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Pharmacognostic and Phytochemical Investigation of *Limnophila rugosa (Roth)* Merr

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Abinash Kumar Sahu*[1], Chhayakanta Panda, Nishigandha Sa, Anjan Kumar Padhan

Department of Medicinal Chemistry. The Pharmaceutical College, Tingipali, Barpali, Bargarh, 768029, Odisha, India

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ABSTRACT

Limnophila rugosa provides an up-to-date information about its botanical classification, chemical constituents as well as pharmacognostical values. **Aim**: The aim of the study is to investigate pharmacognostic and phytochemical screening of petroleum ether, ethanol and water extract of *L.rugosa* with the presence of different secondary metabolites responsible for the therapeutic values of the drug like presence of alkaloids, glycosides, carbohydrate, tannins, proteins and amino acids, and steroids and sterols etc. **Result**: The qualitative phytochemical studies were performed with petroleum ether, alcohol and water extracts to identify its alkaloid, carbohydrate, glycoside, protein and amino acid, etc by using suitable reagents. Alkaloid test showed slightly positive in all reagents. The different extracts collected were petroleum ether yield 3.61%, ethanol 8.36% and water extract 5.74%. The total ash value showed 5.59 %, water soluble ash 1.21% and acid insoluble ash 0.23 % with different extractive values were monitored. The results of total Water soluble extractive were higher as compared to acid insoluble ash. The different fluorescence analysis of ethanolic extract as well as powder of drug seen in day and U.V light to observe different colours. The results of extractive values showed the water extract have higher quantity of extract (7.5%) in comparison to other solvent extracts **Conclusion**: Protein and amino acid were found in all tests. Phenolic, flavonoids, glycosides were abundantly present in all the extracts. Lead acetate test for tanin showed high result in comparison to other tests. Salkowski test showed slight presence of phytosterol and protein and alkaloids, saponin (foam test) is also limited in all the extracts. The characteristics of the powder were observed under daylight and showed different fluorencence colour with different reagents to conform the presence of different chemical constituents in entire plant.

INTRODUCTION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized countries has be entraced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies[1]. The use of medicinal herb, in the treatment and prevention of diseases, is attracting attention by scientists, worldwide. This is corroborated by World Health Organization in it quest to bring primary health care to the people. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants. Among plants of economic importance medicinal and aromatic plants have played a vital role in alleviating human sufferings[2]. Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda and Unani) and unorganized (folk, tribal and native) form. The healing properties of many herbal medicines have been recognized in many ancient cultures[3]. The natural resources how so ever large are bound to diminish hence need effective strategy is needed for sustainable utilization[4]. Cultivation of medicinal and aromatic plants is constrained due to lack of suitable technology, which has led to low yield and poor quality. Consequently, medicinal herbs are predominantly harvested in sufficient quantities from the wild in an unregulated manner[5].

This Limnophila [6-8]. is commonly known as 'Ambulia' and exists in aquatic environments. It is a tropical to subtropical in Australia, Africa as well as Pacific Islands, a perennial from Southeast Asia and has adventives distribution in North America[3] Plants belonging to Limnophila genus are reported to be widely distributed in India, and cover an important position in traditional systems of medicine where a number of species are being used as folk medicines in the treatment of various ailments; crude plant extracts as well as isolated phytochemicals exhibited various significant biological activities.

Limnophila rugosa Roth. Merr. (Bhringaraj) is a ethnomedicinal plant known for its medicinal property belonging to Scrophulariaceae family of Gandhamardan hills, Orissa.

BOTANICAL ASPECTS

Limnophila rugosa is a perennial, 10-50 cm tall. Stems are fascicled, erect and ascending, and mainly unbranched, glabrous. Its leaves are opposite; petiole 1-2 cm, narrowly winged, leaf blade ovate, 3-8 X 1-4 cm, hispidulous along veins, adaxially glabrous or sparsely

