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Ethnomedicinal Studies Of Plants Used by Indigenous Communities

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ABSTRACT

Ethnomedicinal studies provide valuable insights into the traditional knowledge systems of indigenous communities regarding the use of local flora for treating various ailments. This research explores the medicinal plants employed by indigenous groups, documenting their therapeutic applications, methods of preparation, and routes of administration. By conducting ethnobotanical surveys and interviews with traditional healers and community elders, the study identifies a diverse range of species used for primary healthcare needs. These findings not only highlight the cultural and medicinal significance of these plants but also emphasize the urgent need for conservation and sustainable utilization. Furthermore, the documentation of this knowledge serves as a foundational resource for future pharmacological studies and the discovery of novel bioactive compounds.

INTRODUCTION

Nature has provided an excellent store house of remedies to cure all the ailments of mankind. In Ancient days almost all the medicines used were from natural sources, particularly from plants. Plants Continue to be an important source of new drugs even today. The importance of botanical, chemical And pharmacological evaluation of plant derived agents used in the treatment of human ailments has Been increasingly recognized in the last three decades. Plant based remedies are widely used for the Treatment and prevention of various

diseases and often contain highly active multitude of chemical Compounds. Modern research is now focusing greater attention on the generation of scientific Validation of herbal drugs based on their folklores claim. In this modern era, a large Indian population Still relies on the traditional system of medicine, which is mostly plant. Studies are going on throughout the world for the search of protective molecules that would provide Maximum protection of liver as well as other organs and practically very little or no side effects would Be exerted during their function in the body.

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A number of herbs are traditionally used in different countries during drug or toxin induced. Plants Particularly herb have been recently popularized in modern medicine, since many therapeutically Important compounds are derived from them. Extracts prepared from these plants are used in the Treatment of various diseases. *Ficus auriculata* Lour. And *Sarcochlamys pulcherrima* (Roxb.) are the Such plants, which are used extensively in folklore medicine to treat hepatic disorders, microbial Infection, helminthic and other activities. Plants were used widely in the practices of traditional medicine for thousands of years by people in China, India, and many other countries. Some of the earliest records of the usage of plants as drugs Are found in the Atharvaveda, which is the basis for Ayurvedic medicine in India (2000 BC), the clay Tablets in Mesopotamia (1700 BC), and the Eber Papyrus in Egypt (1550 BC). Other famous literature Sources on medicinal plants include “De Materia Medica,” written by Dioscorides between BC 60 And 78, and “Pen Ts’ao Ching Classic of Materia Medica” written around 200 B. These medicines Were used in the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal Formulations. The specific plants to be used and the methods of application for particular ailments Were passed down through oral tradition. The mythological Chinese emperor Shennong is said to have written the first Chinese pharmacopoeia, The “Shennong Ben Cao Jing”. The “Shennong Ben Cao Jing” lists 365 medicinal plants and their Uses- including ephedra (the shrub that introduced the drug ephedrine to modern medicine), hemp, And chaulmoogra (one of the first effective treatments for leprosy). Succeeding generations Augmented on the Shennong Bencao Jing, as in the Yaoxing Lun (Treatise on the Nature of Medicinal Herbs), a 7th century Tang Dynasty treatise on herbal medicine. The earliest known Greek herbals Were those of Diocles of Carystus, written during the

third century BC, and one by Krateuas from the First century BC. Only a few fragments of these works have survived intact, but from what remains, Scholars have noted that there is a large amount of overlap with the Egyptian herbals (Robson and Baek, 2009). Greek and Roman medicinal practices, as preserved in the writings of Hippocrates (e.g. *De herbis et curis*) and especially Galen (e.g. *Therapeutics*), provided the pattern for later western Medicine. Sometime between 50 and 68 AD, the Greek physician Pedanius Dioscorides wrote (commonly known by its Latin title *De Materia Medica*), a compendium of more than 600 plants, 35 Animal products, and ninety minerals. *De Materia Medica* remained the authoritative reference of Herbalism into the seventeenth century. Similarly important for herbalists and botanists of later Centuries was Theophrastus’ *Historia Plantarum*, written in the fourth century BC, which was the first Systematization of the botanical world.

