth, and ulcers, and is used in vaginal infection and skin diseases and increase fertility and potency.
29.	*Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae)	3256	Arachoritita/ Sarpagandha	**Used Part:** Seed, Flower, Leaf **Used in folk medicine:** Root and leaf was brewed as a tea and used in humans to treat hypertension, insanity, snakebite, and cholera.
30.	*Rauvolfia vomitoria* Afzel. (Apocynaceae)	3259	Sarpagandha	**Used Part:** Seed, Flower, Leaf **Used in folk medicine:** Root and leaf was brewed as a tea and used in humans to treat hypertension,

				insanity, snakebite, and cholera.
31.	*Rhus parviflora* Roxb. (Anacardiaceae)	3266	Tamoya	**Used Part:** leaf **Used in folk medicine:** leaf juice used in abdominal pain. Leaves powder used in sex power and sexual transmitted disease.
32.	*Saraca asoca* (Roxb.) Willd. (Leguminosae)	3233	Ashok	**Used Parts :** Stem bark, Flower **Used in folk medicine: Stem** Bark powder is used as astringent, demulcent, styptic and febrifuge. Flower used as primarily in uterine tonic and diabetes for keeping blood sugar under control and other health issue.
33.	*Sida acuta* Burm.f. (Malvaceae)	3264	Tita-phul	**Used Part :** whole plant, leaf, Root **Used in folk medicine:** whole plant decoction is used in treatment for fevers. Juice of leaves treat indigestion. The juice of the leaves is mixed with vinegar to make an anti-inflammatory. Juice of the root is used to treat fevers. Root is chewed to relieve a toothache.
34.	*Solanum viarum* Dunal (Solanaceae)	3236	Siateabole	**Used Part :**Fruit, Seed , Leaf **Used in folk medicine:** fruit used as liver tonic and relives in constipation. Fruit locally used for vegetable and to prevent gastric problem and other ailments.
35.	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Combretaceae)	3235	Arjun gachhi	**Used Part :** Seed, Stem Bark , Flower **Used in folk medicine:** Stem bark used in heart and blood vessels (cardiovascular disease), stem bark seed and flower also used in heart disease and related chest pain, high

				blood pressure, and high cholesterol.
36.	*Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae)	3257	Bahid	**Used Part:** Seed, Stem Bark **Used in folk medicine:** Seeds are used in Gastrointestinal disorders, constipation. Stem bark powder used in externally to cure various ailments.
37.	*Terminalia chebula* Retz. (Combretaceae)	3248	Shilikha/ Harad	**Used Part:** Seed, flower, leaf **Used in folk medicine:** Decoction of seed powder with *Terminalia bellirica* seed help in kidney and heart disorders, homeostatic, diuretic, and laxative. Flower, leaf powder used orally in various ailments.
38.	*Tinospora cordifolia* (Willd.) Miers (Menispermaceae)	3249	Nayam rak /guduchi	**Used Part :** Stem **Used in folk medicine:** Decoction of 6 cm. Stem with water help in malaria, typhoid, dengue, and other fevers.

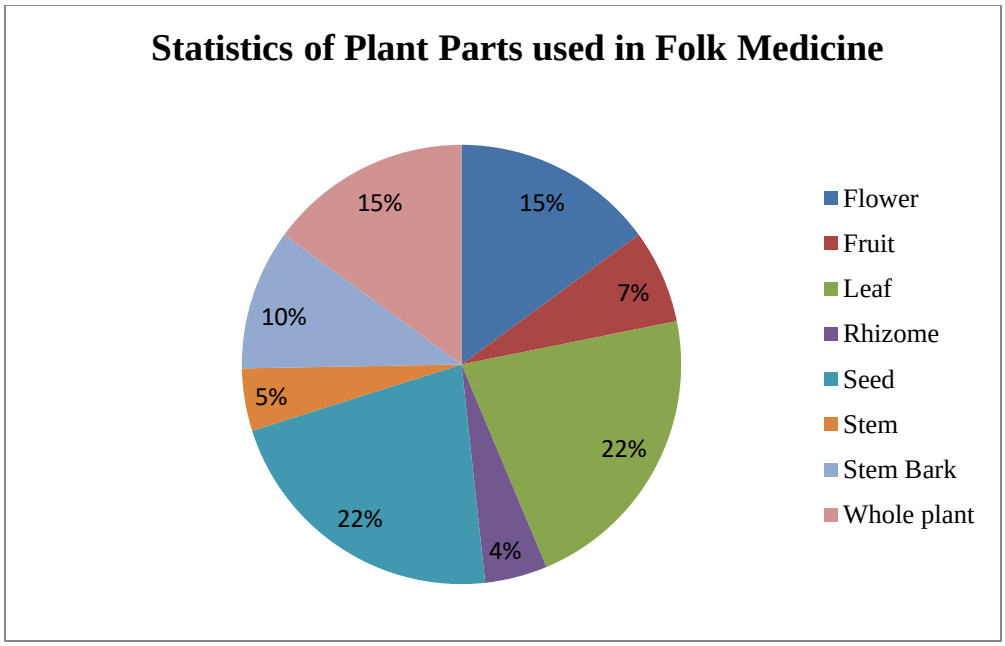


Figure - 7. Statistics of plant parts used in folk medicine

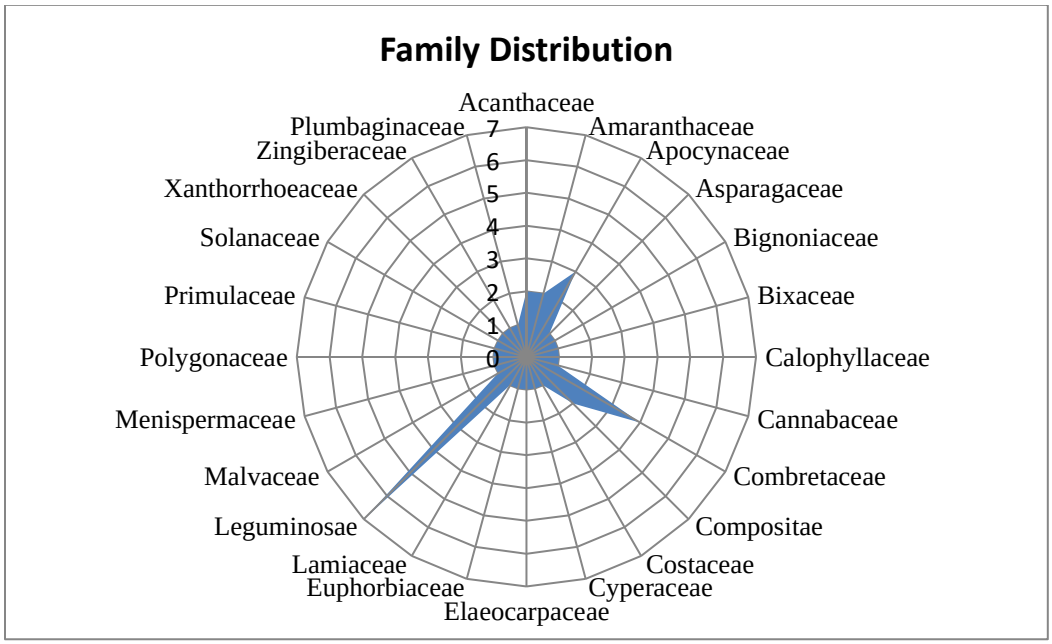


Figure – 8. Statistics of family distribution used in folk medicine

In this study a total of 38 medicinal plants were recorded during survey on the basis of information collected from folk healer, Nyishi tribe of Arunachal Pradesh (Table - 2). These 38 folk medicinal plants belong to 25 families. In six families least 2 to 7 medicinal plant species were recorded represent in (figure No. 8) it revealed that Leguminosae is the highest species family with 7 species followed by Combretaceae and Apocynaceae with 4 and 3 species respectively. Almost all medicinal plant parts were recorded for the folk medicinal treatment (Figure - 7) for the health care management. A total of 38 folk medicinal plants which are used in different disease and nutraceutical supplement by the folk healer of Nyishi tribe are given in (Table -2).

Out of the 38 documented medicinal plants five medicinally important plant species were selected for further study because these five plants species and their parts are frequently used by maximum folk healers. Some of the selected medicinal plants photograph is given in (Figs. - 10-14).

1. *Bauhinia variegata* L.
2. *Bixa orellana* L.
3. *Leucas aspera* (Willd.) Link
4. *Mesua ferrea* L.
5. *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.

Brief enumeration of the medicinal plants selected for the study

Bauhinia variegata L. (Leguminosae)

Bauhinia variegata L. Sp. PI. 375. 1753; Baker in Hk. f. FI. Brit Ind. 2: 284, 1878; Gamble, Man. Ind . Timb. 284. 1902; Brandis. Ind Trees 258. 1906; Kanjilal et al., FI. Assam 2: 140. 1938; Balak. FI. Jowai 1: 172. 1981.

Description

Plant is medium-sized trees with hairy branches. Bark dark gren or grayish brown .Leaves 4.0-12 cm long, lobed one third ways down deeply cordate. Corymbose racemes from leafless axils, Bracts and bracteoles are deltoid. Calyx is 2-2.4 cm long, pubescent, spathaceous, 5-toothed at apex. Petals is 3.7-4.5 cm long, obovate-oblong, clawed, the uppermost darker with purple veins. Stamens 5 in numbers fertile, staminodes absent. Ovary pubescent. Pods 15-30 x 1.5-2.5 cm, flat, glabrous; 10-12 numbers of seed.

Parts Used: Stem Bark, Flower

Flowering: Sept.-March

Fruiting: October-April

Bixa orellana L. (Bixaceae)

Bixa orellana L. Sp. PI. 512. 1753; FL. Brit. Ind. 1: 190. 1872; Fl. As. 1: 83. 1934.

Description

Small evergreen trees, leaves alternate, acuminate, cordate 9 to 18 cm. long Glabrous, shining; petioles slender, 4-6 cm long. Flowerswhite or pink colour 2.0-4.5 cm in diameter, terminal panicles. Ovary is 1-celled. Ovules on 2 parietal placenta. Capsules with 3 to 4 cm long, ovoid, softly echinate.

Parts Used: Stem Bark, Flower

Flowering: July – September

Fruiting: October - November.

Leucas aspera (Willd.) Link (Lamiaceae)

Leucas aspera (Willd.) Link, Enum. Hort. Berol. Alt. 2: 113. 1822; Hook. f., FI. Brit. India 4: 690. 1885. *Phlomis aspera* Willd., Enum. PI. Hort. Berol. 2:621. 1809.

Description

Annual herb, erect or diffuse. Stems 25-60 cm hight, so many braches hispid. Leaves linear oblong-lanceolate, obtuse at apex, narrowed at base, entire or minutely crenate, Flower white in axillary or terminal whorls, bracts linear, ciliate, Calyx tubular, 6-11 mm length, curved, usually smooth and glabrous. Nutlets obovoid-oblong brown.

Parts Used: Stem Bark, Flower

FIowering: August - April

Fruiting: August - April.

Mesua ferrea L. (Calophyllaceae)

Mesua ferrea L. Sp. PI. 515. 175; Anderson in Hk. f. FI. Brit Ind. 1: 277. 1874; Gamble, Man. Ind. Timb. 59. 1902; Brandis, Ind. Trees 55. 1906; Kanjilal *et al.*, FI. Assam 1 (1):11. 1934.

Trees, upto 40 m height; trunk 3 m, often buttressed at base; heartwood dark red. Leaves opposite, decussate, very variable, linear-lanceolate, oblong-lanceolate, lanceolate or elliptic-oblong, obtuse or acute at base, covered with a wax-like white powder beneath. Flowers white, sweet-scented, axillary, tomentose peduncles,

usually solitary, rarely paired, bisexual, showy, 4 - 16 cm in diameter. Pedicels 8 - 15 mm long, rather stout. Sepals 4 in 2 pairs, orbicular, imbricate, fleshy. Petals white with brown or purple veins 4, 2 - 4.5 cm long, obovate or obcordate. Stamens numerous, anthers linear, 2.5 - 3 mm long, golden yellow. Ovary 5 - 7 mm long, ovoid, bilocular, ovules 2 in each locule. Fruits ovoid to globose with a conical point striate. seed 1-4 brown.

Parts Used: Stem Bark, Flower

FIowering: Jan. - March

Fruiting: May – October

Terminalia arjuna (Roxb. ex DC.) Wight & Am. (Combretaceae)

Terminalia arjuna (Roxb. ex DC.) Wight & Am., Prodr. 314. 1834; Clarke in Hook. f., FI. Brit. India 2: 447. 1878. *Pentaptera arjuna* Roxb. ex DC., Prodr. 3: 14. 1828

Description

A large deciduous tree up to 25 m length bark grey, smooth. Leaves sub-opposite, oblong or elliptic-oblong, glabrous, apex obtuse or sub-acute, base rounded or sometimes cordate. Flowers small, yellowish in terminal spike or panicle white. Fruits oblong or ovoid. 2.3 - 3.5 cm long, fibrous-woody, glabrous with 5 hard wings, striated with numerous curved veins.

Parts Used: Stem Bark, Flower

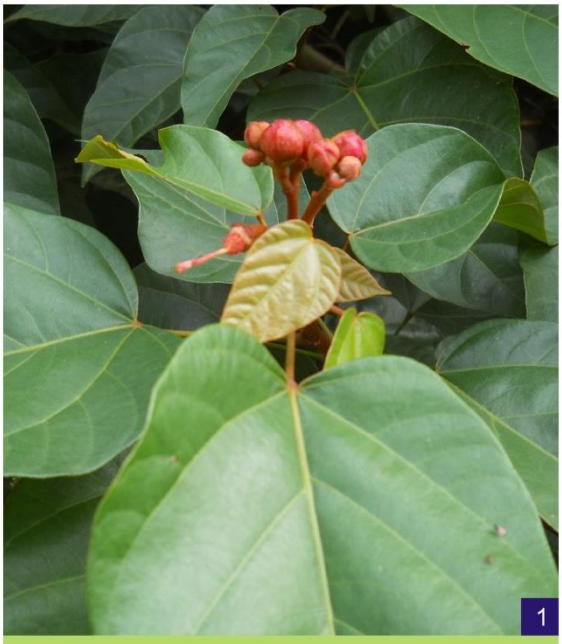
Flowering: April to May

Fruiting: May to June

According to available literature, Kulshrestha *et al.,* (2011) conducted the antimicrobial activity of *Bahunia variegata* L. flower through methanol extract and also analysed the phytochemical screening of the plants sample. Deswal Geeta and Arora Kanika, (2015) also reported that *Bahunia variegata* L. is used in various part of the country in the management of healthcare system and reported the ethnobotanical importance *viz.*, antibacterial, antitumor, hypoglycemic, anti-inflammatory activity.

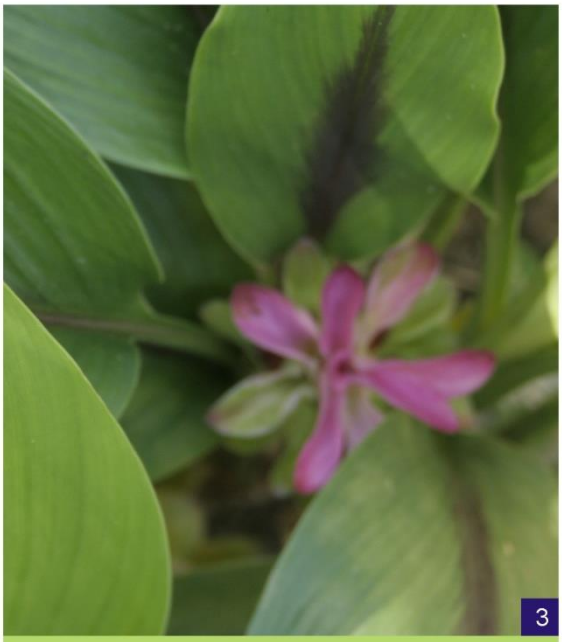
Chaitanya, K. K., (2015) studied the Ethnobotanical and Phytopharmacological study of *Mesua ferrea* L. and reported that this plant is used in the management of asthma, cough, dyspepsia, fever, itchiness, nausea and renal diseases. It was also used as antioxidant, antimicrobial, antiviral, antitumor and immunomodulatory. Kumar *et al.,* (2012) reported the ethnoboanical, phytochemical screening and bioactive compound of the *Bixa orellana* L. plants parts.

Nagarasan *et al.,* (2016) reported that *Leucas aspera* (Willd.) Link was used in folk medicine as insecticide and antipyretic. It has the properties of antioxidant, antibacterial, antifungal and cytotoxic activities and some bioactive compound like hydroxytetratriacontan-4- one, aliphatic ketones, nicotine, farnesene, thujene, and menthol. Shahzad *et al.,* (2014) also studied the bioactive components and antioxidant properties of *Terminalia arjuna* (Roxb. ex DC.) Wight & Am.



Bixa orellana L.

Asparagus racemosus Willd.



Curcuma caesia Roxb.

Amaranthus spinosus L.

Figure- 10 (1-4). Photographs of Medicinal Plant

Sida acuta Burm.f.

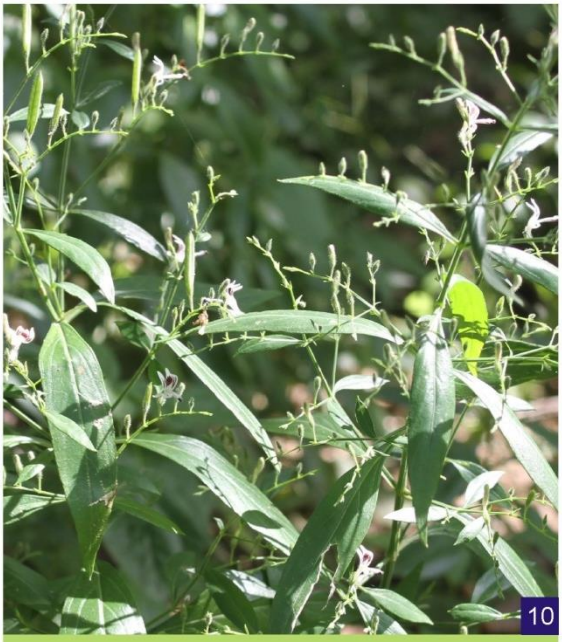
Eclipta alba (L.) Hassk.

Oroxylum indicum (L.) Kurz

Leucas aspera (Willd.) Link

Figure- 11 (5-8). Photographs of Medicinal Plant

Aloe vera (L.) Burm.f.



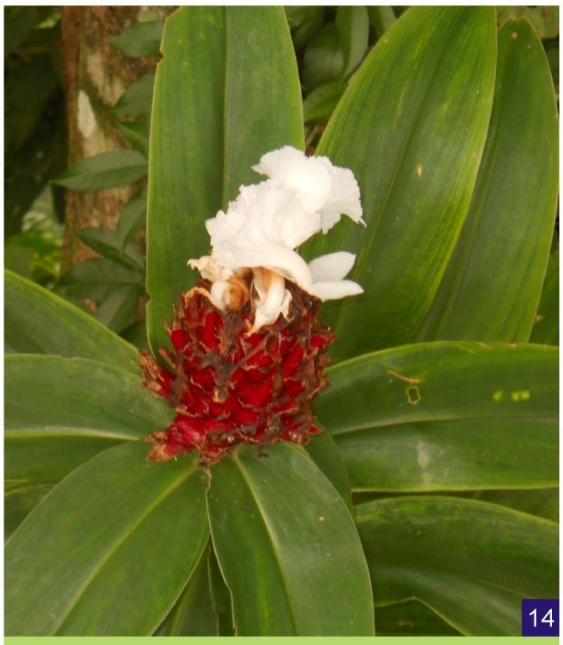
Andrographis paniculata (Burm.f.) Nees

Clitoria ternatea L.