hispidulous bracts subspatulate-oblong, with flattened membranous glands, margin entire or apically undulate-toothed and ciliate. Flowers are axillary, solitary, sessile, usually appearing capitate. Bracteoles absent. Calyx 6-8 mm, without raised veins in fruit or with 5 raised veins and flattened membranous glands, margin ciliate. Corolla is slightly purple-red to blue, to 1.6 cm. Style slender, apically cylindric, and pubescent. Its Capsule are pale brown, ovoid, ca. 5 mm. Leaves simple, thin, oblong, lanceolate, opposite, subsessile, stipulate, measures about 1.5-4 cm × 1-2 cm, serrate, acute, base symmetrical, petiole winged, surface glabrous, Upper dark green, lower light green in colour, covered with fine hairs, venation reticulate, veins – obscure on upper surface, prominent on the lower surface, midrib strong, sub veins 4–5, lateral veins finely divided, astringent bitter odour.

Systematic Classification[9,10,11]

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Asteridae
Order:	Scrophulariales
Family:	Scrophulariaceae
Genus :	Limnophila
Species:	*L. rugosa*
Binomial name:	*Limnophila rugosa* (Roth) Merr

Vernacular Names[12]:

Oriya : Bhringaraja

Hindi : Kala karpur

Marathi : Losodba

Bengali : Kalakapurs

English : Marshrosemerry, sea lavender

Chinese : Da ye shi long wei

French : Herbe a paddy

Spanish : Hierba del arrozal

Fig-1 *Limnophila rugosa*

Synonims:

Limnophila rugosa, Limnophila roxburghii

Habitat:

The plant is distributed in open, wet places at low altitudes in Bangladesh, Bhutan, Burundi, Central African Republic, China, India (Andhra Pradesh, Assam, Bihar, Madhya Pradesh, Manipur, Meghalaya, Orissa, Sikkim, Tripura, Uttar Pradesh, West Bengal), Indonesia, Japan.

Chemical Constituents:

Limnophila rugosa Roth. Merr. (Bhringaraj) found to contain phosphorus (P_2O_5)-0.15%, Calcium (CaO) 0.31%, Iron (Fe_2O_3)- 0.22%[13.]as well as phenolics, flavonoids, terpenoids, amino acids etc.[13] It also contain anethole (24.96 –27.12%), Methyl chavicol (70.79 – 71.00%). Many essential oils are present. It also contains glycosides, little amount of alkaloids and flavonoids, steroids.[14], 5-Hydroxy-6,7,4'-trimethoxyflavone (Salvigenin) from aerial parts and roots[15,16]. The plant contains 5-Hydroxy-7,8,2',4'-tetramethoxyflavone in aerial parts and root.[17]. The plant contains 5,7-Dihydroxy-6,8,4'-trimethoxyflavone

(Nevadensin), 5,7,4'-Trihydroxy-6,8-dimethoxyflavone (Demethoxysudachitin,), 3β-Hydroxy-lup-20(29)-en-27-oic acid[18].

Traditional uses: [19-23]

This leaf of *Limnophila rugosa* (Roth.) Merr. (Scrophulariaceae) is used to prepare hair oil and the infusion of the leaves is used as diuretic, stomachic and digestive tonic [19, 20]. The leaf of *Limnophila rugosa* paste with leaves of tulsi is given orally to cure urinary burning [21]. *Limnophila rugosa* shows many applications in the traditional medicinal uses. It is used to treat elephantiasis. It is also used in diarrhoea, dysentery and dyspepsia, carminative and tonic purposes. The essential oil of this plant also exhibits significant anti-bacterial and anti-fungal activities and is applied as flavouring agent of food and perfuming of hair oils. The plant had been accepted for *Sugandhabala*l as it responded to ayurvedic description of the drug [22]. The plant is also reported to exhibit wound healing activity[23].

MATERIALS AND METHODS:

The different Mayer's, Hager's, Barford's, benedict's and Millon's reagent, Wagner's, Dragendorff's, Fehling's A and B, α-naphthol, ferric chloride, Conc. Sulphuric acid, pyridine, sodium nitroprusside, acetic anhydride, were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, chloroform, and ethanol were purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.