PLANT SECONDARY METABOLITES AS A SOURCE OF DRUGS:

The fascinating structural array of secondary metabolites synthesized by plants in the nature’s tiny laboratories have been an excellent source of medicinal agent for the treatment of diseases both of mankind and animal kingdom since time immemorial. Irrespective of the underlying philosophical premises, the use of plants in the treatment of diseases was exemplified by their use in all the major system of medicine like western medicine with origin in Mesopotamia and Egypt, the Unani, Ayurvedic system centered in western Asia and Indian subcontinent, and those of Orient like China, Japan, Tibet etc. However, information about how and when medicinal plants were first used by mankind in most cases lost in prehistory as because most of such relative information transmitted orally through expertise.



Documentation of uses of plants as medicinal sources are available in Egyptian herbal medicine as early as 2900 BC and Chinese Materia Medica around 1100 BC. Ayurvedic medicine detailing the use of medicinal plants was documented by Charaka, Sushruta and other experts from 1000 BC. Descriptions of thousands medicinal plants in “De Materia Medica” were written by Dioscorides in 78 A.D. In almost all the traditional medicines, the plants play a major role and serves as the backbone of the system. There is still a great wealth of knowledge concerned to medicinal properties of plant that are still transmitted orally from generation to generation by tribal societies all over the world. In recent time, the branch of ethnomedicine gained momentum for harnessing the wealth of information of ethnic groups of people with traditional use of plants around the world. There is an urgent need of conservation and documentation of this valuable knowledge gathered through generations by the tribal and

ethnic people. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This interest of plant origin drugs is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world’s population does not have access to conventional pharmacological treatment, and the general impression that the “natural” products are harmless etc. World Health Organization also encourages, recommends and promotes traditional/ herbal remedies in National Health care programmed because such drugs are easily available at low cost, are comparatively safe and the faith that people have in such remedies.

Table-I.1: Active constituents from plant sources and their therapeutic utility

Active constituents	Plant source	Therapeutic utility
Ajmalicine	<i>Catharanthus roseus</i>	Vasodilator
Berberine	<i>Berberis species</i>	Antidiarrhoeal
Caffeine	<i>Camellia sinensis</i>	CNS stimulant
Cocain	<i>Erythroxylum coca</i>	Local anesthetic
Codein	<i>Papaver somniferum</i>	Antitussive
Colchicine	<i>Colchicum autumnale</i>	Gout
Digoxin	<i>Digitalis lanata</i>	Cardiotonic
Emetine	<i>Cephaelis ipecacuanha</i>	Antiamoebic
Ergometrine	<i>Claviceps purpuria</i>	Oxytocic
Ergotamine	<i>Claviceps purpuria</i>	Vasoconstrictor
Gingkololides	<i>Ginkgo biloba</i>	PAF antagonist
Ginsenosides	<i>Panax ginseng</i>	Adaptogen
Hypericine	<i>Hypericum perforatum</i>	Antidepressant
Morphine	<i>Papaver somniferum</i>	Analgesic
Pilocarpine	<i>Pilocarpus jaborandi</i>	Parasympatholytic
Quinine	<i>Cinchona species</i>	Antimalarial
Quinidine	<i>Cinchona species</i>	Antiarrhythmic
Reserpine	<i>Rauwolfia serpentina</i>	Hypotensive
Rescinnamine	<i>Rauwolfia serpentina</i>	Tranquilizer
Ricin	<i>Ricinus communis</i>	Antitumour
Sophoradin	<i>Sophora subprostrata</i>	Antiulcer

ETHNOMEDICINAL PLANTS USED BY MISHING TRIBE OF ASSAM:

Medicinal plants are extensively used as alternative therapeutic tools for the prevention and curing of various diseases and ailments by various communities. The present study is focused on the utility of certain medicinal plants on the Mishing tribe of various districts of Assam, India. Dhemaji a district located in the bank of the river Brahmaputra is rich in indigenous medicinal plants due to the wide range of habitats. The ethnic people living in the remote areas of the district depend on various plant-based medicine. The Mishings are distributed all over the eleven districts of the state and practised different traditional healing methods. A detailed survey was done with the elderly men or/and women of the community and crosschecking with the information obtained from the local herbalists were carried out. The Mishing are mainly found in Dhemaji, Lakhimpur, Sonitpur, Jorhat, Majuli, Sivsagar, Golaghat, Dibrugarh and Tinsukia districts of Assam. As a riverine tribe, the Mishing people construct their houses in raised platforms about 4-5 feet from the ground and is locally called Chang-ghar. Moreover, due to the lack of proper communication and hospitals, they developed traditional healing practices to protect themselves

from different diseases and are still depend on traditional healing practices. The villages that are mostly inhabited by the Mishings under the study area were surveyed randomly. With prior informing and taking of the personal consent of the local informants' information were collected from the villagers of different age groups and sex of Mising community through conversation and interview using semi-structured questionnaire and other reported sources. Medicinal plant specimens were collected from forest and kitchen gardens following standard manuals and the herbarium was prepared for our selected plants.

The collected plant species were identified by the Botanical Survey of India, Shillong. The collected information of the medicinal uses of the plants was cross checked with the information obtained from the local herbalist and further validation was done through discussion among the local informants. The study revealed that the most common mode of administrations during disease management is decoction, paste and juice for both internal and external applications. In various cases consumption of some plants as a vegetable was also useful in alleviating several ailments. In some cases, the mode of administration is raw (direct internal applications).

Scientific Name	Local name (Mishing)	Family	Plants Parts used	Diseases/ailments
<i>Abelmoschus moschatus</i> Medik.	Ui-sipak	Malvaceae	Root/leaves/ bark	The leaf and stem bark are used for skin diseases and root is used for gastritis.
<i>Acorus calamus</i> L.	Boch	Acoraceae	Rhizome	Rhizome is used in the treatment of diarrhea, dysentery, cough and cold
<i>Aegle marmelos</i> (L.) Corrêa	Bel	Rutaceae	Leaves	Leaf decoction is taken in gastric and fever.
<i>Ageratum conyzoides</i> L.	Namnin eing	Asteraceae	Leaves	The leaf pest is used for and wound healing.
<i>Allium sativum</i> L.	Talab	Amaryllidaceae	Bulb	Cough, fever and malaria.
<i>Alpinia nigra</i> (Gaertn.) Burt	Taling	Zingiberaceae	Rhizome	The root extract is used for treatment in fever, pneumonia etc.
<i>Alstonia scholaris</i> (L.) R Br.	Sotiana	Apocynaceae	Bark	Bark decoction is used for fever and malaria.
<i>Amaranthus spinosus</i> L.	Genak	Amaranthaceae	Root	Root extract with warm water is used for menstruation over bleeding.
<i>Averrhoa carambola</i> L.	Kordoi	Oxalidaceae	Root	The root bark is used in the treatment of jaundice.

TECHNIQUES AND METHODS FOR SELECTING MEDICINAL PLANTS:

In the investigation of medicinal plants, a relevant moment, which can set the course of the work and its impact on all points of view, is the criterion used for the selection of the plant species to study. Guirado and Cuellar (2008) refer to genomics, metabolomics, ecological, botanical, taxonomical and epidemiological based studies. On the other hand Albuquerque and Hanazaki (2006) point out other ways to study the medicinal plants, among which five basic types of approaches are highlighted: the Randomized, the Ecological, the Chemotaxonomic (also known as Phylogenetic or Chemosystematic), the Ethnodirected (or Ethno-oriented) and finally, the exploration of promising Biological test results.