Saraca asoca (Roxb.) Willd.

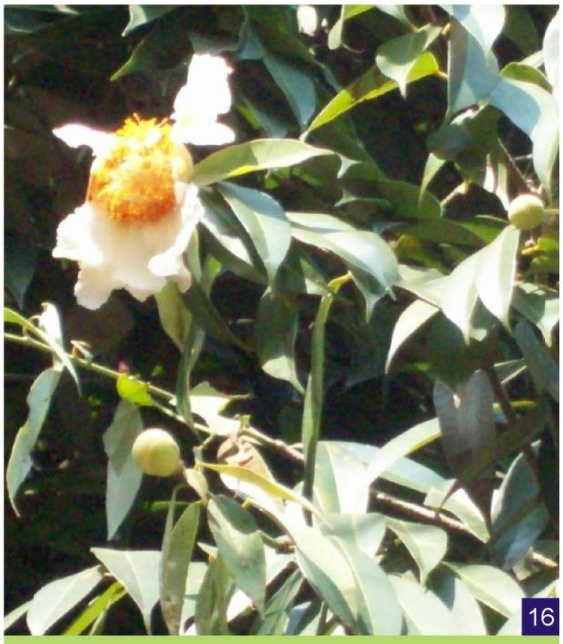
Figure- 12 (9-12). Photographs of Medicinal Plant

Cannabis sativa L.



Costus speciosus (J.Koenig) Sm.

Holarrhena antidysenterica (Roth) Wall. ex A.DC.



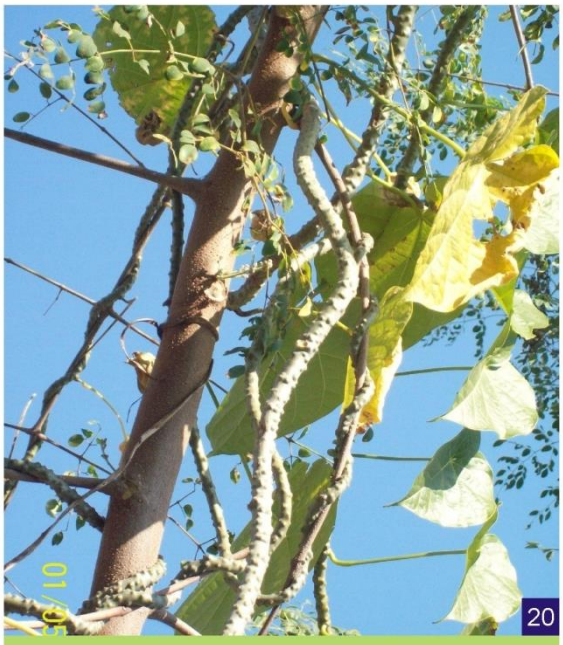
Mesua ferrea L.

Figure- 13 (13-16). Photographs of Medicinal Plant

Plumbago zeylanica L.

Pongamia pinnata (L.) Pierre

Solanum viarum Dunal



Tinospora cordifolia (Willd.) Miers

Figure- 14 (17-20). Photographs of Medicinal Plant

Results and Discussion on Pharmacognostical Evaluation

Present studies of five species have been pharmacognostically evaluated by five pharmacognostic parameters *viz.* Powder microscopy, Fluorescent, ash value, extractive value and phytochemical screening.

Powder Microscopic study

Powder Microscopical study is essential in the identification and characterization of plant sample as well as in identifying small fragments of crude or plant powdered and in the discovery of adulterants as well as identifying the plants by characteristics tissue features. This study is carried out by followed by standard of Ayurvedic Pharmacopoeia of India (Anonymous, API, Vol.III, Part-II, 2010).

***Bauhinia variegata* L. (Flowers)**

Powder is brown yellow colour, smooth to touch, characteristic odour and taste astringent (Fig.-16, 24-b). Abundant pollen grains round or triangular with round edges. Multicellular, uniseriate trichomes, some of them with somewhat bulged cells at tip or base. Some of the trichomes are also filled with light brown coloured material. Fragments of corolla showing papillose surface. Surface view of anther wall. Parenchyma cells, radialy elongated, thin walled, polygonal, with embedded vascular elements and micro rosette crystals of calcium oxalate. The microscopical features of powder are shown in (Fig.- 18, a-e).

Powder Microscopy of *Bixa orellana* L. (Bark)

Powder dull dark brown in colour, fine powder, smooth to touch, not free flowing and lumps forming, characteristic odour and taste astringent (Fig.-15, 22-b). Ray parenchymatous cells in 3 to 4 layers, cells are containing clusters of calcium oxalate

crystal in layers, medullary ray and fragments of fibers with tapering ends. Cork cells shown in 7 to 8 layers. The microscopical features of powder are shown in (Fig.-19, a-e).

Powder Microscopy of *Leucas aspera* (Willd.) Link (Whole plant)

Powder colour is green colour, soft in nature, (fig.-16, 23-b) Abundant multicellular, uniseriate trichomes varying in their size and shape scattered all over the field, three major type of trichomes were identified-Trichomes having short length and with bulbous base, trichomes having moderate length and with pointed ends, trichomes with long length and with hook like curved tip, solitary and groups of irregularly shaped pitted sclerieds with broad lumen, beaker shaped stone cells, round to oval pollen grains with 5 germ pores, fragments of pitted vessels. The microscopical features of powder are shown in (Fig.-20, a-g).

Powder Microscopy of *Mesua ferrea* L. (Stem Bark)

Powder is brown with a mild sweet taste (Fig.-17, 25-b), abundant prismatic crystals of calcium oxalate, aseptate fibres, sclerieds tissue isolated and in groups with thick walls and broad lumen. The microscopical features of powder are shown in (Fig. - 21, a-e).

Powder Microscopy of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark)

Powder is brown colour, little bitter taste (Fig.-15, 21-b). Powder microscopy of Arjuna bark shows the uniseriate medullary rays running straight & parallel, occasionally becoming slightly curved, some cells contain starch grains. Medullary rays and rosette crystals of calcium oxalate in association with fibres (crystal fibre). Cork cell filled with tannins. The microscopical features of powder are shown in (Fig.-22, a-d).

Figure- 15. Photographs of Medicinal Plant (21a-22a) with Powder Sample (21b-22b).

Figure- 16. Photographs of Medicinal Plant (23a-24a) with Powder Sample (23b-24b)

Figure- 17. Photographs of Medicinal plant (25a) with Powder Sample (25b).

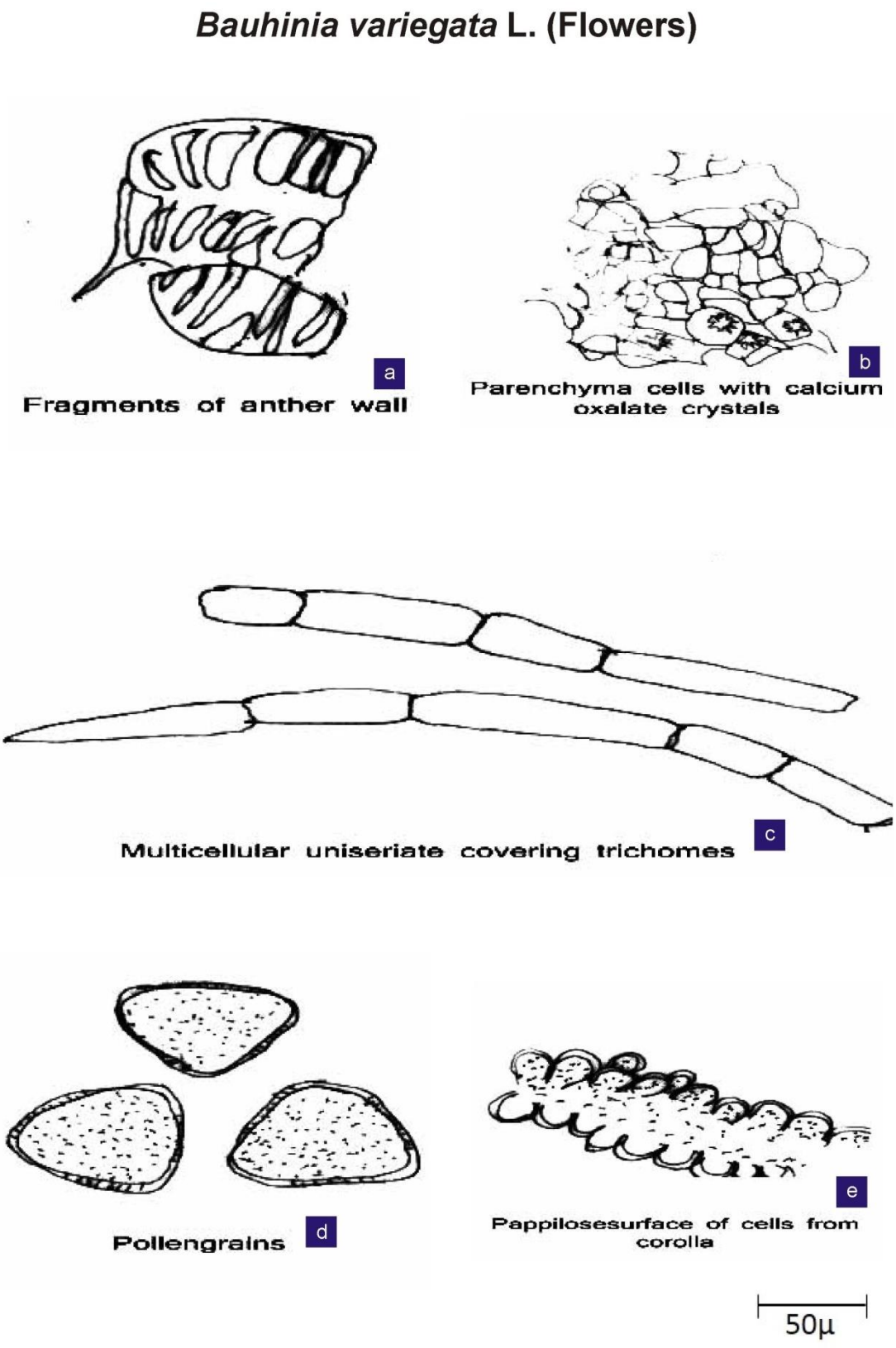


Figure- 18 (a-e). Showing Powder Microscopy of *Bauhinia variegata* L. (Flower).

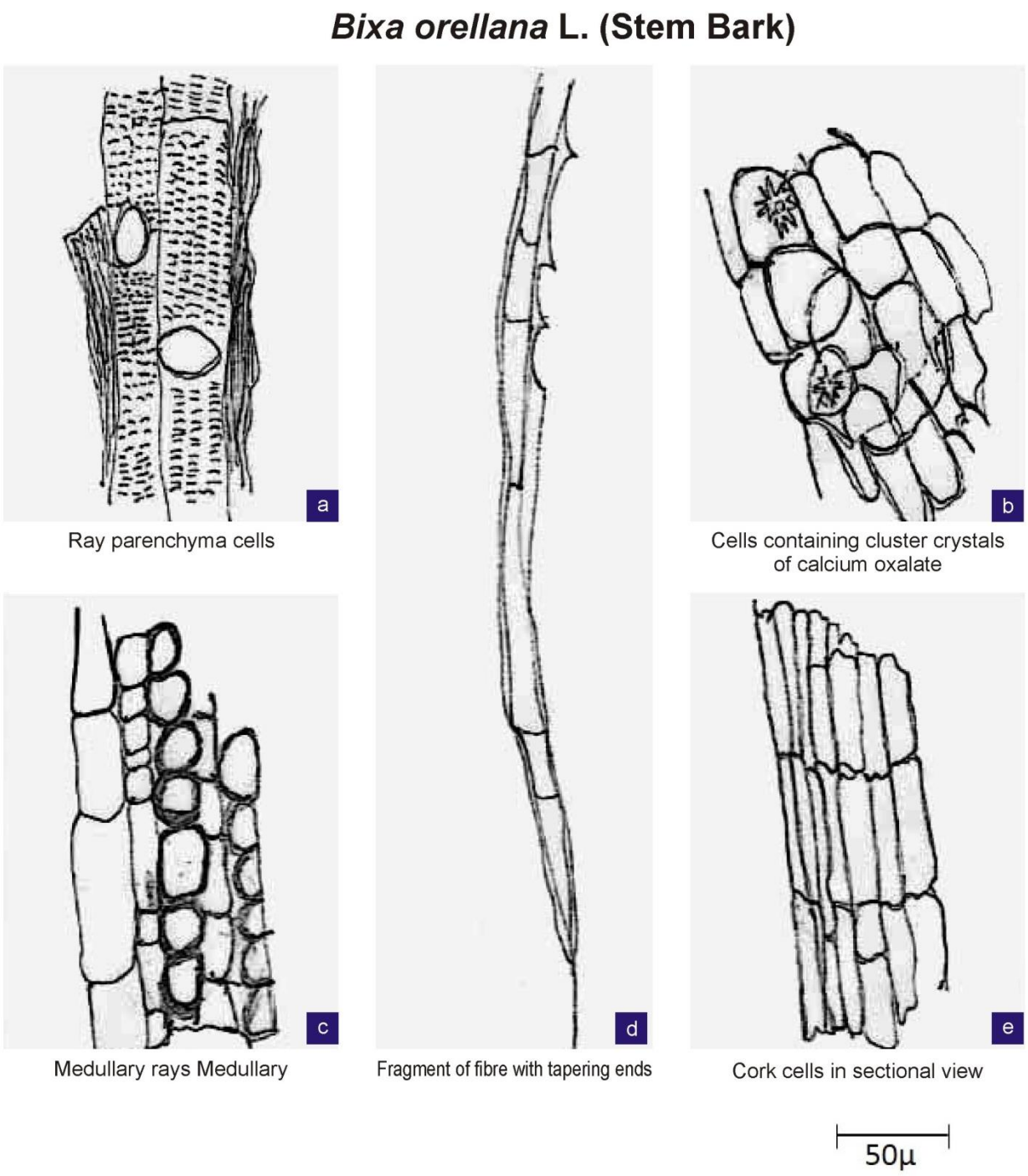


Figure- 19 (a-e). Showing Powder Microscopy of *Bixa orellana* L. (Stem Bark).

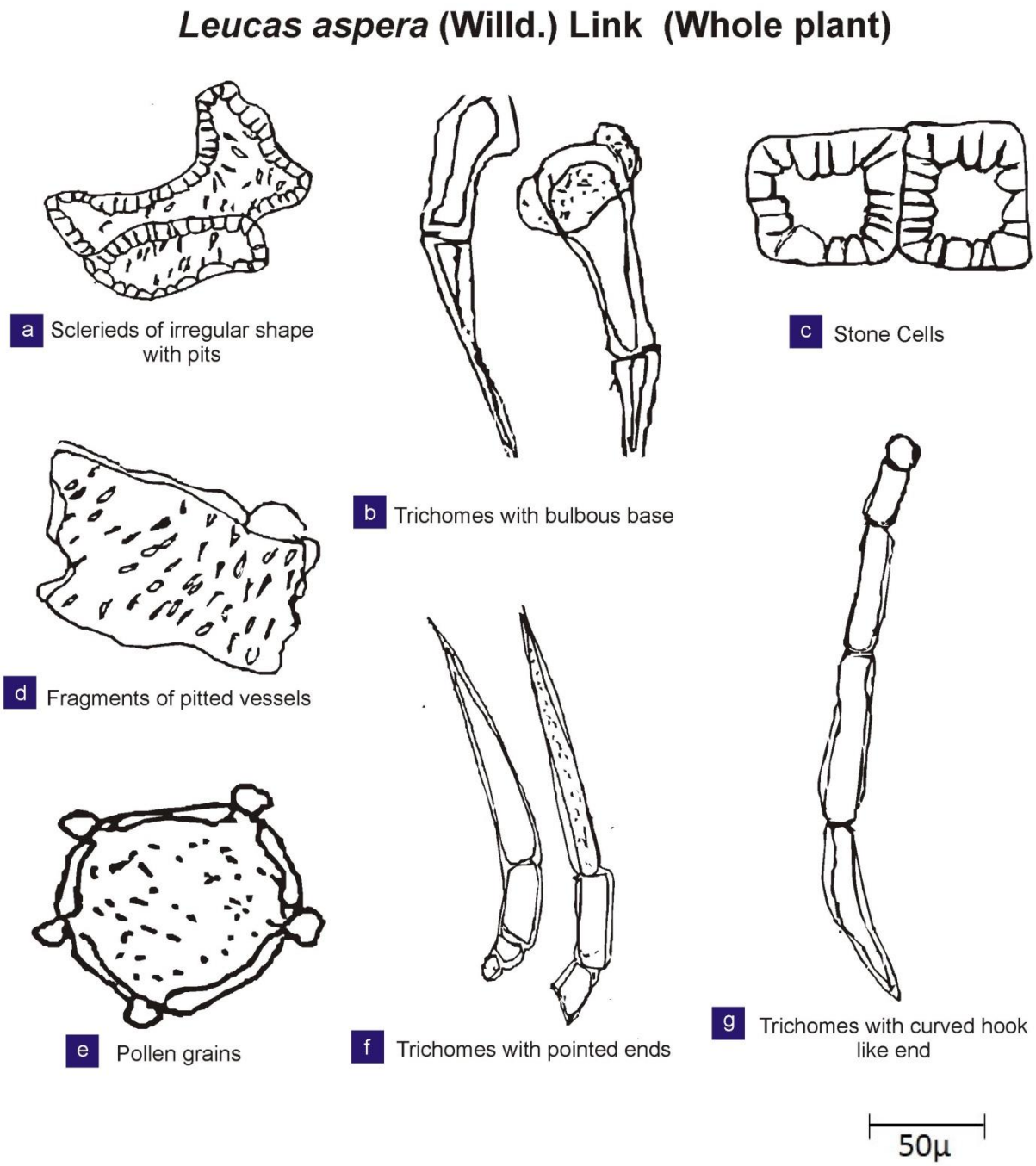


Figure- 20 (a-g). Showing Powder Microscopy of *Leucas aspera* (Willd). Link (Whole Plant).

