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Evaluation of Pharmacognostical parameters and Preliminary Phytochemical Screening of leave of the plant *Myrica esculenta*

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Abstract
In the recent time, the Traditional System of Medicine is giving competitive aspect to the Modern medicine as it's having less side effect. The present investigation is aimed to evaluate the pharmacognostical parameters and find out the bioactive compounds present in the leaves of *Myrica esculenta* belonging to the family Myricaceae. It is an evergreen wild tree which has been used traditionally since time immemorial by the local tribes of Meghalaya and lower parts of Himalayan region for healing various ailments. So, the research on the selected plant include to check if the drug is adulterant or not and to investigate the various phytochemicals present in the extracts in the preliminary level by using methanolic extract of the leaves. The following study will provide a detailed referential information for the correct identification of the crude drug.

Keywords: *Myrica esculenta*, Pharmacognostical, physicochemical, phytochemicals, methanolic extracts

1. Introduction
Nature has always been a complete storehouse of remedies to cure all ailments of mankind. Since time immemorial, humans have used natural products from plants in medicines to alleviate and treat diseases. Traditional medicine is the oldest form of health care in the world and is still practiced in rural and tribal areas. Herbalism (also known as herbal medicine) is the study of botany and use of plants intended for medicinal purposes or for supplementing a diet. Herbal medicines are now in great demand in the developing world for primary health care. This is basically because of the general belief that herbal drugs are without any side effects besides being cheap & locally available. World Health Organisation (WHO) estimates that up to 80 percent of people still rely mainly on traditional medicines. It has recently defined traditional medicine as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today.

Fig 1: Myrica esculenta leaf

In this research work, we have selected the plant Myrica esculenta belonging to the family Myricaceae which is one of the most common plant currently used in Ayurvedic formulations for human health management against various disease like fever, cardiac debility, diarrhoea, dysentery, etc. The present study have been made to evaluate the pharmacognostical and phytochemical parameters of leaves of Myrica esculenta which is important to standardize and also to avoid spurious or adulterated drugs. Phytochemical analysis is also important to find out the presence of active constituents present in the leaves of Myrica esculenta.
Myrica esculenta is a wild tree with edible fruits which is widely used by tribes of sub-tropical Himalayas, Assam and in Khasi, Jaintia, Naga and Lushai hills at an altitude of 900-2100m. The fruits of the plant are edible. They have pleasant sourish-sweet taste and are used in the preparation of refreshing drinks.
Almost all parts of the plant have medicinal properties. Due to overexploitation by the endogenous people for their daily need and commercial income generating value, the species is poorly regenerating in their natural habitat and pose a threat for extinction.

Vernacular names
It is known with different names in different parts of the country. Some of the vernacular names of the species are Kaiphal (Hindi), Somavriksha (Sanskrit), Nogatenga (Assamese), Soh-phie (Khasi), Box-Myrtle (English), Keiphang (Lushai), Kahela (Punjab), Katphala (Nepal), Maruta (Kerala).

2. Materials and method

2.1 Materials

Table 1: List of chemicals used:

Chemicals used	Company name
Benedict's reagent	Fisher Scientific, Mumbai
Ninhydrin	Loba chemie, Mumbai
Dragendroff's reagent	Himedia, Mumbai
Acetic anhydride	Thomas Bker chemicals pvt. Ltd
Sodiumnitropruside purified	E Merck (India) Limited
Mercuric Chloride	Fisher Scientific, Mumbai
Diethyl ether	Fisher Scientific, Mumbai
Barfoed's reagent	Himedia, Mumbai
Benzene	Fisher Scientific, Mumbai
Chloroform	Fisher Scientific, Mumbai
Iodine	S.D. Fine chem. Limited, Mumbai
Gelatin Powder bacto	S.D. Fine chem. Limited, Mumbai
Sodium hydroxide pellets	S.D. Fine chem. Limited, Mumbai
Ferric Chloride	Merck, Mumbai
Phoroglucinol AR	S.D. Fine chem. Limited, Mumbai
Methyl Blue	Merck Limited
Sodium Chloride AR	Universal Laboratories Pvt. Ltd
α-Naphthol	S.D. fine chem. Limited, Mumbai
Lead Acetate LR	S.D. fine chem. Limited, Mumbai
Formaldehyde	S.D. fine chem. Limited, Mumbai
Resorcinol	Himedia, Mumbai
Fehling's Solutions A & B	Fisher Scientific, Mumbai