The Randomized approach:

Several important authors, here mentioned, recognize the randomized approach as an approach without criteria. and others who worked with forest plots, do not identify this form of selecting

plants for research, as random. So, the question here to be discussed is whether it is a type of random selection criterion or whether it should be identified separately as another type of approach? The randomized investigations consist in random selection and collection of plant species for study, according to the plant availability. When carried out in regions with high diversity and endemism the probability of finding novel substances, bioactive or not, is certainly higher in this type of selection. It is an indispensable approach, once it can demonstrate the potential of different plant species that had never been investigated. According to Souza (1996), this type of selection provides an endless source of new structures, since nature is a vast chemical laboratory. However, there are many mistaken views and criticisms about this approach due to its randomness, which does not mean the absence of criteria.

The Ecological approach:

The ecological approach, also known as field observations, consists in observations of



interactions between organisms in their ecological environment, inducing to potential biological activity (antibacterial, antifungal, agrotoxic, pesticide) This approach searches for secondary metabolites and biological activities and it may be performed by the selection of young and mature leaves for a given species, or between different species that are shadow resistant and not shadow resistant, among other characteristics, though little explored, it has achieved excellent results.

The Chemosystematic approach:

When the definition of a plant species, which will be source of a phytomedicine, is based on the structural analogy of the substances present in the plant material, with other known active substances present in different botanical family, genus or even species, it can infer that this strategy is based on chemosystematics, a system created by Professor Otto Richard Gottlieb (1982) to organize and understand the plants. This system consists in identifying groups of chemicals present in plants, considering the taxonomic organization of these plants. To illustrate this topic, consider the use of a plant species containing antiplasmodic indol derivatives as active principle in the development of an antimalarial phytomedicine. Would a plant species from a different genus, containing such substances, give origin to the same phytomedicine? Depending on the results the later plant produces in in vitro biological tests, it may be true. On the other hand, Mikania species (Asteraceae) which contain coumarin in their composition, exhibit different pharmacological activities and alleged popular use, including antimalarial properties. Therefore, the strategy based on chemosystematics can bring in it some uncertainty, once the chemical composition, which can explain the relationship between botanical species, genera and even families, is not decisive enough to guide the development of a

phytomedicinal product and to validate the alleged use, as well as it does not confirm the safety and efficacy of the derivative proposed for the development of the product, requiring indeed the realization of a pharmacological prospection in order to characterize the wanted activity for the phytomedicine.

OBJECTIVES OF THE STUDY:

Considering the usefulness of the plants viz. 1) *Sarcochlamys pulcherrima* Roxb. Family (Urticaceae) 2) *Ficus auriculata* Lour. Family (Moraceae) in the traditional system of medicine, it is thought to be worthy to make a systematic phytochemical and pharmacological investigation of these plants so that the potent bio-active principles could be used either directly as a medicinal agent or as lead compounds for developing newer compounds. Study on ethno botanical and medicinal uses of the *F. auriculata* and *S. pulcherrima* leaves by various ethnic groups are reported by various authors but the phytochemical, pharmacological characterization and scientific validation on most of its therapeutic folklore utility have not yet been reported. Considering the folkloric importance of the both plant leaves, it is thought to be worthy to make a detail study including their phytochemical characters, isolation of phytoconstituents and evaluation of their biological activity. The objective is to throw light on the probable potential therapeutic role of the plant leaves so as to scientifically validate the traditional uses as well as to explore the possibility of getting good therapeutic agent(s) or lead compound(s) for development to use against the indicated ailments. The objective is to study the potential role of the plants on the therapeutic efficacy and to scientifically validate the belief of the ethnic people of Mishing tribe of Assam as well as preparation



of a detail systematic phytochemical and pharmacological record of these indigenous plants. The results recorded from this study will be helpful for the identification and characterization of both plant leaves in future and also will contribute towards establishing pharmacopoeial standards. To assess these properties, the following systematic approach was targeted to design the protocol for the study:

- Properly collection of the plants during their flowering season and their authentication.
- What are the various preliminary processing for storing of the leaves ?
- Preparations of different solvent extracts.
- Development of qualitative and quantitative parameter for the Pharmacopoeial standardization of the plant leaves as well as their leaves extracts.
- Safety aspects of the leaves extracts of *F. auriculata* and *S. pulcherrima* for human use.
- Whether the leaves extracts possess some degree of biological activity like anthelmintic, antioxidant, antibacterial, acute toxicity and hepatoprotective as claimed by ethnic people?
- Which successive solvent fraction of the extracts shows better targeted biological activities?