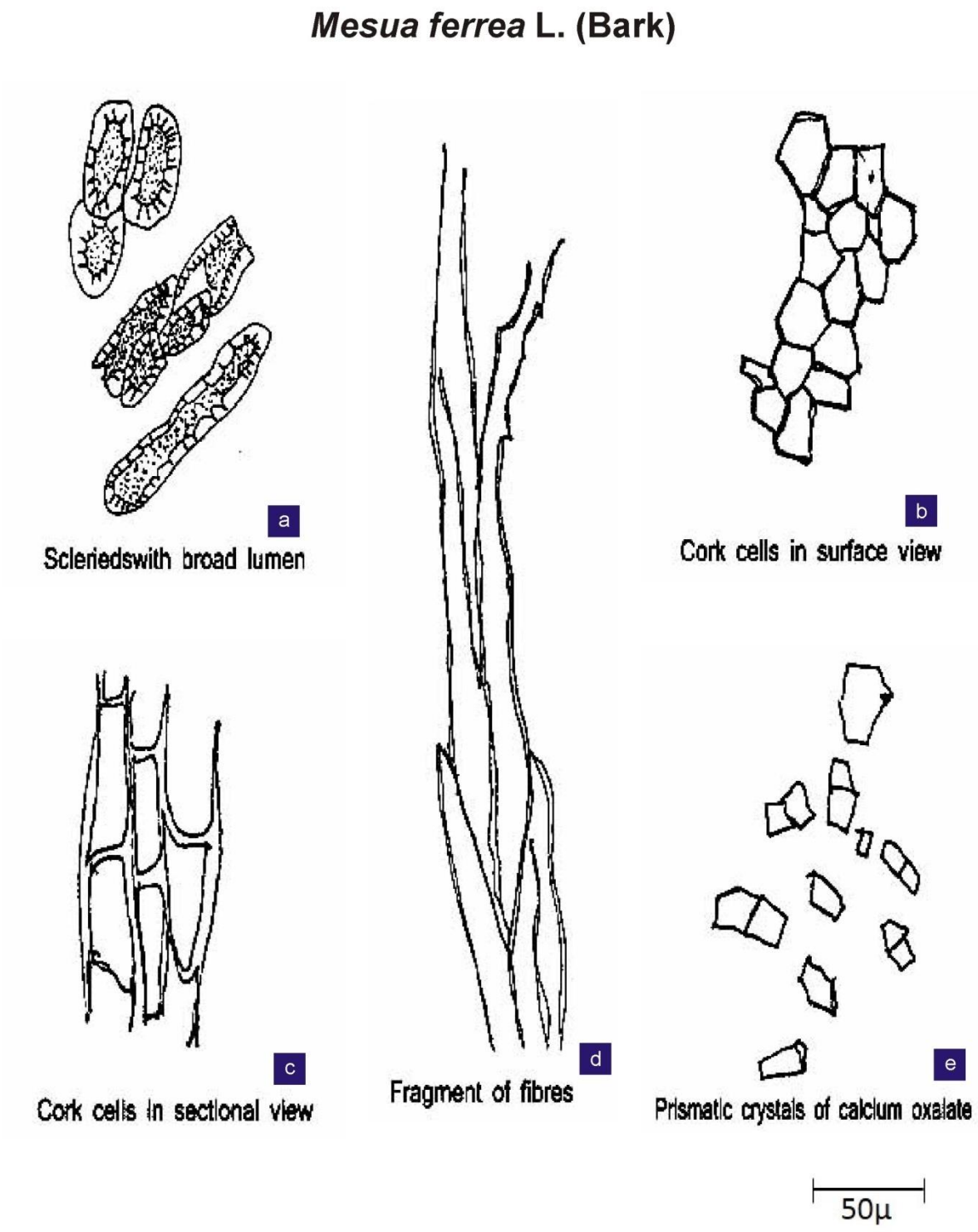


Figure- 21 (a-e). Showing Powder Microscopy of *Mesua Ferrea* L. (Stem Bark).

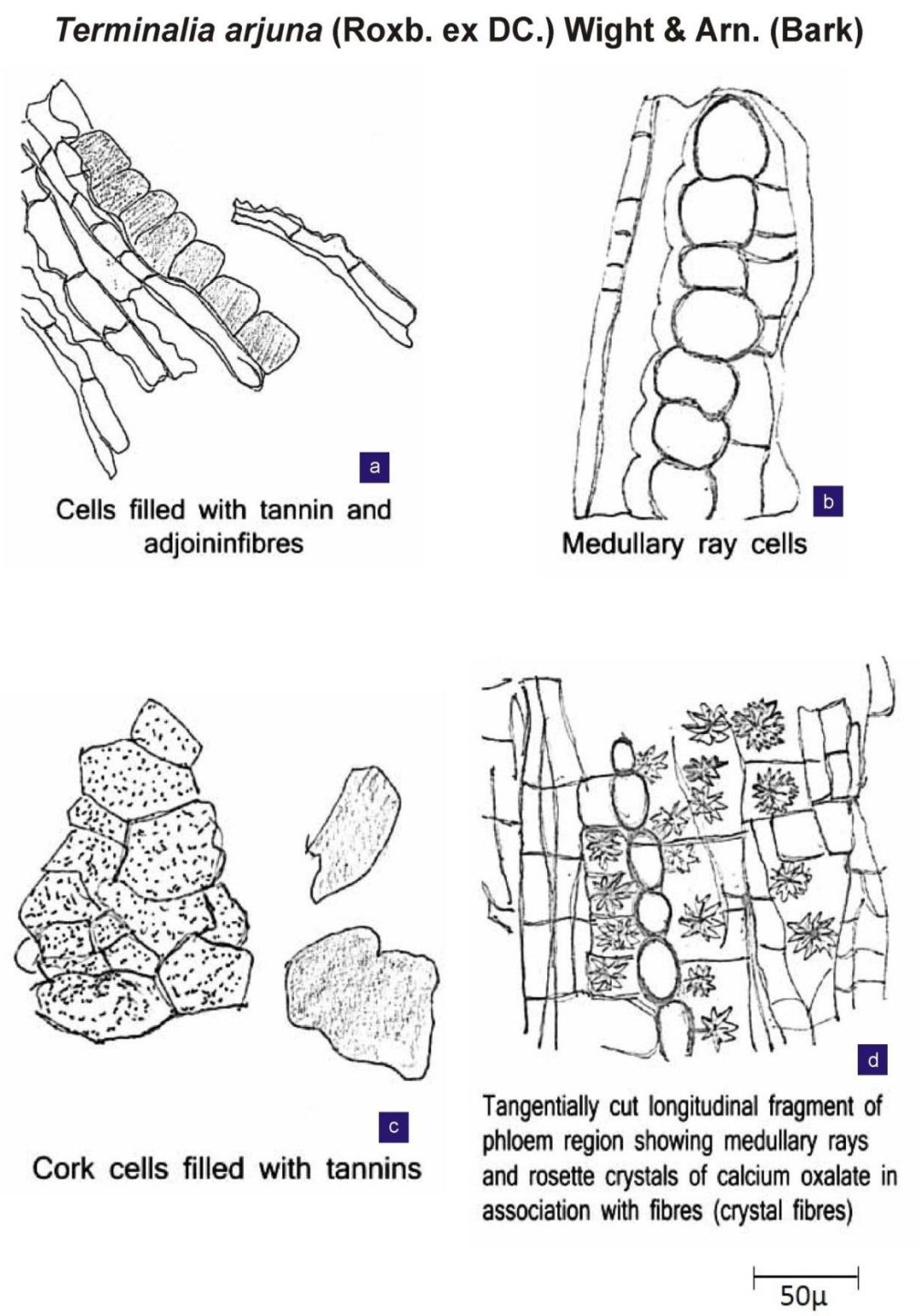


Figure- 22 (a-d). Showing Powder Microscopy of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark).

Fluorescent Analysis of Powder Samples

The Fluorescent study of medicinal plants powder samples were carried out by mixing the powder sample with different chemical reagents and analyzing it under daylight, near UV and far UV. Some of the drugs show characteristics Fluorescent when powder sample is exposed under ultraviolet radiation, and is useful in the identification and authentication of those drugs.

While plant powdered sample is treated with various chemicals reagents, specific color is observed under UV and visible light for specific drug sample. After the chemical reaction different colour was observed. Fluorescent studies of the selected medicinal plants species treated by different chemicals are given (Tables- 3-7).

Table - 3. Fluorescent Analysis of Power Sample of *Bauhinia variegata* L

Sl. No.	Solvents	Fluorescent Colour		
		Under Visible light	UV light (254nm)	UV light (366nm)
1.	Conc. HCl	Brown	Yellow Brown	Light Brown
2.	Conc. H_2SO_4	Reddish Brown	Brown	Brick Brown
3.	Conc. HNO_3	Brown	Yellow	Red
4.	C_3H_6O	Brown	Dark Brown	Pink
5.	Aq. NAOH	Brown	Brown	Pink
6.	NAOH (Alco.)	Light Brown	Green	Brown
7.	5%$FeCl_3$(Aq.)	Dark green	Dark green	Dark green
8.	$FeCl_3$ (Alco.)	Green	Green	Blue
9.	Water	Yellow green	Yellow green	Yellow green

Table - 4. Fluorescent analysis of power sample of *Bixa orellana* L.

S.No.	Solvents	Fluorescent Colour		
		Under Visible light	**UV light (254nm)**	**UV light (366nm)**
1.	Conc. HCl	Light Brown	Brown	Brown
2.	Conc. H_2SO_4	Brown	Black	Radish Brown
3.	Conc. HNO_3	Red Brown	Yellow	Red
4.	C_3H_6O	Brown	Dark Brown	Pink
5.	Aq. NAOH	Red Brown	brown	Red
6.	NAOH (Alco.)	Light brown	Light brown	Pink
7.	5%$FeCl_3$(Aq.)	Dark green	Dark green	Dark Brown
8.	$FeCl_3$ (Alco.)	Dark green	Green	Blue
9.	Water	Brown	Dark Brown	Brown

Table - 5. Fluorescent analysis of power sample of *Leucas aspera* (Willd.) Link.

S.No.	Solvents	Fluorescent Colour		
		Under Visible light	**UV light (254nm)**	**UV light (366nm)**
1.	Conc. HCl	Brown	Dark green	Dark Brown
2.	Conc. H_2SO_4	Brown	Brown	Pink
3.	Conc. HNO_3	Brown	Yellow brown	Red
4.	C_3H_6O	Black	Dark green	Florescent red
5.	Aq. NAOH	Dark green	Brown	Red
6.	NAOH (Alco.)	Dark Brown	Brown	Red (Sindhur)
7.	5%$FeCl_3$(Aq.)	Green	Dark green	Dark green
8.	$FeCl_3$ (Alco.)	Dark Brown	Green	Blue
9.	Water	Green	Green	Green

Table - 6. Fluorescent analysis of power sample of *Mesua ferrea* L.

S.No.	Solvents	Fluorescent Colour		
		Under Visible light	**UV light (254nm)**	**UV light (366nm)**
1.	Conc. HCl	Light Brown	Dark Brown	Brown
2.	Conc. H_2SO_4	Dark Brown	Brown	Red
3.	Conc. HNO_3	Brown	Yellow	Red
4.	C_3H_6O	Light Brown	Yellow	Brown
5.	Aq. NAOH	Brown	Brown	Violet
6.	NAOH (Alco.)	Brown	Brown	Violet
7.	5%$FeCl_3$(Aq.)	Green	Dark green	Green
8.	$FeCl_3$ (Alco.)	Yellow	Green	Blue
9.	Water	Light Brown	Dark Brown	Brown

Table No. 7. Fluorescent analysis of power sample of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.

S.No.	Solvents	Fluorescent Colour		
		Under Visible light	**UV light (254nm)**	**UV light (366nm)**
1.	Conc. HCl	Light Brown	Dark Brown	Brown
2.	Conc. H_2SO_4	Dark Brown	Brown	Red
3.	Conc. HNO_3	Brown	Yellow	Red
4.	C_3H_6O	Light brown	Brown	Brick red
5.	Aq. NAOH	Brown	Brown	Brick red
6.	NAOH (Alco.)	Dark brown	Brown	Brick red
7.	5%$FeCl_3$(Aq.)	Dark green	Dark green	Dark green
8.	$FeCl_3$ (Alco.)	Yellow green	Green	Blue

9.	Water	Brown	Brown	Brown

Ash and Extractive Value Determination

Solvents have the capacity to extracts unlike percentage of chemical constituents from the sample. Based on chemical nature and properties of contents of plant sample, solvents are used for determination of extractive value. Extractive value is one of the qualitative evaluations to authenticate a given sample. The total ash, acid insoluble ash and extractive values of selected species are given as per with Ayurvedic Pharmacopeia of India (API) references (Table 8-12).

Table - 8. Physicochemical parameters of *Bauhinia variegata* (Flower)

S. No.	Parameter	Result	API Reference
1.	Total Ash	3.80%	NMT%
2.	Acid insoluble ash	0.15%	NMT%
3.	Water soluble extractive	09.62%	NLT%
4.	Alcohol soluble extractive	12.08%	NLT%

Table - 9. Physicochemical parameters of *Bixa orellana* L. (Stem Bark)

S.No.	Parameter	Result	API Reference
1.	Total Ash	6.425%	NMT %
2.	Acid insoluble ash	0.39 %	NMT %
3.	Water soluble extractive	18.14 %	NLT %
4.	Alcohol soluble extractive	18.18 %	NLT %

Table – 10. Physicochemical parameters of *Leucas aspera* (Willd.) Link (Whole Plant)

S.No.	Parameter	Result	API Reference
1.	Total Ash	19.02 %	NMT 25 %
2.	Acid insoluble ash	0.34 %	NMT 1 %
3.	Water soluble extractive	26.24 %	NLT 20 %

4.	Alcohol soluble extractive	22.83 %	NLT 20 %

Table - 11. Physicochemical parameters of *Mesua ferrea* L. (Stem Bark)

S.No.	Parameter	Result	API Reference
1.	Total Ash	2.88 %	NMT %
2.	Acid insoluble ash	0.19%	NMT %
3.	Water soluble extractive	12.62 %	NLT %
4.	Alcohol soluble extractive	15.08 %	NLT %

Table – 12. Physicochemical parameters of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark)

S.No.	Parameter	Result	API Reference
1.	Total Ash	15.02 %	NMT 25 %
2.	Acid insoluble ash	0.30 %	NMT 1 %
3.	Water soluble extractive	20.14 %	NLT 20 %
4.	Alcohol soluble extractive	20.83 %	NLT 20 %

Phytochemical Screening

The plant parts contain secondary metabolites such as alkaloids, glycosides, and saponin, flavonoids, phenols etc. which are medicinally very important. Phytochemical screening is an important tool to evaluate the phytochemical composition of drug sample. Phytochemical screenings of the selected species are given in (Table- 13-17).

Table - 13. Phytochemical Screening of *Bauhinia variegata* (Flower)

***Bauhinia variegata* L. (Flower)**	**Observation of plant sample extract reaction to different solvent**				
	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids Test					
Mayer's test	+	-	+	+	+
Wagner's test	-	-	-	-	+
Hager's test	+	+	+	+	-
Flavonoids Test					
Alkaline reagent test	+	-	+	-	+
Phenols Test					
Ferric chloride test	-	-	+	-	+
Gelatin test	-	-	-	+	-
Lead acetate test	+	-	+	+	+
Detection of volatile oil	+	-	+	+	+
Saponins Test					
Foam test	+	+	-	-	+
Glycosides					
Bomtrager's test	+	-	-	-	+
Legal's test	+	-	+	+	-
Carbohydrates					
Molish's test	-	+	+	-	+
Fehling's test	+	-	-	+	-
Barfoed's test	-	+	+	-	+

Table - 14. Phytochemical Screening of *Bixa orellana* L. (Stem Bark)

***Bixa orellana* L. (Stem Bark)**	**Observation of plant sample extract reaction to different solvent**				
	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids Test					
Mayer's test	-	+	-	+	+
Wagner's test	+	+	-	+	-
Hager's test	+	+	+	+	+
Flavonoids Test					
Alkaline reagent test	+	-	+	+	+
Phenols Test					
Ferric chloride test	-	+	+	-	+
Gelatin test	-	-	-	+	-
Lead acetate test	+	+	-	+	+
Detection of volatile oil	+	-	+	+	+
Saponins Test					
Foam test	-	-	-	+	+
Glycosides					
Bomtrager's test	-	-	-	+	+
Legal's test	+	+	+	+	-
Carbohydrates					
Molish's test	+	+	-	-	+
Fehling's test	+	-	-	+	-
Barfoed's test	+	+	-	+	+

Table - 15. Phytochemical Screening of *Leucas aspera* (Willd.) Link (Whole Plant)

***Leucas aspera* (Willd.) Link (Whole Plant)**	**Observation of plant sample extract reaction to different solvent**				
	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids Test					
Mayer's test	-	+	+	-	+
Wagner's test	-	+	-	+	+
Hager's test	+	+	+	+	+
Flavonoids Test					
Alkaline reagent test	+	-	+	+	-
Phenols Test					
Ferric chloride test	-	-	-	-	-
Gelatin test	+	+	+	+	+
Lead acetate test	+	+	+	+	+
Detection of volatile oil	+	-	+	+	-
Saponins Test					
Foam test	-	+	-	+	+
Glycosides					
Bomtrager's test	+	+	-	+	+
Legal's test	+	-	+	+	+
Carbohydrates					
Molish's test	+	+	+	-	+
Fehling's test	-	+	-	+	-
Barfoed's test	+	-	+	-	+

Table - 16. Phytochemical Screening of *Mesua ferrea* L. (Stem Bark)

***Mesua ferrea* L. (Stem Bark)**	**Observation of plant sample extract reaction to different solvent**				
	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids Test					
Mayer's test	+	+	-	-	+
Wagner's test	-	-	-	-	+
Hager's test	+	+	+	+	+
Flavonoids Test					
Alkaline reagent test	+	-	+	+	-
Phenols Test					
Ferric chloride test	-	-	-	-	-
Gelatin test	-	-	-	-	+
Lead acetate test	+	+	+	+	+
Detection of volatile oil	+	-	+	+	-
Saponins Test					
Foam test	-	-	+	+	-
Glycosides					
Bomtrager's test	+	-	+	-	-
Legal's test	-	-	+	+	-
Carbohydrates					
Molish's test	-	-	+	-	+
Fehling's test	-	+	-	-	+
Barfoed's test	+	-	+	+	+

Table - 17. Phytochemical Screening of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark)

***Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark)**	**Observation of plant sample extract reaction to different solvent**				
	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids Test					
Mayer's test	+	+	-	-	+
Wagner's test	-	+	-	+	+
Hager's test	+	+	+	+	+
Flavonoids Test					
Alkaline reagent test	+	-	+	+	-
Phenols Test					
Ferric chloride test	-	+	+	+	-
Gelatin test	+	-	+	+	+
Lead acetate test	+	+	+	+	+
Detection of volatile oil	+	-	+	+	+
Saponins Test					
Foam test	+	-	+	+	+
Glycosides					
Bomtrager's test	+	-	+	+	-
Legal's test	+	+	+	+	-
Carbohydrates					
Molish's test	+	+	+	-	+
Fehling's test	+	+	+	+	-
Barfoed's test	+	-	+	+	+

Pharmacognostic evaluation of a powder drug indicates the identity, quality and purity of a drug. Examination of powder drug means confirmation of its uniqueness and its quality, purity and adulteration (Kakote *et al.,* 2012). Drugs of natural origin can be studied by powder microscopic, physical, chemical parameters. In the present study five species have been pharmacognostically examined by pharmacognostic parameters *viz.*, powder microscopy (Figs.-15-22) ash study, Fluorescent study, extractive value study and preliminary phytochemical screening and results is given in (Tables- 3-17).

From figs.- 18-22, it was observed that in powder sample of *Bauhinia variegata* (Flower), *Bixa orellana* (Stem Bark), *Leucas aspera* (Whole plant), *Mesua ferrea* (Stem Bark) and *Terminalia arjuna* (Stem Bark) the presence of tannins and starch in stem bark is useful in management of various disorders. *Bauhinia variegata* L. (Flowers) contains multicellular, uniseriate trichomes filled with light brown coloured material embedded vascular elements and micro rosette crystals of calcium oxalate. The microscopical features of powder are shown in (Fig. 18). Powder Microscopy of *Bixa orellana* L. (Bark) contains dark brown in colour, fine powder, taste astringent, ray parenchymatous cells in 3 to 4 layers, cells are containing clusters of calcium oxalate crystal in layers, medullary ray and fragments of fibers with tapering ends. The microscopical features of powder are shown in (Fig.19). Powder Microscopy of *Leucas aspera* (Willd.) Link (Whole plant) contains powder green colour, uniseriate trichomes varying in their size and shape and three major types of trichomes were identified. The microscopical features of powder are shown in (Fig.-20). Powder microscopy of *Mesua ferrea* L. (Stem Bark) contains brown colour with a mild sweet taste, prismatic crystals of calcium oxalate, aseptate fibres, sclerieds tissue

isolated and in groups with thick walls and broad lumen. The microscopical features of powder are shown in (Fig. - 21). The presence of fibres, tannin and other cells in samples shows that it is good neuraceutical supplement for the local people. Powder microscopy of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark) powder is brown colour, little bitter taste, medullary rays and rosette crystals of calcium oxalate in association with fibres (crystal fibre). Cork cell filled with tannins. The microscopical features of powder are shown in (Fig.-22).