Plants collection, Identification and processing

The fresh whole plant as *Limnophila rugosa* was collected from local area of Gandhamardana hill ranges of Bolangir district of Odisha, India in the morning hour during the month of September 2018. The plant was authenticated as *Limnophila rugosa* Roth Merr.of family Scrophulariaceae on the basis of the morphological characters of all the parts of the plant by Prof. (Dr.) Santosh Kumar Dash, Retired Professor and H.O.D., P.G Dept. of Biosciences, C.P.S, Mohuda, Berhampur, Ganjam, Odisha. The plant was washed properly with water to remove the mud or dust, and then it was dried in sunlight for one hour and the stem bark was dried under shade and was powdered by the help of mechanical process. The coarse powder have stored in airtight container for further studies.

Physico-chemical analysis [24-27]

The plants parts were subjected for macroscopic study. The different parts of plant were processed with glycerine and saffranine for better visualisation and it was observed under compound microscope (OLYMPUS 100MB, Universal Pvt. Ltd., Mumbai) at magnification of 100 X under daylight**.**

Physical evaluations:

This includes the study of different physical parameters which are rarely constant for crude drugs. The crude powder drug was evaluated for foreign organic matters, extractive values like alcohol and water soluble, ash values like total, water soluble and acid insoluble ash values, determination of swelling index, foaming index and moisture content [19, 20].

Macroscopical studies [28]

Leaves simple, thin, oblong, lanceolate, opposite, subsessile, stipulate, measures about 1.5–4 cm × 1–2 cm, serrate, acute, base symmetrical, petiole winged, surface glabrous, Upper dark green, lower light green in colour, covered with fine hairs, venation reticulate, veins obscure on upper surface, prominent on the lower surface, midrib strong, sub veins 4–5, lateral veins finely divided, astringent bitter odour.

Leaf

Colour : Upper dark green, lower light green in colour,

Odour : Characteristic

Taste : Astringent bitter

Shape : Thin, oblong, lanceolate,

Size : Measures about 1.5–4 cm × 1–2 cm

Figure No. 2: *Limnophila rugosa*

Stem

Colour : Green

Odour : Characteristic

Taste : Bitter

Flower

Colour : Upper portion is purple in colour

Odour : Characteristic

Taste : Astringent bitter

Powder Studies **(Organoleptic properties):**

Colour : Brownish

Odour : Characteristic

Taste : Astringent bitter

Microscopical investigation [29]

This study allows more detailed examination of a drug and it can be used to identify the organized drug by their known histological characters. It is mostly used for qualitative evaluation of organized crude drug in entire and powder form. Microscopic evaluation also covers study of the constituents by application of chemical methods to small quantities of drug in powdered form or to histological section of the drug is called Microchemistry or Chemo-microscopy.

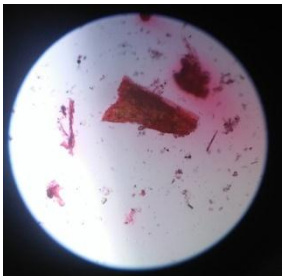
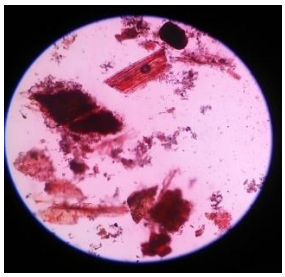
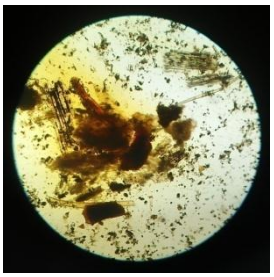
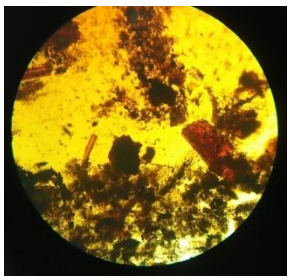

Figure No.3: calicium oxalate Figure No. 4: xylem vessel Figure No. 5: Starch Granules Figure No. 6: Parenchyma

Physio-chemical evaluation [30,31,32-35]

This Physico-chemical analysis includes the study of different physical parameters which are rarely constant for crude drugs. It includes determination of foreign organic matters, extractive values like alcohol soluble and water soluble, determination of ash values like total ash values, water soluble and acid insoluble ash values, determination of swelling index, foaming index and moisture content.