MATERIAL AND METHOD:

Collection and identification of plant material:

The leaves of *Ficus auriculata* Lour. and *Sarcochlamys pulcherrima* (Roxb). were collected in the month of June-July 2011, from Jonai, Dhemaji District, Assam and Rupnagar, Dist. Dibrugarh, Assam respectively. The plants were identified and authenticated at Botanical Survey of India (BSI), Eastern circle, Shillong, Meghalaya,

India. The voucher specimens were deposited at the Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, for future references.

Drying and storage:

The collected leaves were cleaned, washed with water and dried under shade at room temperature with occasional shifting for three weeks. They were then powdered with a mechanical grinder. The powders were passed through sieve number 40 and stored in airtight container for further uses.

Successive solvent extraction:

The dried powdered materials (100 g) were defatted with petroleum ether (60-800C) and the defatted powdered materials thus obtained were further successively extracted using ethyl acetate and methanol as solvent in a Soxhlet apparatus by continuous hot percolation process. Each time, before extracting with next solvent of higher polarity, the marc was kept in hot air oven below 500C for 10 min. The extraction was carried out exhaustively and the solvents were recovered by distillation under reduced pressure using rotary vacuum evaporator. The concentrated extracts kept in refrigerator at 40C for further use. The extractive values of all the solvent extracts were recorded in terms of % w/w as per standard method. The percentage yields of ethyl acetate, methanol and water extract were found to be 0.5%, 0.85% and 3.15% respectively. The extracts were subjected to preliminary phytochemical

analysis to identify the presence of phytoconstituents.

4.1.4 Phytochemical screening of extracts:

Qualitative phytochemical screening:



The qualitative phytochemical screening of the extracts, obtained by successive solvent extraction process was carried out to detect the presence of probable primary as well as secondary metabolites. An amount of 5 g of the extract was dissolved in 100mL alcohol/distilled water and the following tests were performed for identification of different chemical groups present in the extracts.

Preparation of reagents for qualitative phytochemical screening:

The reagents used for the different chemical group tests were prepared as per standard procedure and were as follows:

- Benedict's reagent: 1.73g of cupric sulphate, 1.73g of sodium citrate and 10gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100mL with water.
- Dragendroff's reagent: 1.7 g basic bismuth nitrate and 20 g tartaric acid were dissolved in 80 ml of water. This solution was mixed with a solution containing 16 g potassium iodide in 40 mL of water.
- Fehling's solution A: 34.64 g copper sulphate was dissolved in a mixture of 0.5 mL of sulfuric acid and sufficient water to make up 500 mL.
- Fehling's solution B: 176 g of sodium potassium tartarate and 77 g of sodium hydroxide were dissolved in sufficient water to produce 500 mL. An equal volume of above solutions (Fehling's solution A and B) is mixed at the time of use.
- Hager's reagent: 1% solution of picric acid in water.
- Libermann-Burchard reagent: 5g of acetic anhydride was carefully mixed under cooling with 5mL of concentrated sulphuric acid and this

mixture was added slowly to 50mL of absolute ethanol with cooling.

- Mayer's reagent: Solution of 1.36 g of mercuric iodide in 60 mL of water was mixed with a solution containing 5 g of potassium iodide in 20 mL of water.
- Molish's reagent: 2.5 g of pure α -naphthol was dissolved in 25 mL of ethanol.
- Phloroglucinol solution: 2g of phloroglucin dissolved in 100 mL of 90% alcohol.