Fluorescent analysis Tables- 3-7, the powder sample of *Bauhinia variegata* (Flower), *Bixa orellana* (Stem Bark), *Leucas aspera* (Whole plant), *Mesua ferrea* (Stem Bark) and *Terminalia arjuna* (Stem Bark) powder when treated with the different chemical reagents *viz.* Conc. HCl, Conc. H_2SO_4, Conc. HNO_3 , C_3H_6O, Aq. NAOH, NAOH (Alco.), 5%$FeCl_3$(Aq.), $FeCl_3$ (Alco.) and Water then fluorescence activities is observed and that it may be useful in the authentication and standardization of drug and it also reveals that presence of some phytochemical where fluorescent colour was observed.

Powder of the different parts of the plants sample shows a diagnostics characteristics when observed under day light and UV. Powder as such appears green in day light but appears yellow when observed under UV. Powder treated with NaOH appears dark green in day light as well as under UV; when powder is treated with acetic acid it appears light brown in day light and dark brown under UV, Powder with HNO_3 appears bright orange in day light and light yellow under UV; when treated with H_2S04, it appears light green in day light and dark green under UV; powder with HCl appears dark green in day light and light green under UV light, when treated with $FeCl_3$ it is pale green in day light and light brown under UV light. The characteristics Fluorescent powder activities of the different parts of

the powder sample may be useful in the correct identification and standardization of these species.

In physicochemical parameters Tables-8-12, the highest total Ash value was calculated in *Leucas aspera* (Whole plant) and *Terminalia arjuna* (Stem Bark) i.e. 19.02 % and 15.02% less *in Bauhinia variegata* (Flower) in 3.80% and *Mesua ferrea* (Stem bark) 2.88 %. Highest acid insoluble ash in *Leucas aspera* (Whole Plant) 0.39% and *Bixa orellana* (Stem Bark) 0.34% and lesser in *Mesua ferrea* (Stem Bark) 0.19% and *Bauhinia variegata* (Flower) 0.15%. Highest Water soluble extractive was found in *Leucas aspera* (Whole Plant) 26.24% and *Terminalia arjuna* (stem Bark) 20.14%, and lowest in *Mesua ferrea* (Stem Bark) 12.62% *Bauhinia variegata* (Flower) 09.62%. Highest Alcohol soluble extract was found in *Leucas aspera* (Whole plant) 22.83% and *Terminalia arjuna* (Stem Bark) 20.83, and lowest *Mesua ferrea* (Stem bark) 15.08% *Bauhinia variegata* (Flower) 12.08 % in Bixa *orellana* (Stem Bark) 3.4%.

In Phytochemical Screening Table-13 *Bauhinia variegata* (Flower) sample maximum result were found through methanol extract and revealed that the presence of alkaloids, flavonoids, glycosides, phenols, volatile oil, Saponins and Carbohydrates are present with compare to other solvent.

In Phytochemical Screening Table-14 *Bixa orellana* L. (Stem Bark) maximum result were found through acetone and methanol extract and reavealed that the presence of alkaloids, flavonoids, glycosides, phenols, volatile oil, Saponins and Carbohydrates are present with compare to other solvent.

In Phytochemical Screening Table-15 *Leucas aspera* (Whole plant) maximum result were found through acetone, ethyle acetate, chloroform and methanol extract and

reavealed that the presence of alkaloids, flavonoids, glycosides, phenols, volatile oil, Saponins and Carbohydrates are present with compare to benzene solvent.

In Phytochemical Screening Table-16 *Mesua ferrea* (Stem Bark) maximum result were found through acetone, and methanol extract and reavealed that the presence of alkaloids, flavonoids, glycosides, phenols, volatile oil, Saponins and Carbohydrates are present with compare to other solvent.

In Phytochemical Screening Table-17 *Terminalia arjuna* (Stem Bark) showed maximum positive result were observed in all the solvent i.e. acetone, benzene, ethyl acetate, chloroform and methanol extract and revealed that the presence of alkaloids, flavonoids, glycosides, phenols, volatile oil, saponins and carbohydrates are present. The present study might be useful to provide the information pertaining to the identification tool for the medicinal plants.

Deshmukh *et al.* (2013) reported that phytochemical and pharmacognostical evaluation of *Bixa orellana* L. and found that presence of vascular bundle, collenchymas, spongy parenchyma and palisade cells in leaf. In fluorescence and phytochemicals showed the presence of alkaloids, tannins, triterpenoids, steroids, sterols, saponins, flavones, flavonoids. Modh *et al.,* (2011) also reported the finding of pharmacognostical and phytochemical evaluation of *Bauhinia variegata* L. and reported that total ash is 9.42%, acid insoluble ash is 5.72%, water-soluble extractive value is 3.30% and loss on drying at 105 °C is 6.27%. Present phytochemical analysis revealed the presence of alkaloid, tannin, flavonoid, steroid, triterpenoid and saponin in different extracts. According to available literature in *Bauhinia variegata* L. flower part very few works have been conducted. Sengupta *et al.,* (2014) also reported in their study about total Ash content 15.034% acid

insoluble ash 1.452% water soluble extractive 44.03%, acid soluble extractive 42.05% and phytochemical analysis reveal that presences of alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, steroids, glycosides, carbohydrates and proteins or amino acids in *Terminalia arjuna* (Stem Bark). Kripa *et al.*, (2017) also reported in their study about alcohol soluble 6.5% and water-soluble extractive 9% and in phytochemical screening the presence of alkaloids, flavonoids, glycosides, lignins, phenols, saponins were reported. Overall study reveals that selected medicinal plants parts are found phytochemical significant. This could be the reason why Nyishi people are using these plants in their daily life for folk medicine as well as food.

Results and Discussion on Nutritional Analysis

The nutritional analysis of the five plants undertaken for the work given in Table-18.

Table -18. Nutritional Analysis of the plants species

S.No.	Plant Samples	Nutritional Value			
		Carbohydrate %	**Fat %**	**Protein %**	**Crude Fibre %**
1.	*Bauhinia variegata* (flower)	18.9±1.15	5.6±0.36	11.5±0.15	9.2±1.01
2.	*Bixa orellana* (stem Bark)	4.04±0.77	0.36±.011	5.9±0.39	3.4±0.87
3.	*Leucas aspera* (Whole plant)	9.9±0.52	2.9±0.65	10.6±1.12	10.5±0.99
4.	*Mesua ferrea* (Stem Bark)	13.9±0.76	5.1±0.36	10.5 ±.98	7.9±0.57
5.	*Terminalia arjuna* (stem Bark)	14.5±1.12	4.3±0.54	13.26±1.56	8.9±1.16

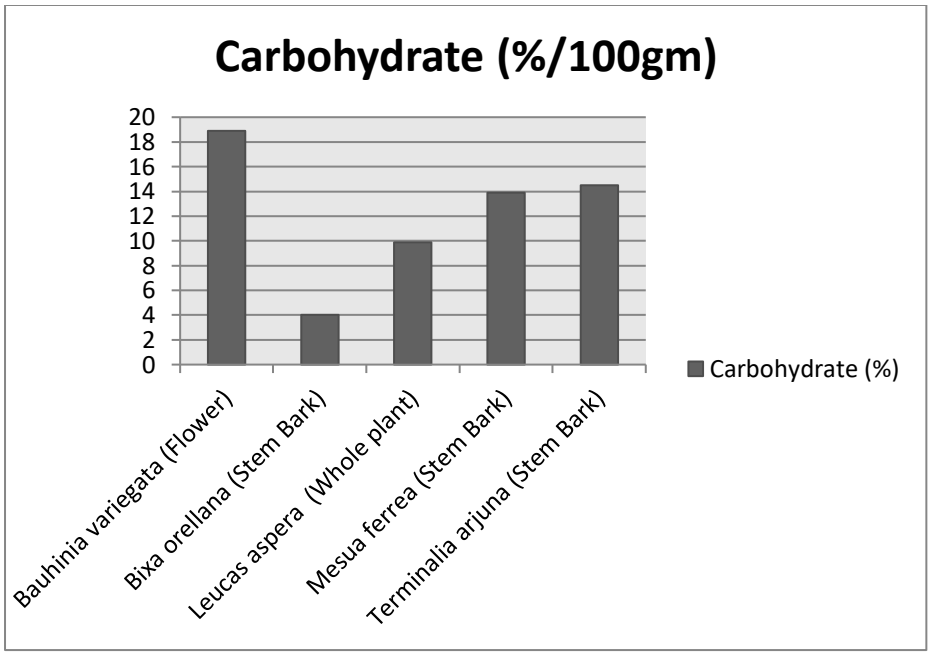


Figure -23. Graphical representation of Carbohydrate content in the plants

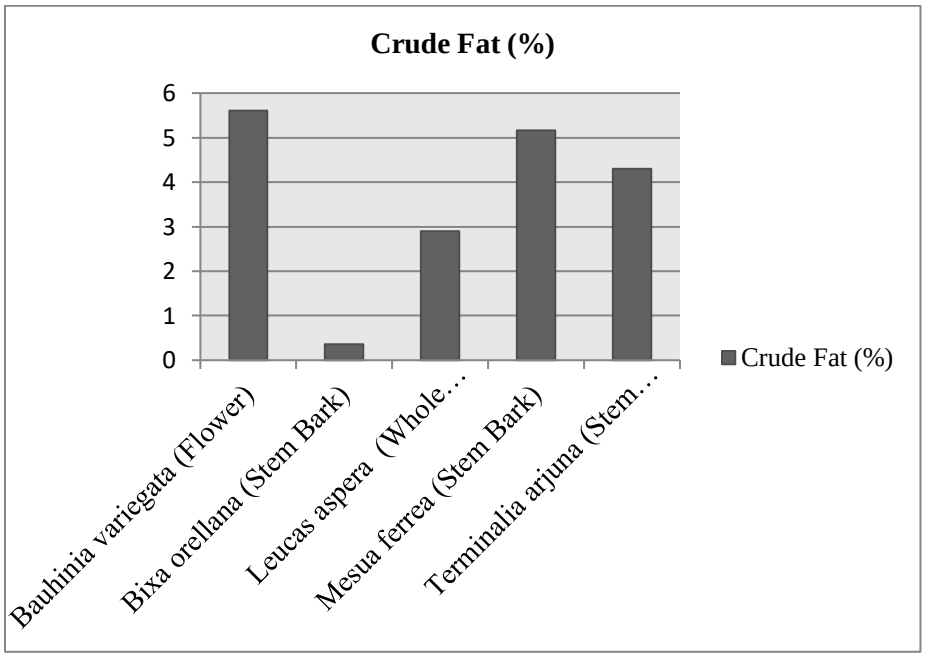


Figure -24. Graphical representation of crude Fat content in the plants

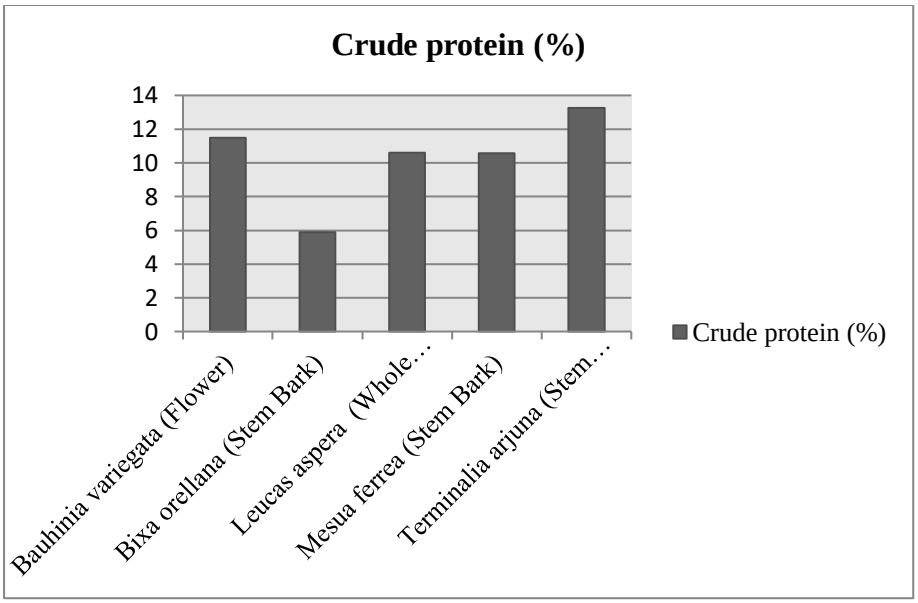


Figure -25. Graphical representation of crude protein content in the plants

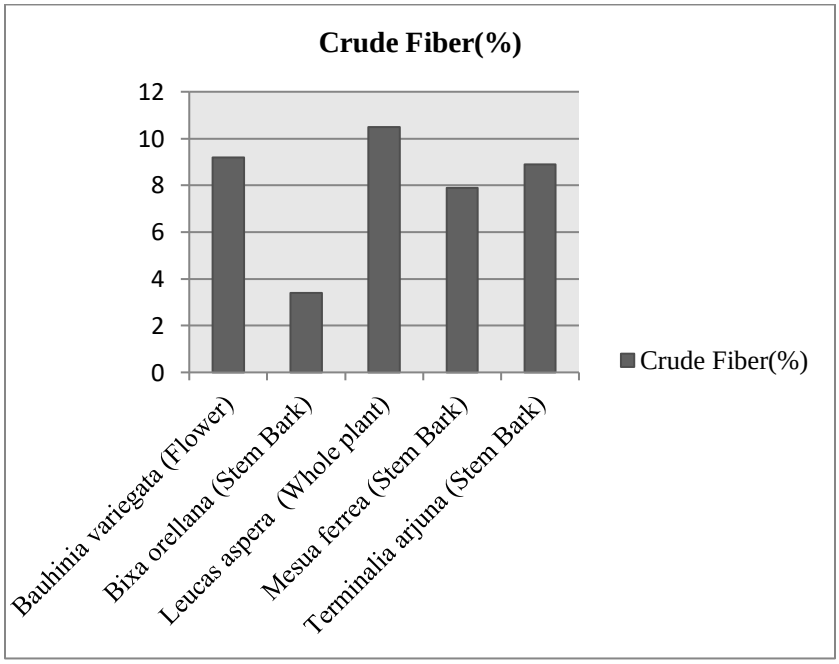


Figure-26. Graphical representation of crude fibre content in the plants

In the present study highest carbohydrate content was found in *Bauhinia variegata* (flower) 18.9% followed by *Terminalia arjuna* (Stem Bark) 14.5%, *Mesua ferrea* (Stem Bark) 13.9%, *Leucas aspera* (Whole plant) 9.9%, and only 4.04% in *Bixa orellana* (Stem Bark).

Crude fat was found to be highest in *Bauhinia variegata* (Flower) 5.6% followed by *Mesua ferrea* (Stem Bark) 5.6%, *Terminalia arjuna* (Stem Bark) 4.3%, *Leucas aspera* (Whole plant) 2.9%, and only 0.36% in *Bixa orellana* (Stem Bark). The protein content is observed to be more or less uniform i.e. *Terminalia arjuna* (Stem Bark) 13.26%, *Bauhinia variegata* (Flower) 11.5%, *Leucas aspera* (Whole plant) 10.6%, *Mesua ferrea* (Stem Bark) 10.5%, except in the case of *Bixa orellana* (Stem Bark) it showed 5.9%.

Crude fibre was found to be highest in *Leucas aspera* (Whole plant) 10.5%, followed by *Bauhinia variegata* (flower) 9.2%, *Terminalia arjuna* (Stem Bark) 8.9%, *Mesua ferrea* (Stem Bark) 7.9%, and *Bixa orellana* (Stem Bark) 3.4%.

With respect to nutritional analysis of *Bixa orillana,* Melissa Alessandra Valério *et al.,* (2015) have reported the content of protein, lipids, fibre and carbohydrate in the seed. Their finding of total carbohydrate content is 28.45% while the present analysis in *Bixa orellana* (Stem Bark) is only 4.04%. This could be attributed to the different plant part used i.e. seed and stem bark. In the same report 2.22% lipids was observed to be minimum which is also similar to the present finding of 0.36% fat content. This is an indication that *Bixa orellana* does not contain much fat on the plant parts. The presence of crude fiber 3.5% is also much lower compared to seed which contains 28.45%.

Another report on *Mesua ferrea* (Seed and Leaves) is available by Sayeed *et al.,* (2004) relating to carbohydrates, protein and crude fibre but the present finding is the first work in *Mesua ferrea* (Stem Bark). The content of carbohydrate 13.9%, Fat 5.1%, Protein 10.5%, and

Crude fibre 7.9% is additional information for *Mesua ferrea* plant part which is used by the folk healer for therapeutic purposes.

In the case of *Bauhinia variegata* (Flower) amount of Carbohydrate i.e. 18.9% is maximum followed by protein 11.5%, and fat 5.6%. This result is almost in conformity with the work done by Liaw & Zhi Xian (2012) except the protein content i.e. 3.24% as against 11.5% in the present evaluation.

The present work conducted on *Bixa orellana* (Stem Bark), *Mesua ferrea* (Stem Bark) Bark), and *Terminalia arjuna* (Stem Bark) is the first investigation on the nutritional value of folk medicinal plants. This work can be taken as future references for researchers in exploring the neutraceutical aspects of plants used by folk healers in tribal area.

Results and discussion on Antioxidant Screening of Plant Samples

To study antioxidant in the plant sample, total phenolic and flavonoid is taken as the base study to correlate antioxidant potential by using free radical scavenging methods of DPPH and ABTS in which Gallic acid, Trolox and Rutin were used as standard as given in Table-19.

Table-19. Result of Antioxidant potential (DPPH, ABTS, TPC, TFC and value)

Sample Name.	**DPPH assay at 517nm (µM/g)**	**ABTS assay at 734 nm (µM/g)**	**TPC at 765 nm (mg GAE/g)**	**TFC at 510 nm (µg RE/g)**
Bauhinia variegata (Flower)	10.4±0.1	47.5±0.7	28.90±0.1	76.5±0.9
Bixa orellana (Stem Bark)	3.1±0.56	35.0±0.5	30.70±0.07	31.9±0.8
Leucas aspera (Whole plant)	9.8±0.1	48.5±0.9	29.92±0.07	124.2±0.4
Mesua ferrea (Stem Bark)	1.3±0.06	50.0±0.5	47.70±0.07	65.9±0.8
Terminalia arjuna (Stem Bark)	6.7±0.58	61.5±0.8	11.67±0.03	52.0±0.2

As shown in table -19, *Bauhinia variegate* (Flower) revealed maximum DPPH assay at 517nm (10.4 µM/g) while *Mesua ferrea* (Stem bark) is having the least (1.3 µM/g). For the remaining species in descending order is *Leucas aspera* (Whole plant) (9.5 µM/g) *Terminalia arjuna* (Stem Bark) (6.7 µM/g) and *Bixa orellana* (Stem Bark) (3.1 µM/g).

The maximum for the ABTS assay at 734nm was found for *Terminalia arjuna* (Stem Bark) with (61.5 µM/g) while minimum was found for *Bixa orellana* (Stem Bark) with (35.0 µM/g). In descending order for the remaning species is *Mesua ferrea* (Stem bark) (50.0 µM/g) then *Leucas aspera* (Whole plant) (48.5 µM/g) and *Bauhinia variegate* (Flower) (47.5 µM/g).