Determination of Ash Value:

Ash values are helpful in determining the qualities and purities of a crude drug. Ash is done to remove all traces of organic matter from the vegetable drugs which otherwise interfere in analytical determination on incineration, crude drug normally leave ash usually contain carbonates, phosphate and silicate of potassium, sodium, calcium and magnesium. A higher limit of acid insoluble ash imposed especially in case where silica may be present or when the calcium oxalate content of drug is varies high.

The percentage of ash value and extractive value [22-24] was calculated with reference to the air dried crude drug and value was recorded in Table no. 1.

Table No. 1: Physico-chemical analysis of *L. rugosa*

Sl. No.	Physical Constants	(%) yield
1. Ash value		
I	Total ash	5.59
II	Water soluble ash	1.21
III	Acid insoluble ash	0.23
IV	Moisture content	8.5
2. Extractive value		
I	Ethanol	2.25
II	Chloroform	11
III	Ethyl acetate	0.6
IV	Pet ether	6.52
V	Water	14

Fluorescence Analysis [36-38]

The results of Fluorescence Analysis are given in Table no. 2 & its extract in Table no. 3.

Table No. 2: Fluorescence Analysis of Drug Powder

Treatment	Daylight	U.V light
Drug powder + 1 N HCL	Light green	Green
Drug powder + 50% HCL	Pale green	Green
Drug powder + 50% H_2 SO_4	Green	Deep green
Drug powder +50% HNO_3	Yellowish brown	Green
Drug powder + 1N NaOH	Yellow	Yellowish green
Drug powder + al. NaOH	Dark green	Brown
Drug powder + water	Green	Green
Drug powder + methanol	Dark green	Green

Table No. 3: Fluorescence Analysis of Ethanolic extract

Treatment	Daylight	U.V light
Ethanol extract + 1N HCL	Light green	Green
Ethanol extract + 50% HCL	Light green	Green
Ethanol extract + 50% H_2SO_4	Pale orange	Crimson
Ethanol extract + 50% HNO_3	Brown	Deep brown
Ethanol extract +1N NaOH	Yellow	Deep yellow
Ethanol extract + al. NaOH	Light yellow	Yellow
Ethanol extract + water	Green	Green
Ethanol extract + methanol	Dark green	green

Phytochemical screening-[35,39,40]

Extraction[35]**:**

The shade dried coarse powder of plant (100 g) was subjected to continuous hot extraction with different solvents i.e. Pet. Ether (60–80^{o}C), Ethanol and Water as per their polarity successively. The results were given in Table no-5.

The extracts were dried by Rotary evaporator, weighed and percentage of yield was calculated in terms of air-dried crude powdered materials and is tabulated in Table no- 4.The extracts were kept in the desiccators for experimentation.

Petroleum ether extract

The powder of the plant was extracted with petroleum ether (60-80^{o}C) by heating in refluxed condenser for 18 hours. The extract was evaporated to dryness under vacuum. The dried extract was stored in vacuum desiccators. The results are given in Table no-5.

Ethanol extract

The marc left after petroleum extract was dried and extracted with methanol by heating in refluxed condenser for 18 hours. The extract was evaporated to dryness under vacuum. The dried extract was stored in vacuum desiccators. The results are given in Table no 5.

Water extract

The marc left after Ethanol extract was dried and extracted with water by heating in refluxed condenser for 18 hours. The extract was evaporated to dryness under vacuum. The dried extract was stored in vacuum desiccators and after these 2 to 3 drops of antifungal agent is used to avoid microbial contamination. The results are given in Table no-5.