Test procedures for qualitative phytochemical screening:

a) Tests for Alkaloids:

- Dragendroff's test: 0.1ml of dilute hydrochloric acid and 0.1 mL of Dragendroff's reagent were added to 2mL solution of extract in a test tube. Development of orange brown colored precipitate suggested the presence of alkaloid.
- Mayer's test: 1 mL of extract was taken in a test tube. 0.2 mL of dilute hydrochloric acid and 0.1 mL of Mayer's reagent were added. Formation of yellowish buff colored precipitate gives positive test for alkaloid.
- Wagner's test: 2mL of extract solution was treated with dilute hydrochloric acid and 0.1 mL of Wagner's reagent. Formation of reddish-brown precipitate indicated the positive response for alkaloid.
- Hager's test: 2mL of extract was mixed with 0.2mL of dilute hydrochloric acid and 0.1 mL of Hager's reagent. A yellowish precipitate suggested the presence of alkaloid.

b) Tests for Amino acids:



- Millon's test: To the test sample 2 mL of Millon's reagent was added, a white precipitate formed which indicated the presence of amino acid.

- Ninhydrin test: Extract solution was treated with Ninhydrin (Triketohydrindene hydrate) solution at the pH range of 4-8. Development of purple color indicated the positive response for amino acids.

c) Tests for Carbohydrates:

- Molisch's test: To 2-3 mL of filtrate few drops of α -naphthal (20 % in ethyl alcohol) was added

L shaken well and about 1ml of concentrated H₂SO₄ was added along the side of test tube. A reddish-violet colored ring at the junction of two layers was shown that the presence of carbohydrate.

- Fehling's test: One mL of Fehling's solution A and 1 ml of Fehling's solution B were mixed properly and boiled for one minute. To this, equal amount of filtrate was added and heated on the boiling water bath for 5-10 minutes and observed for the formation of brick-red colored precipitate which indicated the presence of carbohydrate.

- Benedict's test: Equal volume of Benedict's reagent and filtrate was mixed. The mixture was heated in a water bath for 5 minutes and observed for the formation of yellowish green colored precipitate for indication the presence of carbohydrate.

- Keller-Kilian test (for Deoxy Sugars): Small amount of sugar was dissolved in glacial acetic acid and 2 drops of 5% ferric chloride was added. The solution was added to 2mL concentrated sulphuric acid by the side of the tube. Reddish brown color (which changed to bluish green to dark on standing) at the junction confirmed the presence of deoxy sugars in the sample.

d) Test for Fats and Oils:

- Saponification test: Few drops of 0.5N alcoholic potassium hydroxide was added to a few quantity of the extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Not observed the formation of partial neutralization of alkali which indicated the absence of fixed oils. □

- Spot test: A little quantity of the extract was separately pressed between two filter papers. No appearance of oil stain on the paper indicated the absence of fixed oils.

e) Test for Flavonoids:

- ml of extract solution was hydrolyzed with 10 % v/v H₂SO₄ and allowed to cooled. Then it was extracted with diethyl ether and separately divided into 3 portions in three test tubes. 1 ml of dilute ammonia, 1 mL of dilute sodium carbonate and 1 mL of 0.1 (N) sodium hydroxide were added to the first, second and third test tubes respectively. In each test tube found development of yellow color indicated the presence of flavonoids.

- Shinoda test: The extract was dissolved in alcohol. One piece of magnesium was added followed by concentrated hydrochloric acid drop wise and heated. Appearance of magenta color demonstrated the presence of flavonoids.

- Alkaline reagent test: Addition of increasing amount of sodium hydroxide to the residue showed yellow coloration which decolorized after addition of acid.

- To the small quantity of residue, lead acetate solution was added. A yellow-colored precipitate was formed.



- Ammonia test: Filter paper strip is dipped in alcoholic solution of the extract, ammoniated and observed for color change from white to orange.

f) Test for Glycosides:

Small portion of the extract was hydrolyzed with dilute hydrochloric acid for half an hour in water bath, filtrated and filtrate was then subjected to following tests.

- Keller-Killiani Test (for cardiac glycosides): To 2 mL of filtrate glacial acetic acid, one drop of 5% ferric chloride and concentrated sulphuric acid were added and there was no formation of reddish-brown color at the junction of two liquid layers and upper layer appeared bluish green in the absence of glycosides.

- Legal's Test: To the filtrate, 1 ml of pyridine and 1 ml of sodium nitroprusside solution was added and there was no formation of pink to red color indicated the absence of cardiac glycoside.