The observation for TPC at 765 nm was maximum for *Mesua ferrea* (Stem bark) with 47.5 mgGAE/g while minimum in was for *Terminalia arjuna* (Stem Bark) with 11.67 mgGAE/g. For the rest of the species in descending order it is *Bixa orellana* (Stem Bark) 30.70 mgGAE/g followed by *Leucas aspera* (Whole plant) with 29.92 mgGAE/g and *Bauhinia variegate* (Flower) 28.90 mgGAE/g

Finally TFC at 510nm was also observed to be maximum for *Leucas aspera* (Whole plant) with 124µg RE/g while the minimum was observed for *Bixa orellana* (Stem Bark) 31.9µg RE/g. In descending order of the other species are *Bauhinia variegate* (Flower), *Mesua ferrea* (Stem bark) and *Terminalia arjuna* (Stem Bark) with concentration of 76.5, 65.9, 52.0 µg RE/g respectively.

Present evaluation revealed that *Bauhinia variegata* (Flower) 28.9 mgGAE/g of total phenolic and 76.5 µg RE/g total flavonoid followed with scavenging activity of 47.5 µM/g in ABTS assay and 10.4 µM/g in DPPH assay. Hence, the flavonoid content is more than phenolic content which may be attributed for considerable free radical scavenging activity in ABTS and DPPH study (Table-19).

Hemmalakshmi, S. (2016) reported the antioxidant activity of *Bauhinia variegata* flower with reference to of ethanolic extracts of flower, and DPPH assay result were 84.05

µg/ml with ascorbic acid standard and highest ABTS scavenging activity was observed in ethanolic extracts of flower sample, 53.68 µg/ml with ascorbic acid standard. Sawhney, S.S., (2012) also reported the antioxidant potential of *Bauhinia variegate* plant extracts and found different constituents like phenol, flavonoid etc. very few work has been done relating to *Bauhinia variegata* (Flower).

Bixa orellana (Stem Bark) is recorded to contain 30.70 mgGAE/g total phenolic content, 31.9 µg RE/g total flavonoid content calculated in equivalent to Rutin with scavenging capacity of 35.1 µM/g in ABTS method and scavenging capacity of 3.1 µM/g in DPPH method. The considerable antioxidant potential of this stem bark may be attributed to the flavonoid and phenolic compounds (Table-19). While Giorgi Annamaria *et al.* (2013) recorded on *Bixa orellana,* The TPC of *Bixa orellana* extracts, expressed as Gallic acid equivalent, ranged from 2.82 mg/g to 4.67 mg/g. Many workers are reported antioxidant activity for seed, leaves, fruit and root part of *Bixa orellana* species but no literature is available on the analysis of stem bark.

Leucas aspera (Whole plant) is recorded to contain 29.92 mgGAE/g total phenolic content calculated. 124.2 µg RE/g total flavonoid content calculated in equivalent to Rutin with scavenging capacity of 48.5 µM/g in ABTS method and scavenging capacity of 9.8 µM/g in DPPH method. The considerable antioxidant potential of whole plant may be attributed to the flavonoid and phenolic compounds (Table-19). While Das *et al.*, (2013) have reported antioxidant activity of *Leucas aspera* (Whole plant) with reference to n-hexane extract based DPPH assay recorded 30.79 to 47.5 µg/ml and phenolic compound 15.36 GAE/g that is quite differ from present study because extract solvent is different. It could be because of the use of different extract solvent and also depends on

ecotype of the sample. Emran *et al.*,(2012) also reported antioxidant activity of *Leucas aspera* (Whole plant) with reference to ascorbic acid standard extract based DPPH assay recorded highest scavenging action was 99.58 µg/m and highest scavenging activity of *Leucas aspera* (Whole plant) with ethanolic extract was recorded 86.62% at concentration 800 µg/ml.

Mesua ferrea (Stem Bark) is recorded to contain 47.70 mgGAE/g total phenolic content calculated, 65.9 µg RE/g total flavonoid content calculated in equivalent to Rutin with scavenging capacity of 50.0 µM/g in ABTS method and scavenging capacity of 1.3 µM/g in DPPH method. The considerable antioxidant potential of this stem bark may be attributed to the flavonoid and phenolic compounds (Table-19). While Chaitanya *et al.*, (2015) also reported antioxidant activity of *Mesua ferrea* bark total phenolic content 19.80 mg/gm GAE, total antioxidant capacity of *Mesua ferrea* bark extracts 9.21 µg/ml and ABTS activity is 39.25 µg/ml observed.

Terminalia arjuna (stem Bark) is recorded to contain 11.67 mgGAE/g phenolic content calculated, 52.0 µg RE/g total flavonoid content calculated in equivalent to Rutin with scavenging capacity of 61.5 µM/g in ABTS method and scavenging capacity of 6.7 µM/g in DPPH method. The considerable antioxidant potential of this stem bark may be attributed to the flavonoid and phenolic compounds (Table-18). Chatha, *et al.*, (2014) also reported *Terminalia arjuna* (Stem Bark) extracts have good amount of TPC (6.02-11.00 g/100g, as gallic acid equivalent) and TFC (1.75- 5.96 g/100g, as catechin equivalent).

In all the plant sample maximum flavonoid content were found in *Leucas aspera* (Whole plant) and *Bauhinia variegate* (Flower) i.e. 124.2 and 76.5 µg RE/g and less in *Bixa orellana* (Stem Bark) 31.9 µg RE/g. Highest Phenolic content were found in *Mesua ferrea* (Stem Bark) and *Bixa orellana* (Stem Bark) i.e. 47.70 and 30.70 mg GAE/g and less in *Terminalia arjuna* (Stem Bark) 31.9 µg RE/g. Maximum antioxidant assay result were found through DPPH in *Bauhinia variegate* (flower) and *Leucas aspera* (Whole plant) i.e. 10.4 and 9.8 µM/g and less in *Mesua ferrea* (Stem Bark) 1.3 µM/g. Maximum antioxidant assay result were found through ABTS in *Terminalia arjuna* (Stem Bark) and *Mesua ferrea* (Stem Bark) i.e. 61.5 and 50.0 µM/g and less in *Bixa orellana* (Stem Bark) 35.0 µM/g. (Table-19).

Present investigation of antioxidant potential revealed that the folk healers of Nyishi tribe used the medicinal plants in various ailments also have considerable amount of antioxidant properties. Deficiency of antioxidants, which can quench the reactive free radicals, facilitates the various type of disease in human body (Shahidi *et al.*, 1992). Medicinal plant and vegetable have antioxidant in good amount that prevent various kind of disorders in our body because they contains natural antioxidant (Knekt *et al.*, 1996). These wildly available medicinal plant acts as a defensive medicine for human health care. Based on research studies, antioxidant and rich diet is correlated with the increased longevity and decreased incidence of cardiovascular diseases in populations despite high intake of fat by various researchers (Burr, 1995; de Lange, 2007; Rosenkranz *et al.*, 2002). The antioxidant of many flavonoids are stronger than so many vitamins (Prior and Cao, 2000).

Result and Discussion on Thin Layer Chromatography

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents on basis of their band and R_f value. The chromatographic profile may serve as a characteristic fingerprint for qualitative evaluation of fruits. The details of each five plant species TLC profile result is given in Figs. - 27-32 and Tables-20-24.

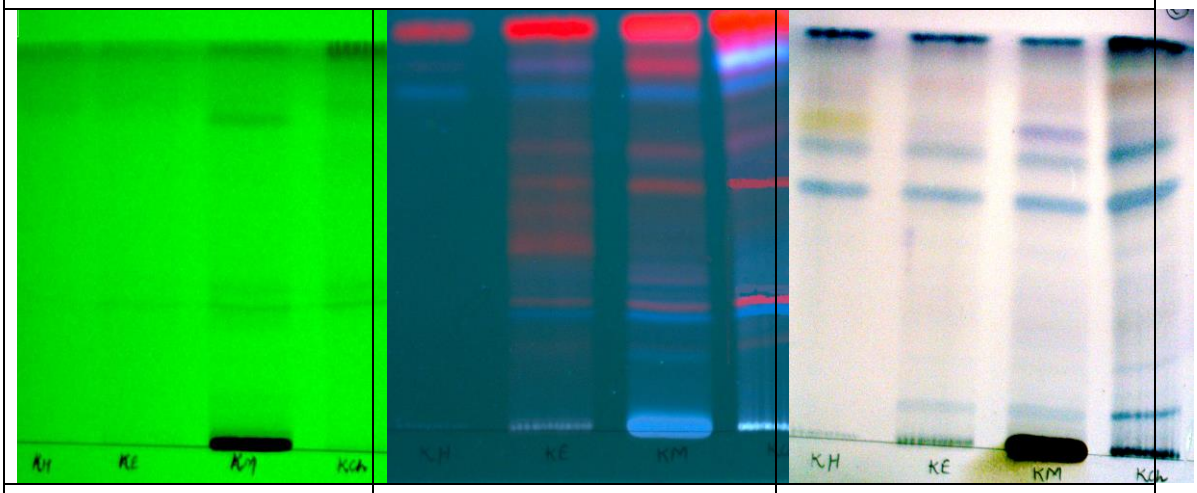
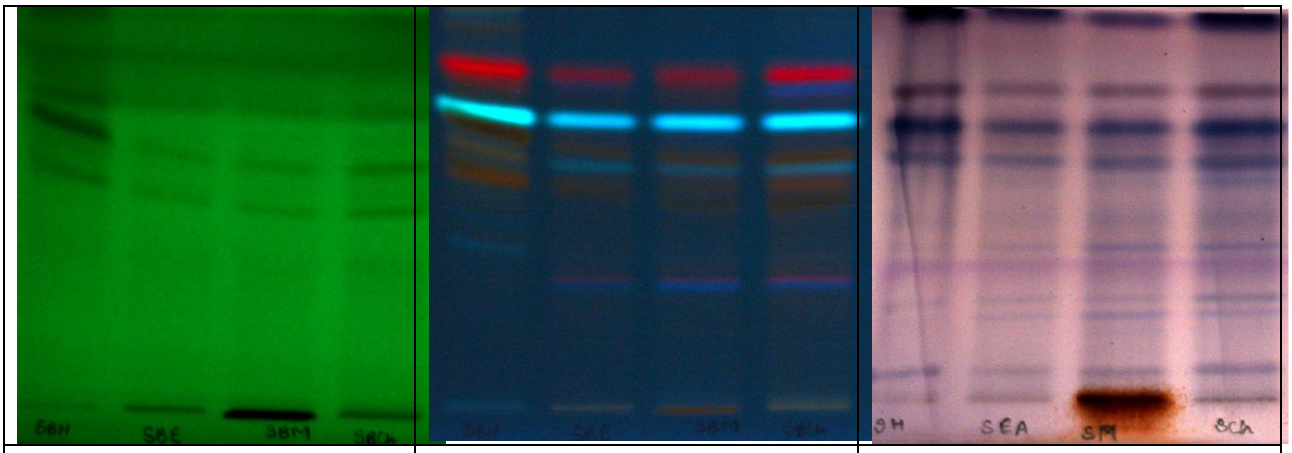
Thin Layer Chromatographic (TLC) Profiles of *Bauhinia variegata* L. (Flowers)												
												
T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	
254nm				366nm				Derivatized				

Figure - 27. TLC Profiles of *Bauhinia variegata* L. (Flowers)

Table - 20. R_f value of TLC Profiles of *Bauhinia variegata* L. (Flowers)

Extract tract	Retention factor (R_f)		
	254nm	366nm	Derivatized
T1 (KH)	0	0.74 (BLUE), 0.80, (VIOLET), 0.90 (FLUORESCENT RED)	0.42(BLUE), 0.49(VIOLET), 0.56(YELLOW), 0.68(VIOLET), 0.75 (BLUE)
T2 (KE)	0.28, 0.70	0.26 (BLUE), 0.28 (RED), 0.41 (RED), 0.47 (RED), 0.54 (RED), 0.63 (RED), 0.76 (BLUE), 0.82 (BLUE), 0.88 (FLUORESCENT RED)	0.06 (BLUE), 0.14 (BLUE), 0.22 (BLUE), 0.41 (BLUE), 0.56 (BLUE), 0.67 VOILET, 0.76 (VIOLET)
T3 (KM)	0.09, 0.28, 0.32, 0.66	0.15 (BLUE), 0.17 (BROWN), 0.25 (BLUE), 0.27 (RED), 0.33 (RED), 0.54 (RED), 0.61 (RED), 0.71 (VIOLET), 0.76 (BLUE), 0.82 (RED), 0.89 (FLUORESCENT RED).	0.10 (BLUE), 0.14 (BLUE), 0.22 (BLUE), 0.26(BLUE),0.41(BLUE),0.46 (VIOLET), 0.57 (BLUE),0.69(VIOLET), 0.76 (VIOLET)
T4 (KCH)	0.28, 0.32, 0.71	0.20 (ORANGE), 0.28 (BLUE), 0.30 (RED), 0.40 (BLUE), 0.56 (RED), 0.65 (VIOLET), 0.75 (VIOLET), 0.77 (BLUE), 0.82 (BLUE), 0.91 (RED)	0.15(BLUE), 0.24(BLUE), 0.26(VIOLET), 0.42(BLUE), 0.57(BLUE), 0.65(VIOLET),0.76(VIOLET), 0.88 (VIOLET)
Thin Layer Chromatographic (TLC) Profiles of *Bixa orellana* L.			



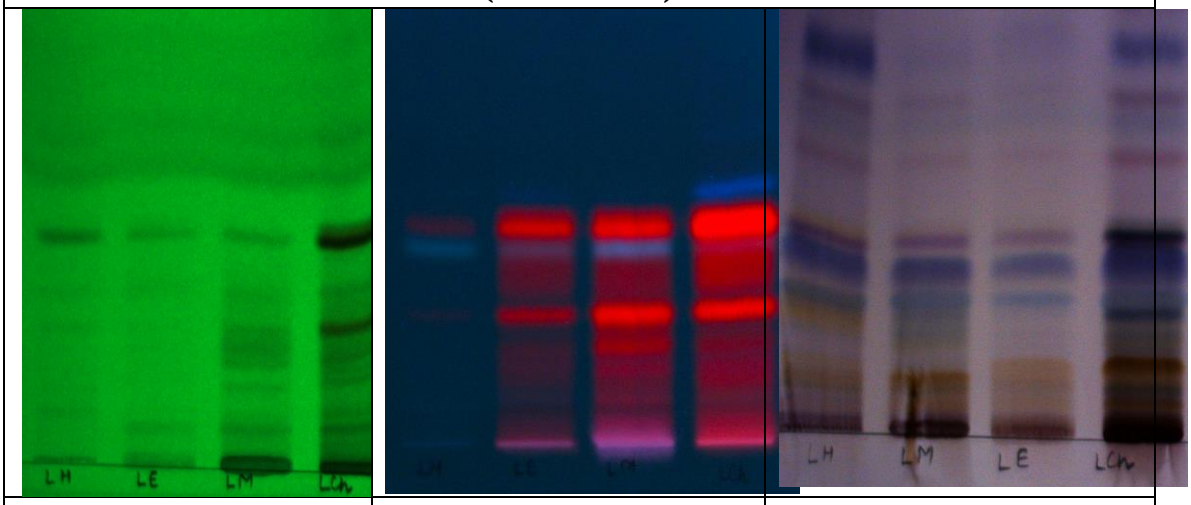
T_1 T_2 T_3 T_4	T_1 T_2 T_3 T_4	T_1 T_2 T_3 T_4
254nm	**366nm**	**Derivatized**

Figure- 28. TLC Profiles of *Bixa orellana* L. bark

Table- 21. R_f value of TLC Profiles of *Bixa orellana* L. bark

Extract tract	Retention factor (R_f)		
	254nm	254nm	254nm
T1 (SH)	0.59, 0.72, 0.79, 0.89	0.40 (BLUE), 0.48 (BLUE), 0.56 (ORANGE), 0.63 (YELLOW), 0.68 (RED), 0.73 (FLUORESCENT BLUE), 0.84 (RED)	0.29 (BLUE), 0.57 (BLUE), 0.61 (BLUE), 0.71 (BLUE), 0.80 (VIOLET), 0.87 (BLUE)
T2 (SBE)	0.53, 0.63, 0.76, 0.86	0.30 (BLUE), 0.33 (RED), 0.55 (ORANGE), 0.59 (BLUE), 0.63 (BROWN), 0.72 (FLUORESCENT BLUE), 0.83 (RED)	0.28 (BLUE), 0.77 (VIOLET)
T3 (SBM)	0.50, 0.61, 0.75	0.29 (BLUE), 0.31 (RED), 0.55 (ORANGE), 0.58 (BLUE), 0.60 (BROWN), 0.71 (FLUORESCENT BLUE), 0.80 (RED)	0.29 (BLUE), 0.66 (BLUE), 0.77 (VIOLET)
T4 (SCH)	0.50, 0.56, 0.63, 0.75	0.29 (BLUE), 0.33 (RED), 0.50 (RED), 0.55 (ORANGE), 0.59 (BLUE), 0.62 (BROWN), 0.71 (BLUE), 0.78 (FLUORESCENT BLUE), 0.81 (RED)	0.11 (BLUE), 0.28 (BLUE), 0.56 (BLUE), 0.60 (BLUE), 0.75 (VIOLET)

Thin Layer Chromatographic (TLC) Profiles of *Leucas aspera* (Willd.) Link (Whole Plant)



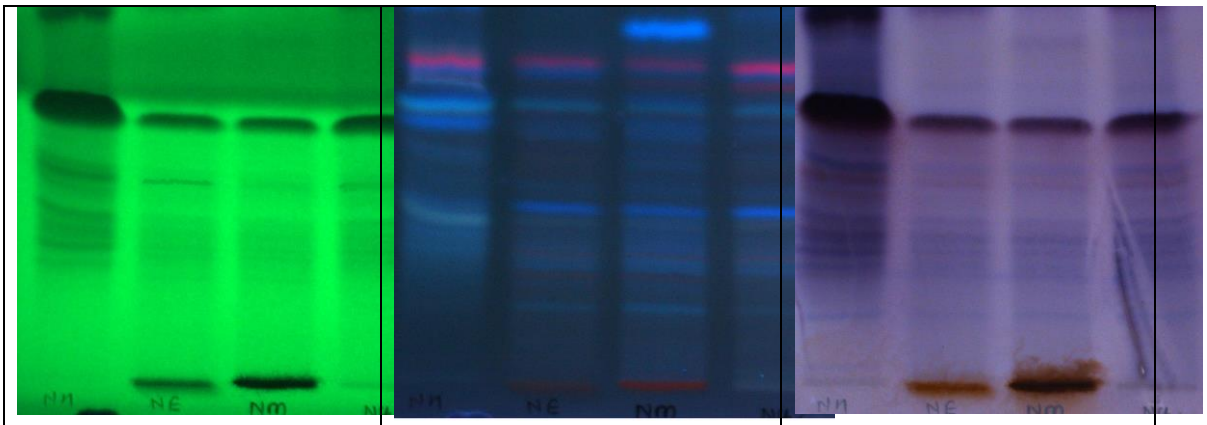
T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
254nm				**366nm**				**Derivatized**			

Figure - 29, TLC Profiles Profiles of *Leucas aspera* (Willd.) Link (Whole Plant)