Table 2: List of general instruments used

Instruments	Model
Hot air oven	Indosati complete laboratory furnishers, Haryana
Digital Balance	Indosati complete laboratory furnishers,m Haryana
Automated Rotary Shaker	Indosati complete laboratory furnishers, Haryana
Muffle furnace	Indosati complete laboratory furnishers, Haryana
Grinder	Picasho, Delhi
Microscope	Indosati complete laboratory furnishers, Haryana
Water bath	Indosati complete laboratory furnishers, Haryana

2.2 Methods

1. Collection of plant: Leaves of the plant were collected from Umsning, a small village in Meghalaya, during the month of September-October and washed in running water to separate the soil and other extraneous materials and the field data of the leaf was noted in the notebook. It was then shade dried and then pulverized.
2. Authentication of the plant: The selected plant was collected in the flowering condition and was then transformed in the form of herbarium and submitted to the Botany Department of Guwahati University and authentication of submitted plant was carried out by the authorised person of Department of Botany, University of Guwahati, Assam, and India.
3. Macroscopical evaluation: The macroscopical observation of the leaf were carried out as per performed by the standard methods to determine the shape, size, taste, colour and odour.
4. Microscopical evaluation: Microscopical evaluations were done on qualitative basis.
   - Transverse section of leaves: The leaves of the tree was finely sectioned by using a new blade. Then on a clean glass slide a drop of glycerine water was placed at the centre of the slide. The cover-slip was held between the finger and the thumb of the left hand and the edge of the cover-slip was made to rest on the slide at the left hand edge of the drop. Then a dissecting needle was inserted under the right hand edge of the cover-slip and the latter was made to rest on the needle. Then the cover-slip was lowered on to the drop slowly, taking enough care that the drop of the liquid exactly filled the space between the slide and the cover-slip without any air bubbles being trapped inside. Then the slide was placed on the stage of microscope and was observed by using 10X and 45X lens.
   - Powdered microscopy: For powder analysis the shade dried leaves were pulverized and passed through sieve no. 40. This was further subjected with different reagents like phloroglucinol, conc. HCl (1:1) and safranine for the presence of the components like stomata, fibres, starch and calcium oxalate crystals.
5. Physicochemical parameters
   - Determination of Ash Value: Ash value is generally the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug and also those which has been added for the purpose of adulteration. The main objective is to remove all the traces of organic matter which may otherwise interfere in an analytical determination. Procedures given in Indian pharmacopoeia were used to determine the different ash values like total ash, acid insoluble ash and water soluble ash.

     % of Total ash= ((x-y)/n) X 100

     Where,
     X: weight of the dish + ash obtained
     Y: weight of empty dish
     N: weight of the crude drug taken
   - Determination of moisture content: The presence of moisture in a crude drug can lead to its deterioration due to activation of certain enzymes or due to growth of microbes. It is determined by heating the drug at 100o-105oC in an oven to a constant weight.
   - Determination of extractive value: Sometimes the active chemical constituents of a crude drug cannot be determined by normal procedures. Therefore, extractive values are used to determine the amount of active constituents extracted with solvent. Thus nature of the chemical constituents can be estimated.
   - Fluorescence analysis: Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. The powdered drug was examined under U.V and ordinary light with different reagents taken in a petridish. The different wavelengths used for U.V were 254 nm and 365 nm.
6. Preliminary phytochemical screening: For preliminary phytochemical studies, 100 gm. of powdered material was extracted with successive solvent extraction in soxhlet apparatus with methanol-water (7:3) and water successively. Extracts were dried in water bath until it turned to a sticky mass allowing the alcohol to evaporate

leaving the extract alone on the petry dish. It was then weighed. The extractive values for each extract is shown in table 4. Further test were performed for various phytoconstituents like alkaloids, glycosides, tannin, steroids, saponins etc. with methanol-water extract by usual methods prescribed in standard text.

3. Results

3.1 Macroscopical characters

Colour of Myrica esculenta leave is green on both side. Size is (10-13 × 3.5) cm and its shape is lanceolate. They have a smooth surface with somewhat light green colour on abaxial surface as compared to adaxial surface. The leaves are with fairly prominent midrib with serrate or entire margin. The venation of the leaf is pinnately reticulate. Leaves are odourless.