- Test for Saponins glycosid

1. Foam test: Small amount of residue is diluted with distilled water up to 20 ml and shaken vigorously in a graduated cylinder for 15 minutes and observed for the formation of 1 cm layer of foam which is stable for 10 minutes.

2. To the alcoholic extract few drops of sodium bicarbonate was added and shaken well. The solution

becomes honey comb like frothing confirmed the presence of saponins.

3. 1mL extract was treated with 1% lead acetate solution. Formation of white precipitate indicated the presence of saponins.

g) Test for Gums:

- Molisch's test: 2ml of concentrated H₂SO₄ was added to 2ml of extract solution. Then it was treated with 15% α -naphthol in ethanol (Molisch's reagent). Formation of a red violet ring at the junction of two layers indicated the presence of gums.

- Test solution was hydrolyzed using dilute HCL and Benedict's or Fehling's test was performed and observed for formation of red colour.

h) Test for Lignin:

A small quantity of the extract was treated with alcoholic solution of phloroglucinol and hydrochloric acid, there was no appearance of red colour showed the absence of lignin.

i) Test for Mucilage:

- A small quantity of the extract was added separately to 25mL of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties.

j) Test for Proteins:

- Biuret test: One ml of 40% sodium hydroxide solution was added to two drops of 1% CuSO₄ solution till a blue color produced, and then add to the 1 ml of the extract. Formation of pink or purple colour and violet color indicates the presence of proteins.

- Xanthoproteic test: To 1ml of the extract, 1 ml of concentrated nitric acid was added. A white precipitate was found and it was boiled and cooled. Then 20% of sodium hydroxide was added and observed the formation of orange color for presence of proteins.

k) Test for Steroids:



- **Libermann-Burchard's Test:** 10mg of extract was dissolved in 1 ml solution of chloroform. To this 1ml of acetic anhydride was added following the addition of 2 ml of concentrated H₂SO₄. Formation of reddish violet color indicated the presence of steroids.

- **Salkowski Test:** 1mL of concentrated sulfuric acid was added to 10mg of extract dissolved in 1mL of chloroform. A reddish-blue color exhibited by chloroform layer and green fluorescence by the acid layer suggested the presence of steroids.

I) Tannins and Phenolic Test:

- **Ferric chloride test:** About 5ml solution of extract was allowed to react with 1m of 5% ferric chloride solution. Greenish black color formed which indicated the presence of tannins.

- **Test for Potassium dichromate:** About 5m of the extract solution was treated with 1 ml of 10%

aqueous potassium dichromate solution. Yellowish brown precipitate colour was formed which indicated the presence of tannins.

- **Test for Lead acetate:** About 5ml of the extract was treated with 1 ml of 10% lead acetate solution in water. Yellow color precipitation formed and indicated the positive test for tannins.

RESULTS AND DISCUSSION:

The two isolated compounds each from methanolic extract of *Ficus auriculata* Lour. coded as KD and LD were identified as Norwogonin 8-O glucoside i.e. 5, 7-Dihydroxy 8-O- β -D-glucopyranosylflavone and 1-(hydroxymethyl)-piperidine-2,3,4,5,6-pentanol respectively on the basis of analysis of spectral data obtained from UV-spectra, FT-IR, NMR (1H, 13C) Spectroscopy and Mass spectrometry. By considering UV

spectra, we can assume that the isolated compound contains flavonoid. It shows a strong peak at 288nm and a shoulder of lower intensity at 335nm. From the FTIR spectra peaks we can say that the isolated compound coded as KD contains flavonoid structure. Comparing the UV spectra, FTIR spectra, NMR (1H, 13C) and LC-MS/MS of the isolated compounds, we found that both isolated compounds contained flavone and are Norwogonin 8-O-glucoside i.e. 5, 7-Dihydroxy 8-O- β -D-glucopyranosyl flavones and 1-(hydroxymethyl)-piperidine-3,4,5,6-pentanol. The structural formula of the compound is C₂₁H₂₀O₁₀ and C₆H₁₃N and the molecular weight is 434 and 195 respectively

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