Table - 22. R_f value of TLC Profiles of *Leucas aspera* (Willd.) Link

Extract tract	Retention factor (R_f)		
	254nm	**366nm**	**Derivatized**
T1 (LH)	0.09, 0.29, 0.35, 0.44	0.28 (ORANGE), 0.42 (BLUE), 0.46 (FLUORESCENT RED)	0.32(BROWN),0.46 (BROWN), 0.32 (BLUE), 0.32 (BLUE), 0.67 (VIOLET), 0.55 (BLUE), 0.76 (VIOLET)
T2 (LE)	0.08,0.34, 0.44	0.10 (BROWN), 0.12 (BLUE), 0.21 (RED), 0.28 (RED), 0.34 (ORANGE), 0.41 (BLUE), 0.45 (FLUORESCENT RED), 0.53 (BLUE)	0.06 (BLUE), 0.14 (BLUE), 0.22 (BLUE), 0.41 (BLUE), 0.56 (BLUE), 0.67 VOILET, 0.76 (VIOLET)
T3 (LM)	0.06,0.14, 0.19, 0.22, 0.27, 0.33, 0.43	0.01 (BROWN), 0.17 (RED), 0.21 (RED), 0.29 (RED), 0.34 (RED), 0.42 (BLUE), 0.47 (FLUORESCENT RED)	0.32(BROWN), 0.46 (BROWN), 0.32 (BLUE), 0.32 (BLUE), 0.67 (VIOLET), 0.55 (BLUE), 0.76 (VIOLET)
T4 (LCH)	0.07, 0.11, 0.11,0.17,0.21, 0.27, 0.27, 0.33, 0.44	0.09 (VIOLET), 0.16 (ORANGE), 0.23 (RED), 0.30 (RED), 0.37 (RED), 0.42 YELLOW, 0.46 (FLUORESCENT RED), 0.53 (BLUE)	0.72(BROWN), 0.66 (BROWN), 0.32 (BROWN), 0.32 (BLUE), 0.67 (VIOLET), 0.55 (BLUE), 0.76 (VIOLET)

Thin Layer Chromatographic (TLC) Profiles of *Mesua ferrea* L.(Stem Bark)



T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
254nm				366nm				Derivatized			

Figure-30, TLC Profiles of *Mesua ferrea* L. (Stem Bark)

Table-23. R_f value of TLC Profiles of *Mesua ferrea* L. (Stem Bark)

Extract tract	Retention factor (R_f)		
	254nm	366nm	Derivatized
T1 (NH)	0.31, 0.35, 0.41, 0.45, 0.55, 0.59, 0.70	0.43 (BLUE), 0.64 (DARK BLUE), 0.68 (GREEN), 0.75 (GREEN), 0.78 (BLUE), 0.78 (FLUORESCENT RED)	0.17 (BLUE), 0.30 (BLUE), 0.34 (VIOLET), 0.36 (BLUE), 0.38 (VIOLET), 0.42 (BLUE), 0.48 (ORANGE), 0.52 (VIOLET), 0.56 (BLUE), 0.66 (VIOLET), 0.81 (BLUE)
T2 (NE)	0.27, 0.41, 0.52, 0.68	0.20 (BLUE), 0.29 (BLUE), 0.43 (BLUE), 0.61 (BLUE), 0.69 (GREEN), 0.77 (BLUE), 0.80 (FLUORESCENT RED)	0.17 (BLUE), 0.27 (BLUE), 0.33 (BLUE), 0.37 (VIOLET), 0.42 (VIOLET), 0.51 (ORANGE), 0.55 (BLUE), 0.65 (VIOLET)
T3 (NM)	0.53, 0.67	0.19 (RED), 0.30 (BLUE), 0.40 (GREEN), 0.45 (BLUE), 0.55 (BLUE), 0.61 (BLUE), 0.68 (GREEN), 0.76 (BLUE), 0.79 (ORANGE), 0.87 (FLUORESCENT BLUE)	0.17 (BLUE), 0.31 (VIOLET), 0.37 (VIOLET), 0.40 (BLUE), 0.50 (ORANGE), 0.53 (BLUE), 0.66 (VIOLET)
T4 (NCH)	0.42, 0.44, 0.52, 0.65	0.19 (BLUE), 0.29 (LIGHT BLUE), 0.40 (BROWN), 0.43 (BLUE), 0.61 (BLUE), 0.68 (GREEN), 0.74 (RED), 0.78 (FLUORESCENT RED), 0.90 (BLUE)	0.18 (BLUE), 0.29 (BLUE), 0.34 (VIOLET), 0.38 (VIOLET), 0.40 (BLUE), 0.47 (ORANGE), 0.51 (ORANGE), 0.55 (BLUE), 0.68 (VIOLET), 0.75 (BLUE), 0.89 (BLUE)

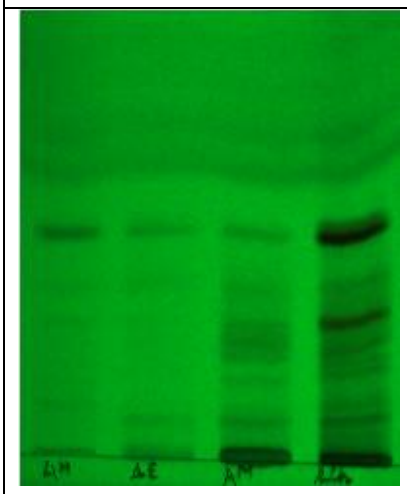
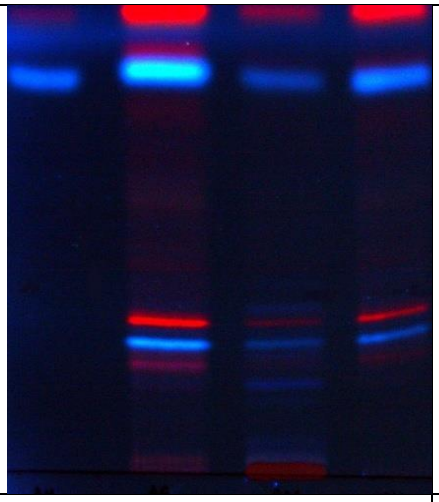
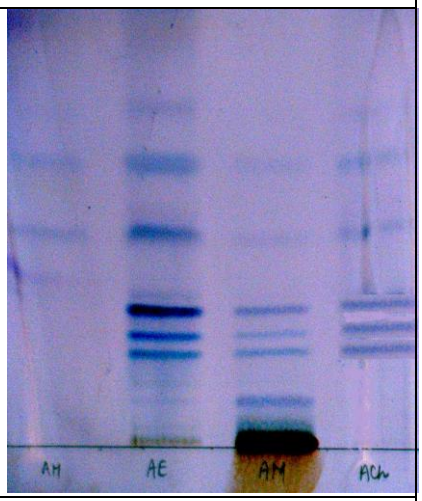
Thin Layer Chromatographic (TLC) Profiles of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark)		
		
T_1 T_2 T_3 T_4	**T_1 T_2 T_3 T_4**	**T_1 T_2 T_3 T_4**
254nm	**366nm**	**Derivatized**

Figure -31. TLC Profiles of *Terminalia arjuna (*Roxb. ex DC.) Wight & Arn. (Stem Bark)

Table-24. R_f value of TLC Profiles of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark)

Solvent tract	Retention factor (Rf)		
	254nm	366nm	Derivatized
T1 (AH)	0.0.88	0.0.81 (BLUE), 0.93 (RED)	0.82(BLUE), 0.91(VIOLET)
T2 (AE)	0.72, 0.88	0.23 (RED), 0.27 (FL. BLUE), 0.73 (RED), 0.83 (FL. BLUE), 0.86 (RED), 0.93 (FL. RED)	0.09(BLUE),0.13(VIOLET),0.32(BLUE),0.41(BLUE),0.51(BLUE),0.63(BLUE),0.79(BLUE),0.87(BLUE),0.98(BROWN)
T3 (AM)	0.18, 0.35, 0.74, 0.94	0.19 (BLUE), 0.28 (BLUE), 0.31 (RED), 0.81 (BLUE), 0.95 (RED),	0.08(BLUE),0.11(ORANGE),0.23(BLUE),0.62 (VIOLET), 0.77 (BLUE), 0.86 (BLUE),
T4 (ACH)	0.76, 0.92	0.24 (RED), 0.30 (BLUE), 0.34 (RED), 0.82 (FL. BLUE), 0.96(FL. RED)	0.11 (BLUE), 0.31 (BLUE), 0.40(BLUE),0.50 (VIOLET), 0.63(BLUE), 0.70 (VIOLET), 0.79 (BLUE), 0.85 (BLUE)

Visualization *Bauhinia variegata* L. (Flowers)

The plate was dried and visualized under UV 254nm and then at 366 nm and photo-documented using CAMAG Reprostar 3. R_f values were recorded at both wavelengths (figure- 27 & Table -20). Now developed TLC plate was sprayed with anisaldehyde-sulphuric acid reagent and heat at 105° C for 5-10 minutes. After derivatization, plate was visualized under white light and R_f values of each colour band was recorded (Figure 27 & Table -20).

Evaluation

1. **Track 1(T_1-n-Hexane extract)** – 0 bands under 254 nm, 3 bands under 366 nm and 5 bands under white light.
2. **Track 2(T_2- Ethyl acetate extract)** – 2 bands under 254 nm, 10 bands under 366 nm and 7 bands under white light.
3. **Track 3(T_3- Methanol extract)** – 4 bands under 254 nm, 11 bands under 366 nm and 09 bands under white light.
4. **Track 4(T_4- Chloroform extract)** – 03 bands under 254 nm, 10 bands under 366 nm and 08 bands under white light.

After comparative study of extracts it is concluded that maximum extraction of constituents are observed in methanol extracts as T_3 has maximum number of bands

Visualization *Bixa orellana* L. (Stem Bark)

The plate was dried and visualized under UV 254nm and then at 366 nm and photo-documented using CAMAG Reprostar 3. R_f values were recorded at both wavelengths (Figure 28 & Table 21). Now developed TLC plate was sprayed with anisaldehyde-sulphuric acid reagent and heat at 105° C for 5-10 minutes. After derivatization, plate was

visualized under white light and R_f values of each colour band was recorded (figure 28 & table 21).

Evaluation

1. **Track 1(T_1-n-Hexane extract)** – 4 bands under 254 nm, 6 bands under 366 nm and 6 bands under white light.
2. **Track 2(T_2- Ethyl acetate extract)** - 4 bands under 254 nm, 7 bands under 366 nm and 2 bands under white light.
3. **Track 3(T_3- Methanol extract)** – 4 bands under 254 nm, 7 bands under 366 nm and 3 bands under white light.
4. **Track 4(T_4- Chloroform extract)** – 4 bands under 254 nm, 9 bands under 366 nm 5 and bands under white light.

After comparative study of extracts it is concluded that maximum extraction of constituents are observed in Chloroform extract extracts as T_4 has maximum number of bands.

Visualization *Leucas aspera* (Willd.) Link (Whole Plant)

The plate was dried and visualized under UV 254nm and then at 366 nm and photo-documented using CAMAG Reprostar 3. R_f values were recorded at both wavelengths (Fig.-29 & Table-22). Now developed TLC plate was sprayed with anisaldehyde-sulphuric acid reagent and heat at 105° C for 5-10 minutes. After derivatization, plate was visualized under white light and R_f values of each colour band was recorded (Fig.-29 & Table-22).

Evaluation

1. **Track 1(T_1-n-Hexane extract)** - 4 bands under 254 nm, 3 bands under 366 nm and 7 bands under white light.
1. **Track 2(T_2- Ethyl acetate extract)** - 3 bands under 254 nm, 8 bands under 366 nm and 7 bands under white light.
2. **Track 3(T_3- Methanol extract)** - 7 bands under 254 nm, 7 bands under 366 nm and 7 bands under white light.
3. **Track 4(T_4- Chloroform extract)** – 9 bands under 254 nm, 8 bands under 366 nm and 7 bands under white light.

After comparative study of extracts it is concluded that maximum extraction of constituents are observed in methanol and chloroform extract extracts as T_4 and T_3 has maximum number of bands.

Visualization *Mesua ferrea* L. (Stem Bark)

The plate was dried and visualized under UV 254nm and then at 366 nm and photo-documented using CAMAG Reprostar 3. R_f values were recorded at both wavelengths (Fig-30& Table-23).Now developed TLC plate was sprayed with anisaldehyde-sulphuric acid reagent and heat at 105° C for 5-10 minutes. After derivatization, plate was visualized under white light and R_f values of each colour band was recorded (Fig- 30 & Table-23).

Evaluation

2. **Track 1(T_1-n-Hexane extract)** - 7 bands under 254 nm, 6 bands under 366 nm and 11 bands under white light.
3. **Track 2(T_2- Ethyl acetate extract)** - 4 bands under 254 nm, 7 bands under 366 nm and 8 bands under white light.
4. **Track 3(T_3- Methanol extract)** - 2 bands under 254 nm, 10 bands under 366 nm and 7 bands under white light.
5. **Track 4(T_4- Chloroform extract)** - 4 bands under 254 nm, 9 bands under 366 nm and 11 bands under white light.

After comparative study of extracts it is concluded that maximum extraction of constituents are observed in methanol and n- hexane extracts as T_1 and T_3 has maximum number of bands.

Visualization *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.(stem Bark)

The plate was dried and visualized under UV 254nm and then at 366 nm and photo-documented using CAMAG Reprostar 3. R_f values were recorded at both wavelengths (Fig.-31 & Table-24). Now developed TLC plate was sprayed with anisaldehyde-sulphuric acid reagent and heat at 105° C for 5-10 minutes. After derivatization, plate was visualized under white light and R_f values of each colour band was recorded (fig.-31 & table-24).

Evaluation

1. **Track 1(T_1-n-Hexane extract)** – 1 bands under 254 nm, 2 bands under 366 nm and 2 bands under white light.
2. **Track 2(T_2- Ethyl acetate extract)** – 2 bands under 254 nm, 6 bands under 366 nm and 9 bands under white light.
3. **Track 3(T_3- Methanol extract)** – 3 bands under 254 nm, 5 bands under 366 nm and 6 bands under white light.
4. **Track 4(T_4- Chloroform extract)** – 2 bands under 254 nm, 5 bands under 366 nm and 8 bands under white light.

After comparative study of extracts it is concluded that maximum extraction of constituents are observed in Ethyl acetate extract and Chloroform extract as T_2 and T_3 has maximum number of bands.

Modh *et al.,* (2011) HPTLC fingerprinting for flavonoids revealed presence of two flavonoids rutin and Kaempferol-3-glucoside in *Bauhinia variegata* (Flower). Chakraborty *et al.,* (2017) analyzed the TLC study of *Bixa orellana* seed and found 8 separated spots with different Rf values meaning that the sample contains a minimum of 8 different compounds in the plants sample. When the R_f values spots were compared with the R_f values standard substances, it indicate that the sample contains stearic acid, palmitic acid, oleic acid, linoleic acid, myristic acid, cholesterol but no linolenic acid. TLC of the sample shows more spot at the top of the solvent which indicates that the sample may contain some other components which are having different Rf values other than standards used.

Vellaichamy *et al.,* (2017) study chromatographic fingerprint analysis on flavonoids compounds in *Leucas aspera* (Willd.) Link leaf by HPTLC. Different R_f value of the extracts were found to be 0.61, 0.66, 0.67, 0.70, 0.76 and 0.77 of the peaks. Out of

10 spots 6 were confirmed as flavonoid compounds. Same value was found in present plant sample TLC of spot *Leucas aspera* (Willd.) Link it indicates the presences of flavonoid in the sample.

Rasheed *et al.,* (2017) reported that HPTLC analysis of various solvent extracts in which acetone extracts outcome are very significant and acetone extracts of *Terminalia arjuna* indicates that the sample has 8 spots at R_f values 0.04, 0.20, 0.28, 0.32, 0.37, 0.51, 0.81, 0.89 where as the reference market sample has shown 9 spots at R_f values 0.02, 0.11, 0.20, 0.29, 0.33, 0.39, 0.52, 0.81, 0.90. both sample exactly coinciding with the Rf values resulting authenticity of the sample.

Beena *et al.,* (2014) reported that R_f values and colour spots in UV-L light for *Mesua ferrea* bark giving 6 bands with different R_f value is characteristic for a given stationary phase and solvent combination and conformation for the presence of 6 chemical compound. In our result *Mesua ferrea* of TLC band reported so many spot with different R_f values it indicates that each colour band represents different chemical constituent which may or may not have similar characteristics in the plant sample. Comparative TLC of similar part of plant sample in same extracts and solvent system may be used for authentication of samples in future, if the tested samples have same bands with same R_f values, then the sample is said to be authentic.

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may serve as a characteristic fingerprint for qualitative evaluation of plant samples. In another hand

phytochemical screening and florescent study also revealed the presence of particular chemical compound in plant species.

Phytochemistry (TLC) study of five medicinal plants sample study reveals that on the basis of TLC spectrum or band spot observed in different solvent with various Rf value may indicate presence of bioactive compound which is directly or indirectly helpful for human health care. This will also help in correct identification of raw drugs as a reference on the basis of their TLC profile.

Conclusion

The Indian indigenous system of medicine is purely based on medicinal plants for the healthcare management. The health benefits on the use of these folk medicine and medicinal plants used among the folk healers by Nyishi tribe is quite significant for their folk healing practices which have been practicing from generation to generation.

On the basis of survey in Papum Pare area and interaction with different folk healers through questionnaires and interview, a total of 38 medicinal plants were recorded (Table-2). These medicinal plants are helpful in various kinds of disease and nutritional supplement as per information given by folk healers.

These ethnic medicinal plants are organic, naturally grown and contains nutraceutical supplements. From the present study of five medicinal plants *viz. Bauhinia variegata* L. (Flower), *Leucas aspera* (Willd.) Link (whole plant), *Bixa orellana* L., *Mesua ferrea* L. *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Stem Bark) clearly supports the folk healing practice, knowledge and system of "Medicinal plants as medicine as well as food" among Nyishi tribe people. The nutraceutical importance of medicinal plants is observed and scientifically found to be significant.

The micro morphological study of powder sample from these plants showed the presence of adequate amounts of proximate in the studied medicinal plants. Considerable amount of total phenolic and flavonoid contents in these medicinal plants is a good indication that the folk medicinal plant can be further investigated to explore the antioxidant ingredients. There are many medicinal plants available in the forest used by the tribals in their daily life and it is expected that these edible plants will have some health benefits to human mankind. Therefore, there is large scope to explore them

scientifically and use commercially for the sustainable development.

Phytochemical analysis on carbohydrate, protein, fat, fibre, alkaloids, flavonoids, glycosides, phenols, volatile oil, and saponins showed that the folk medicinal plants also contain nutritionally valuable compounds. This could be the reason why many of the plants in the survey were also found to be used as vegetable. Hence, there is also an avenue to explore the wild vegetables used by the Nyishi tribe.

The additional information from present study on Powder Microscopy, TLC, Physicochemical study of *Bixa orellana* L. (Stem Bark), *Mesua ferrea* (Stem bark), *Bauhinia variegata* L. (flower) has not been listed in Ayurvedic Pharmacopeia of India (API) till date. The results may be taken as reference for researcher. It will provide as a base reference for conducting further research.

From the present study, it is evident that the tribal people do have rich knowledge about the use of botanical natural resources available to them in the study area. Their age old knowledge about the plant resources for various purposes is highly appreciable which is also supported from the laboratory results of this study. This data of medicinal plants will be helpful for the National Medicinal Plant Board (NMPB) for developing database on medicinal plants and folk practices. Nyishi tribe which is one of the major tribes in Arunachal Pradesh have rich folk healing practices and were found to be very systematic and scientific.

References

Abitogun, A. S. (2010). Nutritional and chemical compositions of ripe and unripe *Vitex gladifolia*. *Journal of Research in National Development;* 8(1): 1-3.

Addis, G., Asfaw, Z., Singh, V., Woldu, Z., Baidu-Forson, J. J. & Bhattacharya, S. (2013). Dietary Values of wild and semi-wild edible plants in Southern Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development;* 13 (2): 7485-7503.