Table No. 4: Percentage yield of leaf extract of *Limnophila rugosa*

Extract	% Yield	Colour	Consistency
Petroleum ether	3.61%	Light Brown	Sticky
Ethanol	5.36%	Dark green	Greasy
Water	7.74%	Light greenish yellow	Greasy

Table No. 5: Qualitative Phytochemical Screening of *L. rugosa*

Sr. No.	Phytochemical test	Pet. Ether	Ethanol	Water
1. Alkaloids				
1	Mayer's test	Absent	Present	Absent
2	Wagner's test	Absent	Present	Absent
3	Hager's test	Present	Present	Present
4	Dragendorff's test	Present	Present	Present
2. Carbohydrates & Glycosides				
1	Molish's test	Present	Present	Present
2	Fehling's test	Present	Present	Absent
3	Barfoed's test	Present	Present	Present
4	Benedict's test	Absent	Present	Present
5	Borntrager's test	Present	Absent	Absent
3.Saponins				
	Foam test	Absent	Present	Absent
4. Proteins & amino acid				
1	Millon's test	Absent	Absent	Present
2	Ninhydrin test	Absent	Absent	Present
5. Phenolic compounds & flavonoids				

1	Ferric chloride test	Present	Present	Absent
2	Lead acetate test	Present	Present	Present
3	Alkaline test	Present	Present	Absent
4	Shinoda test	Absent	Present	Present
6. Phytosterol				
1	Salkowski test	Absent	Absent	Present
2	Liebermann-Burchard	Absent	Absent	Present
7. Tannins				
1	lead acetate sol	Present	Absent	Present
2	Ferric chloride test	Present	Present	Absent
8. Test For Fixed Oils & Fats				
1	Spot Test	Present	Present	Present

Powder Drug with Chemical Reagents

A small amount of powder was placed in white porcelain dish and treated with several chemical reagents; changes in behaviour were noted and reported & tabulated in Table no- 6.

Table No. 6: Powder Analysis with Chemical reagents

Reagents	**Colour observed**
Powder as such	Brown
Powder + Conc. HCL	Light Green
Powder + Conc. HNO_3	Brown
Powder + Conc. H_2SO_4	Brownish
Powder + Glacial CH_3COOH	Light black
Powder + 5% NaOH sol.	Reddish black
Powder + 5% KOH sol.	Red
Powder + 5% $FeCl_3$ sol.	black
Powder + Picric acid	Yellowish green
Powder + Ammonia	Brownish black
Powder + Iodine sol.	Brownish blue

RESULTS AND DISCUSSION

Macroscopical characters finding shows, *L. rugosa* is a herb having greenish in colour, characteristics odour and taste is astringent. The characteristics of the powder of *L. Rugosa* were observed with different chemical reagents under Daylight using powder of the drug. The result showed that different fluorescence colour was seen by different reagent. That gives idea about presence of different chemical constituents in entire plant (Table no-5).In the study of physical constant, ash values, moisture contents and extractive values were monitored. The results of total ash value showed 5.59 %. Water soluble extractive was higher as compared to alcohol. The results of moisture content showed 8.5%. The p^H of the powder showed 7.8 in

10% powder solution (Table no-1). The dried powder plant material was extracted Pet. Ether, Ethanol and water by hot continuous percolation (soxhlet extraction) method with increasing order of their polarity. The extracts were evaporated in a distillation unit. The results of extractive values showed, the water extract have higher quantity of extract in comparison to other solvent extracts. The extractive value was recorded in Table no 1. Macroscopical characters finding shows, *L. rugosa* is a herb having greenish in colour, characteristics odour and taste is astringent. The dried powder plant material was extracted Pet. Ether, Ethanol and water by hot continuous percolation (soxhlet extraction) method with increasing order of their polarity. Qualitative phytochemical studies were performed on its pet.ether, alcoholic and water extracts to identify its Alkaloid, Carbohydrate and Glycoside, Saponin, Protein & Amino acid, Phenolic compounds & Flavonoids and Phytosterols by using suitable chemicals and reagents. Alkaloid test results of all extract showed slightly positive in all four tested reagents. Qualitative phytochemical studies of Carbohydrate & Glycoside showed a good characteristic colour and precipitate in all five tested reagent. Slight presence of Saponin was confirmed by foam test in all extracted solvents. Protein and amino acid was found present in all tests. Phenolic compounds and Flavonoids were abundantly present in all the extracts. However, lead acetate test showed a high result in comparison to other two tests. Salkowski test showed slight presence of Phytosterol in all the extracts. The above qualitative phytochemical screening showed that the whole plant is a rich source of Glycosides, Phenols & Flavonoids. However, presence of protein and alkaloids is limited in whole plants. The observations were recorded in the Table no- 5.

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