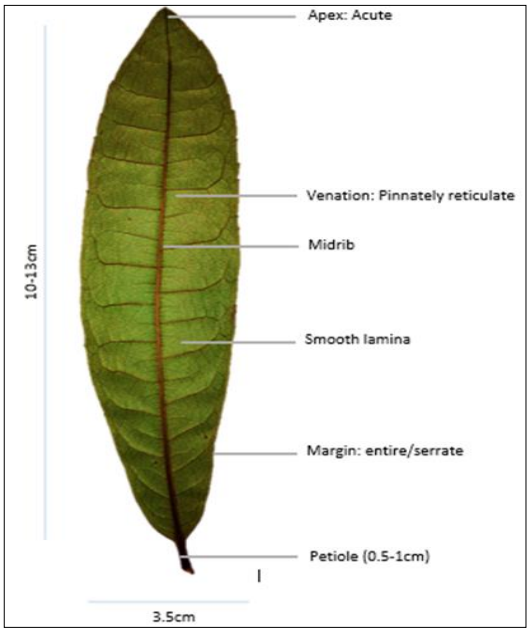

Fig 2: organoleptic and morphological features of *Myrica esculenta* leaf

3.2 Microscopical evaluation

- Transverse section of leaf: The fresh leave was transversely sectioned through the midrib region and was mounted with safranine, phloroglucinol, iodine solution and was observed which showed upper and lower epidermis with unicellular trichomes. Below epidermis, palisade cell along with spongy parenchyma and vascular bundles were seen.

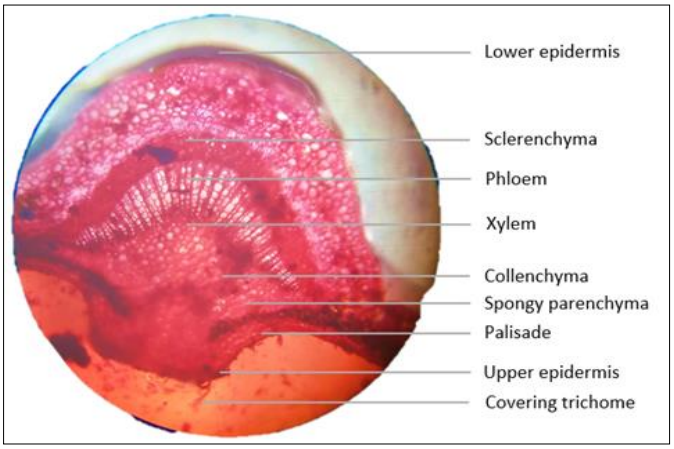

Fig 3: T.S of *Myrica esculenta* leaf with safranine

- Powdered microscopy: The powdered microscopy of the leaf when mounted with safranine, phloroglucinol and HCl showed the presence of Fibres, Oil glands, calcium oxalate crystals.

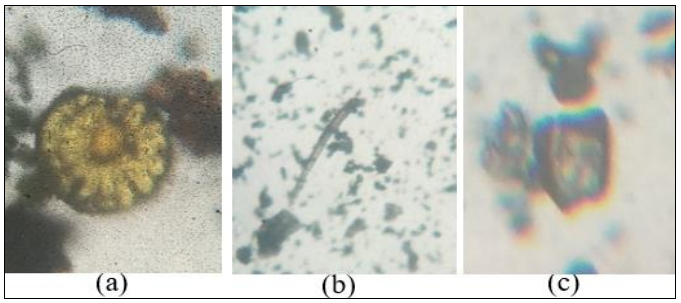

Fig 4: Powdered microscopy of *Myrica esculenta* leaf (10X view). (a) Oil gland (b) trichome (c) Ca-oxalate crystal

3.3 Physicochemical evaluation

The results of physicochemical parameters are as follows:

Table 3: Ash value of the powdered leaf

Sl. No.	Ash value	% (w/w)
1.	Total ash	2.6
2.	Acid insoluble ash	0.4
3.	Water insoluble ash	0.82

Table 4: Extractive value of the leaf

Sl. No.	Type of extract	% (w/w)
1.	Alcoholic extract(90% methanol)	12.6
2.	Water extract	10

Table 5: Moisture content of the leaf

1st observation	2nd observation	3rd observation	Average value
0.53% (w/w)	1.60% (w/w)	1.23% (w/w)	1.12% (w/w)

Fluorescence analysis

The behaviour of the powdered leaf sample with different chemical reagents and their fluorescence property were obserseved and are tabulated below. Observation was done under different wavelenghts i.e., visible rays and ultraviolet rays (254 nm and 365 nm).