Ahmad, M., Khan, M.A., Zafar, M., Arshad, M, Sultana, S., Abbasi, B.H. (2010). Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines. *J Med Plant Res*.; 4:1244–52.

Altieri, M. A., Anderson, M. K. & Merrick, L. C. (1987). Peasant Agriculture and the Conservation of Crop and Wild Plant Resources. *Conservation Biology*; 1 (1): 49- 58.

Anokwuru, C.P, Esiaba, I, Ajibaye O, Adesuyi AO. (2011). Polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx. *Res J Med Plant.* 5: 557–566.

Anonymous, (2010). The Ayurvedic Pharmacopoeia of India, CCRAS, Department of AYUSH, M/o Health & Family Welfare, Government of India,; Part-II, Vol. III.

Anonymous. (2010). The Ayurvedic Pharmacopoeia of India, CCRAS, Department of AYUSH, M/o Health & Family Welfare, Government of India, Part-I, Vol. II.

Aoshima, H., Tsunoue, H., Koda, H., & Kiso, Y. (2004). Ageing of whiskey increases 1, 1-diphenyl-2-picryl hydrozyl redical scavenging activity. *J. Agr. Food Chem;* 52(16):5240-5244.

Arunkumar, S. (2009). Muthuselvam, Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens, *World J. Agril., Sc.;* 5(5), 572-576.

Attanayake, A. P. & Jayatilaka. K. A. P. W. (2016). Evaluation of antioxidant properties of 20 medicinal plant extracts traditionally used in Ayurvedic medicine in Sri Lanka. *Indian Journal of Traditional Knowledge*; 15 (1):50-56.

Ayyanar, M., & Ignacimuthu, S. S. (2013). Plants as Food and Medicine: An Ethnobotanical Survey among Kanikaran Community in Southern India. *European Journal of Nutrition & Food Safety;* 3 (4): 123-133.

Babu, K., Gokal S. S., & Rai, S. (2010). Comparative pharmacognostic studies on the bark of four *ficus species. Turk. J. Bot*; 34: 215-224.

Balunas, M. J. & Kinghom, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences*; 78 (5): 431-41.

Balunas, M. J. & Kinghom, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences*; 78 (5): 431-41.

Baskar, V., Murugamani, V., Anusha. & Indraja, C. H. N. (2012): Preliminary Pharmacognostical Evaluation of *Givotia moluccana* leaves. *International Journal of Pharmacognosy and Phytochemical Research;* 4(2): 74-78.

Beena, C., Kanakamany, T., Sindhu, P.V. (2014). Chemical investigation on the stem bark of *Mesua ferrea* Linn., an ethno medicinally important plant. *life Sciences International Research Journal* .; 1(1) 118-120.

Bhalla, S. & Chand, T. (2007). Traditional Foods and Bevarages of Himachal Pradesh. *Indian Journal of Traditional Knowledge;* 6 (1). 17-24.

Bharali, P., Sharma, M., Sharma, C. L. & Singh, B. (2017). Ethnobotanical survey of spices and condiments used by some tribes of Arunachal Pradesh. *Journal of Medicinal Plants Studies*; 5(1): 101-109.

Bhattachaijee, P. (1977. Flora of Mebo. *Arunachal News;* 4 (2): 5-8.

Binu, S. (2010). Wild edible plants used by the tribals in Pathanamthitta district, Kerela. *Indian Journal of Traditional Knowledge;* 9 (2): 309-312.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature;* 181: 1199-1200.

Bose, N. M. F. J., Aron, S., & Mehalingam, P. (2014). An ethnobotanical study of medicinal plants used by the Paliyars aboriginal community in Virudhunagar district, Tamil Nadu, India. *Indian Journal of Traditional Knowledge,* 13(3): 613-618.

Bourguad, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: A historical perspective. *Plant Science*; 161: 839-851.

Buchanan, B. B., Grissem, W., & Jones, R.L. (2000). Biochemistry and molecular biology of plants, *I.K.International Pvt Ltd*.; 1250-1309.

Burchifield, R.W. (1972). *A supplement to the Oxford English dictionary. Vol. I.* Oxford University Press; 980.

Calapai, G. (2008). European legislation on herbal medicines: A look into the future. *Drug Saf*. 2008:31: 428-431.

Campbell, D. & Burgess, C. P. (2011). Potential primary health care savings for chronic disease care associated with Australian Aboriginal involvement in land management. *Health Policy*; 99:83–89.

Chaitanya, K. K., Rao, K. K., Sastry, Y. N., Padal, S. B., Rajyalakshmi, A. & Rao, D. G. (2015). Anti-Inflammatory, Antioxidant and Phytochemical Analysis of *Mesua ferrea* bark extracts. *IJCTP* 3(3): 891–902.

Chakraborty, T, & Das M. K. (2017). Oil of *Mesua ferrea* L. Seed as a Promising Pharmaceutical Excipient in Lipid Based Nanoformulation. *Journal of Applied Pharmaceutical Science* ; 7 (07):133-141

Chakraborty, T., Saha, S., Bisht, N. S. (1998). First Report on the Ethnopharmacological Uses of Medicinal Plants by Monpa Tribe from the Zemithang Region of Arunachal Pradesh, Eastern Himalayas and India. *Plants;* 6(1): 13.

Cho, H.Y. & Kleeberger, S. R. (2007). Genetic mechanism of susceptibility to oxidative lung injury in mice. *Radical Biology and Medicine;* 42(4): 433-445.

Chopra, R. N., Chopra, I. C., Handa, K. L. & Kapur, L. D. (1958). *Indigenous Drugs of India,* 2nd edn. UN Dhar and Sons Pvt. Ltd, Kolkata.

Chopra, S. L. & Kanwar, J. S. (1991). In Analytical Agricultural Chemistry, Kalyani Publications, New Delhi (4): 297.

Cordell, G. A (1995). Changing strategies in natural products chemistry. *Phytochemistry;* 40: 1585-1612.

Cragg, G. M. & Newman, D. J. (2002). Drugs from nature: Past achievement, future prospects. Advances in Phytomedicine Vol.I. In. Iwu and Wooton (edn.) *Ethnomedicine and drug discovery,* Elseviers: 23-24.

Cragg, G. M. & Newman, D.J. (2001). *Drugs from nature: Past achievement, future prospects. Advances in Phytomedicine Vol.I.* In. Iwu and Wooton (edn.) *Ethnomedicine and drug discovery,* Elseviers; 23-24.

Das, K., Das, B., Arpita, F. K., Morshed, M. A., Uddin, A., Bhattacherjee, R. & Hannan J.M.A. (2011). Phytochemical screening and antioxidant activity of *Leucas aspera*. *IJPSR;* 2(7): 1746-1752.

De Pasquale, A. (1984). Pharmacognosy: The oldest modem science. *Journal of Ethnopharmacology;* Vol.11 (1): 1-16.

De Pasquale, A. (1984). Pharmacognosy: The oldest modem science. *Journal of Ethnopharmacology;* 11(1): 1-16.

Deshmukh, S & Rathod, V. (2013). Nutritional evaluation of some wild edible tuberous plants. *Asian Journal of Pharmaceutical and Clinical Research;* 6 (2): 58-60.

Deswal, G. & Arora, K. (2015). Ethnobotany and phytopharmacology of *Bauhinia variegata, Int J. Pharm. Drug. Anal*.; 3(9):261-263.

Dhami, N. (2013). Trends in pharmacognosy: A modem science of natural medicines. *Journal of Herbal Medicines;* 3 (4): 123-131.

Dharamsi, S, S. (2009). Quantitative and qualitative research: received and inter pretivist views of science. *Can Fam Physician*; 55(8):843-4.

Edoga, H.O. Okwu D.E. Mbaebie, B.O. (2005). Phytochemicals constituents of some Nigerian medicinal plants, *Afr. J. Biotechnol*.; 4(7), 685-688.

Emeka, I.N. & Elizabeth, E.E. (2009). Justification for the use of *Ocimum gratissimum* L. in herbal medicine and its interaction with disc antibiotics.

Emran, T. B. Md. Rahman, A., Zahid Hosen, S. M., Saha, D. & Dey, T. K. (2012). Antioxidant property of ethanolic extract of *Leucas aspera* Linn. *Bulletin of Pharmaceutical Research*; 2(1):46-9.*Ethnomusicology,* 9: 39-42.

Fabricant, D. S., Farnsworth, N. R (2001). The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect;*109 (Suppl. 1): 69-75.

Fabricant, D. S., Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect;* 109 (Suppl. 1): 69-75.

Flyman, M.V., & Afolayan, A. J. (2006). The Suitability of wild vegetables for alleviating human dietary deficiencies. *South African Journal of Botany*; 72: 492-497.

Ford. R. Inter (1978). The Nature and Status of Ethnobotany. Mus. Anthrop; University of Machigan.

Geissman, T. A. (1963). Flavonoid compounds, tannins, lignins and related compounds. In M. Florkin and E. H. Stotz (ed.). Pyrrole pigments, isoprenoid compounds and phenolic plant constituents. *Elsevier. New York;* 9: 265.

Giorgi, A., Pietro De, M., Giuseppe, G., Chiesa L. M., & Panseri, S. (2013). "Secondary Metabolite Profile, Antioxidant Capacity, and Mosquito Repellent Activity of *Bixa orellana* from Brazilian Amazon Region," *Journal of Chemistry*; 1-10.

Gokhale, S. B and Kokate, C. K (2011): *Practical Pharmacognosy*. Nirala Prakashan, Pune; 411002.

Gopalkrishnan, B. & Shimpi, S. N. (2012). Pharmacognostical studies on stem bark of *Madhuca longifolia* (Koen.) Macbr. var *latifolia* (Roxb.) A. Cheval. *Indian Journal of Natural Products and Resources;* 3(2): 232-236.

Griffith, W. (1836). *Journey of a trip to the Mishmi Mountains.* In Elwin (edn.) *India's North-East Frontier in the nineteenth century,* (3rd ed. 1972). Oxford University Press: pp 313.

Gupta, P. (2014). Healing Traditions of the Northwestern Himalayas. *Springer Briefs in Environmental Science;* 21-35.

Gurib-Fakim, A. (2006). Medicinal Plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*; 27: 1-93.

Gurib-Fakim, A. (2006). Medicinal Plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*; 27: 1-93.

Hadjichambis, A. C. H., Paraskeva, D., Della, A., Giusti, M., De Pasquale, A., Lenzarini, C., Censorii, E., Gonzales-Tejero, M. R., Sanchez-Rojas, C. P., Ramiro- Gutierrez, I , Skoula, M., Johnson, C. H., Sarpakia, A., Hmomouchi, M., Jorhi, S., El-Demerdash, M., El-Zayat, M., & Pioroni, A. (2008). Wild and semi-domesticated food plant consumption in seven circum-Mediterranean areas. *Int J Food Sci Nutr;* 59:383-414.

Hahn N. I. (1998). Is Phytoestrogens Nature's Cure for What Ails Us? A Look at the Research. *Journal of the American Dietetic Association*; 98: 974- 976.

Halliwell, B., & Gutteridge, J. C. (1995). The definition and measurement of antioxidants in biological systems. *Free Radical Biol Med*; 18:125–6.

Harbome, J. B. (1999). *Phytochemical Methods,* Chapmann and Hall Ltd. London: 60-66.

Harbome, J. B. (2009). *Phytochemical Methods- A guide to modern techniques of plant analysis,* Springer (India) Private limited; New Delhi.

Haridasan, K & Bhuyan R. L. (2002). *Medicinal Plants distribution and farming potential in Arunachal Pradesh.* SBRDT Workshop cum Trai. Bull. SFRI, Itanagar.

Haridasan, K. (2001). Ethnobotanical Studies in Arunachal Pradesh-A status report. Ethnomedicine of North East India. *National institute of science Communication and Information Resources;* CSIR New Delhi. 154-168.

Hasler C. M, & Blumberg J. B. (1999) Symposium on Phytochemicals: Biochemistry and Physiology. *Journal of Nutrition*; 129: 756S-757S.

Heimler, D., Isolani, L., Vignolini, P., Tombelli, S. & Romani, A. (2009). Polyphenol content and antiradical activity of *Cichorium intybus* L. from biodynamic and conventional farming. *Food Chemistry;* 114: 765-770.

Heinrich, M. (2005). Undserstanding local Mediterranean diets: A multidisciplinary pharmacological and ethnobotanical approach. *Pharmacological Research;* 52: 353- 366.

Heinrich, M., Barnes, J., Gibbons, S., & Williamson, E. M. (2004): Fundamentals of Pharmacognosy and Phytotheraphy. Churchill Livingstone Publication, Edinburgh.

Hemmalakshmi, S., Priyanga, S., Vidya, B., Gopalakrishnan, V. K., & Devaki, K. (2016). Screening of the Antioxidant Potential of the Leaves and Flowers Extract of *Erythrina variegata* L. : A Comparative Study. *Int. J. Pharm. Sci. Rev. Res*.; 40(2): 186-191.

Hooker, J. D. (1872-97). *Flora of British India.* Vol. I -VII (Rep. 1973). Bishen Singh Mahendra Pal Singh, Dehradun.

Hooker, J. D. (1894). In: *Flora of British India.* L Reeve and Co., Ltd, The Oast Houde, Brook, Ashford Kent, Vol. VI.: pp 341.

Hyun, D.H., Hernandez, J.O., Mattson, M.P., & de Cabo, R (2006). The plasma membrane redox system in ageing. *Ageing Research Reviews*; 5(2): 209-220.

Inti, M. & Faoro, F. (2006). Grape phytochemicals: a bouquet of old and new nutraceuticals for human health Medical Hypotheses. 67: 833-838.

Isaac, K. A., Abraham, Y. M., Antonia, O., Merlin, L. M., & Jato J. (2014). Pharmacognostic standardisation of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae) *Asian Pac J Trop Biomed*; 4(12): 941-946.

Iswaran, V. (1980). A Laboratory Handbook for Agricultural Analysis, *Today and Tomorrow's Printers and Publishers*, New Delhi.

Jain, C. P., Dashara, A, Garg, R., Kataria, U. & Vashistha, B.(2008). Animal selfmedication through natural resources. *Natural Product Radiance;* 7: 49-53.

Jain, S. K (1976). Plants in India Medicine and Folklore associated with healing of bones. *Indian Journ. Orthop;* 1: 95-104.

Jain, S. K. & C. R. Tarafder, C.R. (1970). Medicinal Plant-Lore of the Santals: (A Revival of P. 0. Bodding's Work). *Economic Botany;*24 (3): 241-278.

Jain, S. K. & Rao, R. R. (1977). *Handbook of Field and Herabarium Methods* `New Delhi.

Jain, S. K. (1963a). Studies in Indian Ethnobatany-Plants used in medicine by the tribes of Madhya Pradesh. *Bull. Region. Res. Lab. Jammu;* (1&2): 126-128.

Jain, S. K. (1963b). Wild Edible plants of Bastar. *Procec. Indian Science Cong.* 3:446.

Jain, S. K. (1963c) Studies in Indian Ethnobotany-Less Known uses of Forfty plants from the tribal areas of Madya Pradesh. *Bull. Bot. Surv. India;* 5: 223-226.

Jain, S. K. (1963d). Observation on Ethnobotany of the tribals of Madhya Pradesh.*Vanyajati;* 11:177-183.

Jain, S. K. (1964). The Role of Botanist in Folklore Research. *Folklore;* 5(4): 126-128.

Jain, S. K. (1964). Wild plants food of the tribals of Baster (M.P) *India. Proc. Nat.Inst. Sci. India,* 30 B: 56-80.

Jain, S. K. (1965a). Wooden Musical Instruments of the gonds of central India.

Jain, S. K. (1965b). Medicinal Plants lore of the tribals of Bastar. *Eco. Bot;* 19: 236-250.

Jain, S. K. (1967). Ethnobotany, Its Scope and Study. *Indian Mus;* 2: 39-43.

Jain, S. K. (1967). Ethnobotany, Its Scope and Study. *Indian Mus;* 2: 39-43.

Jain, S. K. (1981a). *Observation on Ethnobotany of the Tribals of central India.* In S.K Jain (eds.) *Glimpses of Indian Ethnobotany* Oxford IBH Publ. Co. New Delhi. 13-36.

Jain, S. K. (1981b). *Glimpses of Indian ethnobotany.* Oxford and IBM Publ. Co. New Delhi.

Jain, S. K. (1987). *A Manual of Ethnobotany.* Scientific Publications; Jodhpur.

Jain, S. K. (1989). *Methods and Approaches in Ethnobotany.* Society of Ethnobotanists; Lucknow.

Jain, S. K. (1991). *Dictionary of Indian Folk-medicine and Ethnobotany.* Deep Publication, New Delhi.

Jain, S. K. (1991). Medicinal Plants among the certain Adivasis in India. *Bull. Bot. Surv. India;* 15: 85-91.

Jain, S. K. (1996): *Ethnobotany in Human Welfare.* Deep Publ. New Delhi.

Jain, S.K. (Edn. 1989). *Methods and Approaches in Ethnobotany.* Society of Ethnobotanist, Lucknow.

Jain. S. K. & Borthakur, S.K. (1980). Ethnobotany of the Mikirs of India. *Economic Botany;* 34(3): 264-272.

Jayanthy, A., Sulaiman, C. T. & Rema Shree, A. B. (2011). Pharmacognostical and Phdytochemical Comparison of Roots of *Solanum* species Used in Ayurvedi Formulations. International Journal of Pharmacognosy and Phytochemical Research; 4(1): 28-37.

John, A. S., Kuruvilla, G. R., Krishnamurthy, K. V., Nagarajan, M. P. & Venkatasubramanian. (2013). Pharmacognostic and phytochemical studies on Ayurvedic drugs Ativisha and Musta. *Brazilian Journal of Pharmacognosy;* 23(3):398-409.

Joseph, J. (1977). Quest of Medicinal Plants and Re-establishment of their medicinal Virtues. *Cult. & Utilization of Mech and Arom. Plants:* 454-461.

Kala, C. P. (2005). Ethnomedicinal botany of the Apatani in the Eastern Himalayan Region of India: *Journal of Ethnobiology and Ethnomedicine:* 1:11.

Kanjilal, U.N. (1934-40). *Flora of Assam:* Vol. I-IV, Omsons Publications, New Delhi.

Kaplan, M. N. (2007). Interpreting people as they interpret themselves: narrative in medical anthropology and family medicine. *Can Fam Physician*.; 53:1268–9.

Kar, A. (2004). Common Wild Vegetables of Aka Tribe of Arunachal Pradesh. *Indian Journal of Traditional Knowledge.;* 3 (3). 305-313.

Kaul, K. N. & Haridasan, K. (1987). Forest types of Arunachal Pradesh: A preliminary study. *Journal of Eco. Tax. Bot.;* 9 (2):379-389.

Kaur, R., Jaiswal, M. L., & Jain, V. (2012). Preliminary Pharmacognostical & Phytochemical investigation of Bark & Leaves of *Lannea coromandelica* Houtt. Merrill. *International Journal of Pharmacognosy and Phytochemical Research;* 4 (3): 82-88.

Kinghom, A. D. (2001). Pharmacognosy in the 21st century. *Journal of Pharmacy and Pharmacology;* 53(2): 135-148.

Kinnula, V. L & Crapo, J. D (2004). Superoxide dismutase in malignant cells of human tumours. *Free Radical Biology and Medicine;* 36(6). 718-744.

Kokate, C. K, Purohit, A. P., Gokhale, S. B (2012). *Pharmacognosy* (47th edn.). 2011; I, Nirala Prakshan; Pune.; 6.22.

Kripa, K. G, R. & Chamundeeswari, S. D. (2016). Pharmacognostical and physicochemical evaluation of the plant *Leucas aspera. Asian J Pharm Clin Res*; 9(2), 263-268.

Krishnaiah, D., Sarbatly, R., & Nithyanandam, R (2011). A review of the antioxidant Potential of medicinal plant species. *Food and Bioproducts Processing;* 89(3): 217-233.

Kulshrestha P. K., Mishra, A. K., Pal, Vishesh, K., Pandey, S., Tripathi, D., & Yadav, P. (2011). Antimicrobial activity of *Bahunia variegata* L. Flower extract (methanolic) *Asian J Pharm Clin Res.;*4, Suppl 1: 46-47.

Kumar, S., Tripathy, P. K. & Jena, P. K. (2012). Ethnobotany and bioactive compounds in leaf of *Bixa orellana* L. and its toxicity to *Artemia salina* L. *Plant Science Research*; 34 (1&2): 93-96, 2012.

Kumari, M,, Gupta, S., & Prakash, A. (2004). Iron bioavailability in green leafy vegetables cooked in different utensils. *Food Chemistry;* 86(2): 217-222.

Kunwar, R. M., Mahat, L., Acharya, R. P., & Bussmann, R. W. (2013). Medicinal plants, traditional medicine, markets and management in far-west Nepal. *Journal of Ethnobiology and Ethnomedicine;* 9:24.

Kunwar, R.M. & Adhikari, N. (2005). Ethnomedicine of dolpa district Nepal: The plants their vernacular names and uses. *Lyonia*., 8: 43-49.l (54): 176-186.

Liaw, Zhi Xian (2012) Nutritional composition of edible plant Bauhinia Variegata. *School of Food Science and Nutrition*. 2014

Like, R.C., & Steiner, R.P. (1986). Medical anthropology and the family physician. *Fam Med.* 18(2):87-92.

Liya H., Zhiyong, G., Huang, K., Shanjun W., Liu, B., Shaowu, M. & Chunlin L. (2015). Ethnobotanical study on medicinal plants used by Maonan people in China. *Journal of Ethnobiology and Ethnomedicine* ;11-32.

Lu, J. M., Lin, P .H., Yao, Q., & Chen, C., (2010). Chemical and molecular mechanisms of Antioxidants: Experimental approaches and model systems, *J Cell Mol Med (Berl)*; 14: 840-860.

Maheshwari, J. K. (1993). *Ethnobotany of India.* Sc. Publ. Jodhpur.

Maheswari, J. K. (1987). *Interdiscplinary approaches in Ethnobotany.* In S.K Jain (ed). *A manual of Ethnobotany.* Sci Publ., Jodhpur: 23-32.

Marimuthu, K. & Dhanalakshmi, R. (2014). A Study on Phytochemicals in *Bauhinia purpurea* l. Leaf and Flower. *Int. J. Pharm. Sci. Rev. Res*.; 29(2), 72-76.

Mbaebe, B.O., Edeoga, H.O., & Afolayan, A. J. (2012). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pac J Trop Biomed*; 2(2):118–124.

McBumey, R. P. Griffin, C., Paul, A. A., & Greenberg, D. C. (2004). The nutritional composition of African wild food plants: From compilation to utilization. ***J*** *Food Comp Anal;* 17: 277-289.

Meenakshi, S., Umayaparvathi, S., Arumugam, M., & Balasubramanian, T., (2011). In vitro antioxidant properties of FTIR analysis of two sea weeds of Gulf of Mannar. *Asian Pac J Trop Biomed*; 1(Suppl 1):S66–S70.

Misra, S. &. M. K. (2014). Nutritional evaluation of some leafy vegetable used by the tribal and rural people of south Odisha, *India J. Nat. Prod. Plant Resour*.; 4 (1):23-28.

Moa, M., Zemede, A., Ensermu, K., Abebe, B., & Bizuneh, W. (2015). An ethnobotanical study of medicinal plants in Wayu Tuka District, East Welega Zone. *Journal of Ethnobiology and Ethnomedicine* ; 11:32 - 34.

Modh, K. M., Parmar, P. T., Panigrahi, B., Singh, I. A., & Patel, C. N. (2011). Pharmacognostical and Phytochemical Evaluation of Leaves of *Bauhinia variegata* Linn. *Pharmacognosy Journal;* 3(24).

Mohammed, M. I. & Sharrif, N. (2011). Mineral composition of some leafy vegetables consumed in Kano, Nigeria. *Nigerian Journal of Basic and Applied Science*; 19 (2):208-211.

Mohan, V. R. & Kalidass, C. (2010). Nutritional and antinutritional evaluation of unconventional wild edible plants *Tropical and Subtropical Agroecosystems;* 12: 495- 506.

Mukheeije, P. K. (2001). Evaluation of Indian Traditional Medicine. *Drug biformation Journal;* 35: 623-632.

Mukheeije, P. K. (2001). Evaluation of Indian Traditional Medicine. *Drug biformation Journal;* 35: 623-632.

Mukheijee, B. (1953). *The Indian Pharmaceutical codex, Vol. I, Indigenous Drugs.* Council of Scientific and Industrial Research; New Delhi.

Mukheijee, G. N. (1974). *History of Indian Medicines.* In: Chaudhuri & Sarkars (edn.) *Mega-diversity conservation-flora, fauna and medicinal plants of Indian hotspots.* Daya Publishing House, New Delhi: 222-224.

Murtem, G & Das, A. K (2005). Traditional medicinal plants of Nyishi tribes of Arunachal Pradesh. *Arunachal Forest, News;* 21 (1&2):3M3.

Murtem, G. & Chaudhry, P. (2016). An Ethnobotanical Study of Medicinal Plants Used by the Tribes in Upper Subansiri District of Arunachal Pradesh, India. *American Journal of Ethnomedicine*; 3: 35-49.

Murtem, G. (2005). *Ethnobotanical Studies in Upper and Lower Subansiri Districts of Arunachal Pradesh.* PhD thesis submitted to Rajiv Gandhi University, (formerly Arunachal University), Unpublished.

Nagarasan, S., (2016). Perspective Pharmacological Activities of *Leucas aspera*: An Indigenous Plant Species. *Indo American Journal of Pharmaceutical Research*. 6(09).

Namsa, N. D., Manabendra, M., Sumpam, T. & Subhash C. M. Ethnobotany of the Monpa ethnic group at Arunachal Pradesh, India. Journal of Ethnobiology and Ethnomedicine 2011, 7:31.

Newman, D. J., Cragg, G, M., Snader, & K. M (2000). The influence of natural products upon drug discovery. *Natural Product Reports;* 17(3):215-34

Ni, M. (1995). *The Yellow Emperor's Classic of Medicine.* A New Translation of the Neijing Suwen with Commentary Shambhala Publications, Boston, MA. *Official methods of analysis* (1990): Association of Official Analytical Chemists 16th ed., Washington DC, U.S.A.

Pereira, C., Barros, L., Carvalho, A. M. & Ferreira, I.C.F.R. (2011). Nutritional Composition and bioactive properties of commonly consumed wild greens. Potential sources for new trends in modern diets. *Food Research International;* 44: 2634-2649.

Pharmacognosy and Phytotheraphy. Churchill Livingstone Publication, Edinburgh.

Philipso, J. D. (1995). A matter of some sensitivity. *Phytochemistry*; 38: 1319-1343.302.

Phillipson, J. D. (2007). Phytochemistry and Pharmacognosy. *Phytochemistry;* 68: 2960-2972.

Pope, C. (2005). Conducting ethnography in medical settings. *Med Educ*.; 39(12):1180–7.

Pridham J.B. (1960). Phenolics in Plants in Health and Disease, *Pergamon Press*, New York; 34-35.

Prior, R. L. & Cao, G.H. (2000). Analysis of botanical and dietary supplements for antioxidant capacity. *Journal of AO AC International;* 83 (2000): 950-956.

Rajasab, A. H. & Ishaq, M. (2004). Documentation of folk knowledge on edible wild plants of North Karnataka. *Indian Journal of Traditional Knowledge;* 3 (4):4I9- 429.

Raman, N. (2008). *Phytochemical Techniques*, New India Publishing Agency.

Rao, R. S. & Joseph, J. (1965). Observation on the flora of Siang Division, NEFA. *Bull. Bot. Surv. India*; 7: 138-161.

Rao, R. S. (1972). Botanical works in Arunachal Pradesh. *Arun Prad Bull.* (Independence silver Jubilee Issue): 49-76.

Rasheed, N.M.A., Shareef, M.A., Rehana, A., Waheed, M.A., Arfin, S. & Aminuddin. (2014). Phytochemical and HPTLC fingerprint analysis of *Terminalia arjuna* (Roxb.) Wight & Arn. in different solvent extracts *Indo American Journal of Pharmaceutical Research,*; 4(1), 457-463.

Ravi Subbiah, M.T. (2007). Nutrigenetics and nutraceuticals: the next wave riding on personalized medicine. *Translational Research*. 149: 55-61 (2007).

Rawat, M. S. & Shankar, R. (2005). Folk medicinal plants in Arunachal Pradesh. *Arunachal Forest News;* 21 (l&2):59-64.

Re Roberta, N., Pellegrini, A., Proteggente, A., Pannala, M. Yang & C. Rice-Evans (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med,* 26: 1231-1237.

Ribnicky, D. M., Poulev, A., Schmidt, B., Cefalu, W. T., & Raskin, I. The science of botanical supplements for human health: A view from the NIH botanical research centers: Evaluation of botanicals for improving human health. *Am J Clin Nutr.;* 2008:87:472S-475S.

Rice-Evans, C. & Miller, N. J. (1994). Total antioxidant status in plasma and body fluids. *Methods in Enzymology;* 234:279-93.

Rice-Evans, C.A., Miller, N.J., Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med,* 20:933-56.

Sahoo, N., Manchikanti, P., & Dey, S (2010). Herbal drugs: Standards and Regulations. *Fitoterapia*; 81: 462-471.

Sahreen, S. M. K. & Khan, R. A. (2010). Evaluation of antioxidant activities of various solvent extracts of Carisa apaca fruits. *Food Chem;* 122:1205-1211.

Saikat, S. & Chakraborty, R. (2016). Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future, *J Tradit Complement Med.*; 7(2): 234–244.

Sawhney, S. S., Amin, M. M., & Kumar S. (2012). Phytochemical Screening and Antioxidant properties of Bauhinia variegate Int. *J. Res. Phytochem. Pharmacol*., 2(1), 21-24.

Sayeed, M. A., Abbas, A. M., Sohel, F. I., Khan, G. R. M. & Yeasmin, S. (2004). Physico-chemical characteristics of *Mesua ferrea* seed oil and nutritional composition of its seed and leaves Bull. *Chem. Soc. Ethiop*.; 18(2), 157-166.

Schmidt, B., Ribnicky D. M., Poulev, A., Logendra, S., Cefalu, W. T, & Raskin I. (2008). A natural history of botanical therapeutics. *Metabolism*; 2008:57: S39.

Schultes, R. E. (1978). The kingdom of plants. In W. A. R. Thomson (ed.). Medicine from the Earth. McGraw- Hill Book Co. New York; 208.

Sengupta, R. Ahmed A. B. & Kumar B. J. (2014). Pharmacognostical evaluation of *Terminalia arjuna* bark on different marketed samples. *Der Pharmacia Lettre*; 6 (3):299-303.

Shah, G., Chawla, A., Baghel, U.S., & Rahar, S (2013): Pharmacognostic stardardisation of leaves of Melaleuca leucadendron. *Pharmacognosy Journal*; 5: 143-148.

Shah, N. C. (1981*). The role of Ethnobotany in relation to medicinal Plants in India.* In S.K Jain (ed.) *Glimpses of Indian Ethnobotany,* Oxford & IBH Publ Co., New Delhi: 69-80.

Shahidi, F., Janitha, P.K & Wanasundara, P.D. (1992). Phenolic antioxidants. *Crit Rev Food Set Nutr;* 32: 67-103.

Shahzad, A., Hussain, A. I., Asad, R, Mudasir Majeed & Nosheen Aslam. (2014). Bioactive Components and Antioxidant Properties of *Terminalia arjuna* L. Extracts. J *Food Process Technol* ; 5:2.

Shukla, Y. & Singh, M. (2007). Cancer preventive properties of ginger: a brief review. *Food and Chemical Toxicology*; 45: 683-690.

Singh, R., Tiwari, S. S., Srivastava, S. & Rawat, A. K. S. (2012). Botanical and phytochemical studies on roots of *Berberis umbellata* Wall, ex G. Don. *Indian Journal **of** Natural Products and Resources;* 3(1): 55-60.

Singh, S. P. (2005). Collection and Utilization of Medicinal and Aromatic plants in Arunachal Pradesh. *Arunachal Forest News;* 21 (l&2):65-68.

Singleton, V. L & Rossi, J. A (1965). Colorimerty of total phenolics with phosphor molybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture;* 144-158.

Singleton, V. L. & Rossi, J. A. (1965). Colorimerty of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture;* 144-158.

Sinha, R. & Lakra, V. (2005). Wild tribal food plants of Orissa *Indian Journal of Traditional Knowledge;* 4(3): 246-252.

Stockwell, C. (1988) *Nature's Pharmacy*, Century Hutchinson Ltd London, United Kingdom.

Sumpam, T.N.D., Namsab, C. A. L. (2011). An ethnobotanical survey of medicinal plants in the Eastern Himalayan zone of Arunachal Pradesh, India. *Journal of Ethnopharmacology* ;134(1) :18-25.

Swati, N., Shrivastava, B., Sharma, P., Jain, H. K. & Ganesh, N., (2013). Pharmacognostical and Phytochemical Investigation of leaves of *Bixa orellana* L., *Int. J. Pharm. Sci. Rev. Res.;* 22(1): 247-252.

Tag, H. & Das, A. K. (2004). Ethnobotanical Notes on the Hill Miri Tribe of Arunachal Pradesh. *Indian Journal of Traditional Knowledge;* 3 (l):80-85.

Tag, H., Das, A. K. & Kalita, P. (2005). Plants used by the Hills miri tribe of Arunachal Pradesh in ethno fisheries. *Indian Journal of Traditional knowledge;* 4(1): 57-64.

Tesch, R. (1990). Qualitative research: analysis types and software tools. Philadelphia, PA: Routledge Falmer.

Thothatri, K. & Paul, C. B. (1978). Some medicinal plants from District Tirap of Arunachal Pradesh. *Indian J. Pharm. Sci;* 40. 206-208.

Tilbert, J. C., & Kaptchuk, T. J. (2008). Herbal Medicine Research and Global Health: An ethical analysis. *Bulletin of the World Health Organizations;* 86 (8): 577-656.

Tripathi, A.k., Limasenla., & Shankar, R. (2017). Ethno-Medicinal Plants Used By Nyishi Tribe of Arunachal Pradesh, India. *WJPPS;* 6(5): 1246-1253.

Umesh K., S. & Sunita D. R. (2017). Macroscopic and Microscopic Study of *Terminalia arjuna* . *WJPMR*; 3(9), 261-264.

Uthayakumari, F., & Sumathy, M. (2011). Pharmacognostical studies on the endemic medicinal plant- *Jatropha maheswarii* Subr. & Nayar (Euphorbiaceae), *International journal of Pharm Tech Research;* 3 (4): 2169-2174.

Valério, M. A., Ramos, M. I. L., Braga N. J. A. & Macedo, M. L. R. (2015). An seed residue (*Bixa orellana* L.): nutritional quality Food Sci. Technol, Campinas; 35(2): 326-330.

Vedavathy, S. (2002). Tribal Medicine Real Alternative. *Indian Journal of Traditional Knowledge;* 1(1) 25-31.

Vellaichamy, M. A., Sundaram, R. S. & Swaminathan, G. (2017). Phytochemical Evaluation and Chromatographic Fingerprint Analysis on Flavonoids Compounds in *Leucas aspera* (Willd.) Link Leaf by HPTLC. *Pharmacogn J.:* 9(6): 942-946

Wallis, T. E. (1985). *Text book of Pharmacognosy,* 5th edition, CBS Publishers & Distributors, New Delhi: Pp-1.

Wallis, T. E. (2011). *Practical Pharmacognosy.* Pharma Med Press, sultan Bazar, Hydrerabad : PP-1.

WHO/AFRO. (1976). 'African Traditional Medicine'. Brazzaville: Technical report series, No. 1; pp. 3–4.

Wu, Y.Y., Li, W., Xu, Y., Jin E.H., & Tu, Y.Y. (2011). Evaluation of the antioxidant effects of four main tha eflavin derivative through chemiluminescence and DNA damage analyses. *J Zhejiang Univ Sci B*. 12(9):744–751.

Yamagishi, S. & Matsui, T. (2011). Nitric oxide, a Janus-faced therapeutic target for diabetic microangiopathy - friend or foe? *Pharmacol Res.* 2011; 64:187–194.

Yemm, E.W., & Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, *57*(3): 508–514.

Yonggam, D. (2005). Ethnomedico-botany on the Mishing tribe of East Siang District of Arunachal Pradesh. *Arunachal Forest News;* Vol.21 (l&2):44-49.

Young, I. S & Woodside, J. V. (2001). Antioxidants in health and disease *J d in Pathol*;

Zaman, K. & Pathak, K. (2013). Pharmacognostical and Phytochemical Studies on the leaf and stem bark of *Annona reticulata* Linn. *Journal of Pharmacognosy and Phytochemistry*; l (5): 1-7.

ANNEXURE-I

PROFORMA FOR DOCUMENTATION OF FOLK MEDICINAL PANTS AND MEDICINE INFORMATION

S.No.	Content	Details Information
1.	Record No:	
2.	Name of Folk Healer	
3.	Tribe	
4.	Address and Locality	
5.	Age	
6.	Sex	
7.	Profession and Educational Qualification	
8.	Plant collection No.	
9.	Name of the specimen	
10.	Local/Hindi Name	
11.	English	
12.	Botanical Name	
13.	Family	
14.	Indications	
15.	Medicine	
16.	Used in vegetable or Food	
17.	Other uses, if any	
18.	Time duration	
19.	Mode of administration	
20.	Remark	
21.	Information Recorded by	

List of Publications

1. Tripathi Ak, Limasenla, Shankar R. Ethno-Medicinal Plants Used By Nyishi Tribe of Arunachal Pradesh, India. *WJPPS* (2017); 6(5): 1246-1253.

2. Tripathi Ak, Limasenla, Shankar R. Devesh Tewari. Nutritional Assessment of Some Important Medicinal Plants Used By Nyishi Tribes of Arunachal Pradesh. *Fazl Ali College Journal* (2017); 70:75.

List of Seminar Conference Attended

1. National Workshop on Bio informatics tools & its Application held during June 19-20, 2013 organized by BIF Center Nagaland University Lumami.
2. National Workshop on Data Base Desiging for Biologists organized by BIF Center Nagaland University Lumami on September 9-11, 2014.
3. National Workshop on "Conservation, Cultivation and Exploration of Therapeutic Potential of Medicinal Plants" in North Eastern States Organized by ARRI, Itanagar under CCRAS, Ministry of AYUSH, Govt. of India. (10-11 March, 2014).
4. National Workshop on Traditional Healing Practices in North Ease India. Organized by ARRI, Itanagar under CCRAS, Ministry of AYUSH, Govt. of India. (02-03 December, 2014).
5. Workshop on Emerging Trends in Research & Health care organized by CCRAS, Ministry of AYUSH, Govt. of India. (23rd July, 2015).
6. National Seminar on Dravyaguna Organised by Gujrat Ayurved University, Jamnagar, Gujarat. (21st July, 2017 at Jamnagar).
7. National Conference on Diseases & Drugs: Emerging Trends & Challenges January 31 to February 1, 2018.Organized by Department of Zoology, Zakir Husain Delhi College, University of Delhi.