Table 6: Fluorescence analysis of the leaf:

Sl. No.	Treatment of powder	Visible light	Short wavelength UV rays (254 nm)	Long wavelength UV rays (365 nm)
1.	Powder as such	Brownish green	Greenish brown	Green
2.	Powder + 50% H_2SO_4	Brown	Greenish black	Green
3.	Powder + 50% HNO_3	Brown	Brownish green	Green
4.	Powder + 5% KOH	Green	Black	Dark green
5.	Powder + methanol	Brown	Greenish brown	Green
6.	Powder + 1N HCl	Brown	Black	Green
7.	Powder + cold water	Brown	Brownish green	Green
8.	Powder + picric acid	Green	Dark green	Light green
9.	Powder + hot water	Brown	Brownish green	Green
10.	Powder + $FeCl_3$	Blackish green	Black	Blackish brown
11.	Powder + 1 N NaOH	Yellowish green	Black	Dark green
12.	Powder + Formaldehyde	Green	Dark green	brown
13.	Powder + calcium chloride	Dark green	Dark brown	Brown
14.	Powder + ammonium hydroxide	Greenish yellow	Dark green	Brownish yellow
15.	Powder + diethyl ether	Dark green	Dark brown	Green

3.4 Phytochemical screening

For phytochemical screening the obtained extracts were concentrated and percentage of yield was calculated (table 7). The concentrated extracts were redissolved in respective solvents and then subjected to various chemical test as per standard methods for the identification of various phytoconstituents. The phytochemical screening for hydro alcoholic extract showed positive for carbohydrate, protein, steroids, tannins & phenols, glycoside, saponins, iodine and flavonoids (table 8).

Table 7: Successive solvent extraction & Nature of extracts

Sl. No.	Solvent	Color	Consistency	% of Yield (w/w)
1.	Methanol + water	Dark green	Sticky	39.77
2.	Water	Dark brown	Dry	0.4

Table 8: Phytochemical evaluation of Myrica esculenta leaf extact with methanol-water as a solvent

Phytoconstituents	Test performed	Results
Akaloids	Mayer's test	-
	Dragendorff's test	-
	Wagner's test	-
Fixed oil	Spot test	+
Protein	Biuret test	+
	Millon's test	+
Amino acids	Xanthoproteic test	+
Carbohydrates	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
	Barfoed's test	+
Steroids	Liebermann burchard's test	+
Tannins & phenols	Ferric chloride test	+
	Gelatin solution	+
	Lead acetate test	+
	Aqueous bromine test	+
Cardiac glycosides	Killer lillani test	+
Anthraquinone gycosides	Borntrager's test	-
Saponins	Foam test	+
Flavonoids	Alkaline reagent test	+
Terpenoids	Salkowaski test	+

(+) present, (-) absent

4. Discussion

The following research work was carried out for the standardization of the leaves of Myrica esculenta. According to the World Health Organization, the macroscopic and microscopic description of a plant is the first step to establish the identity and degree of purity of such materials and should be carried out before any tests are undertaken. As a part of standardization, the macroscopical examination of leaves was

studied which can serve as a diagnostic parameter. In the present study the leaves are found to be with entire/serrate margine with acute apex and a smooth lamina. The venations are pinnately reticulate. The microscopical studies of transeverse sections showed the presence of covering trichomes. Evaluation of parameters such as total ash helped us to know about the amount of organic and inorganic matters present in the sample as acid insoluble ash determines the amount of silica, carbonates etc. Percentage of extractives in different solvent helped us to determine the quantity and nature of active phytoconstituents in the extract. The water soluble extractive value indicates the presence of sugar, acids and inorganic compounds and alcohol soluble extractive values indicates the presence of polar constituents like phenols, steroids, glycosides and flavonoids. Determination of moisture content of the drugs is important as excessive moisure may favor fungal growth or may cause micro-organism contamnation which may deteriorate the drug. Fluorescence is an important phenomenon exhibited by various chemical consituents present in plant material. The observation made on powdered leaves under UV and visible light served as primary fingerprints for the sample as different radiations were emitted by it. The extracts obtained by successive solvent extractions were subjected to priliminary phytochemical analysis to find out the presence of phytoconstituents.

5. Conclusion

Verifiable knowledge about medicinal plants plays a vital role in primary health care and has great potential for the discovery of new herbal drugs. Here in this study, an attempt has been made to evaluate the pharmacognostical and preliminary phtochemical parameters of leaves of Myrica esculenta. The pharmacognostical parameters reported here can be considerd as distinctive enough to identify and prevent its adulterations in herbal industry. The datas thus obtained will provide relevant information which will be used in authentication of the crude drug and check adulteration for quality control measures. Preliminary phytochemical screening was also performed to find out the biochemical or bioactive compounds present in the leaves of the plant. The observed parameters will add to the existing knowledge of Myrica esculenta and will be useful in preparation of crude drug's formulation for treating various ailments. In conclusion, the present study may be useful to provide information with regards to its identification and standardization and also in carrying out further research of its use in the Ayurvedic System of Medicine.

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