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Research Article

PRELIMINARY PHYTO-CHEMICAL STUDY ON THE LEAF OF AN ETHNO-MEDICINAL PLANT *Limnophila rugosa* Roth. (Merr.)

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Abstract

Limnophila rugosa Roth. (Merr.) (Scrophulariaceae), an ethno medicinal plant, is used as Bhringaraja by traditional practioners of Odisha. In the present study the leaves of *L. rugosa* were characterized for its physico-chemical properties and High Performance Thin Layer Chromatography, following standard procedures. The ethanol & aqueous extracts were subjected to various chemical tests and different types of functional groups like alkaloid, tannin, triterpenoids (Steroid), flavonoid, and phenols were detected. In Chromatography, HPTLC method, at different R_f, 16 spots were detected under both long and short UV. The results, being reported could be useful in the identification and standardization of the crude drug.

Key words: *Limnophila rugosa*; Phytochemistry; Bhringaraja; leaf drug.

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INTRODUCTION

A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance.[1] The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds.[2]

The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries. [3][4] People of small villages and native communities use folk medicine for the treatment of common infections.[5] Rural communities, in particular Gandhamardana hill area tribes, depend on plant resources mainly for herbal medicines, food, forage, making household implements, for fire and shade. *Limnophila rugosa* Roth. Merr. (Scrophulariaceae), an ethno-medicinal plant, locally called as Bhringaraja, in the hair oil preparation, by traditional practitioners of this area of Odisha. The plant also shows numerous medicinal applications in the traditional system. The juice of the plant is rubbed over the body in pestilent fever. It is applied on elephantiasis with coconut oil. It is administered in diarrhoea, dysentery and dyspepsia. It is used as carminative and tonic. The essential oil is used as flavouring agent in food and as perfuming agent in hair oils. The essential oil of this plant also exhibits significant anti-bacterial and anti-fungal activities. Infusion of leaves is used as diuretic and stomachic in the Philippine Islands and more or less throughout India.[6][7][8] Some terpenoids, essential oils and flavonoids were reported in this plant.[9] The phytochemical research based on ethno pharmacological information is generally considered as an effective approach in the discovery of new anti-infective agents from higher plants[10] and the development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants.[11] Classical text of Ayurveda describes three types of Bhringarajaa (White, yellow and blue), among which botanical identity of blue is still awaited. *L. rugosa*, having blue coloured flowers, is not reported yet for phyto-chemical characters of its leaves of and hence the present study was carried out to find out the same.

MATERIAL AND METHODS

Collection and authentication of plant material:

The plant, locally known as Bhringaraja,[12] growing in Gandhamardana hill ranges, Balangir of Odisha district of India[13] was identified as *Limnophila rugosa* Roth. Merr. (Scrophulariaceae) by studying the morphological characters of various parts of the plant and comparing them with the various characters mentioned in various floras.[13][14][15] The plants were shaken to remove adherent soil, dirt and washed with water. The herbarium specimen was prepared (Herbarium No. 6003) and stored in the Pharmacognosy department of the institute, for further documentation. Leaves was washed with running fresh water and few leaves were stored in solution of FAA (70% Ethyl alcohol: Glacial acetic acid: Formalin) in the ratio of (90:5:5).[16] The remaining leaves were dried under the shade and then were subjected for 60# powdering for further study.

The physicochemical evaluation such as determination of loss on drying at 110º C, total ash, acid insoluble ash, water and alcohol soluble extractive values was carried out as per the Ayurvedic Pharmacopoeia of India (API) [17] and values are depicted in Table 1.

Preparation of extract

5 g of *L. rugosa* leaf powder was extracted with methanol (100 ml) by keeping it for

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overnight with initial occasional shaking up to 6 hrs, and then set aside. After 24 hours it was filtered and alcoholic extract was collected. Similarly water extract were prepared and collected.[17]

Preliminary phyto-chemical tests

The presence of different phytoconstituents viz. alkaloid, triterpenoids (Steroid), flavonoid, tannin and phenol compounds were determined by standard procedure.[18]

Chromatographic study

Methanolic extract of *Limnophila rugosa* leaf were used for the study . The solvent system used was Chloroform : Methanol : Acetic acid in the ratio (8:2:1) . The application mode was camag linomat V and the development Chamber used was camag twin trough chamber. The plates used were precoated silica gel $_{GF254}$ plates. The chamber saturation duration, development time and development distance was 30 min, 30 min and 8 cm respectively. The scanner used was camag scanner III. For detection deuterium lamp and Tungsten Lamp were used. Win cats software was used.

After the scanning done by the Camag Scanner III, the area under the curve of the methanolic extract of *Limnophila rugosa* whole plant was studied.[19]

HPTLC study:

HPTLC study was carried out by standard method. Spray reagent used in this study was Vanillin-Sulphuric acid.

RESULT AND DISCUSSION

The Physico-chemical parameters of the leaves viz. foreign matter, loss on drying, total ash, acid insoluble ash were found to be 0.00 % w/w, 3.4 % w/w, 7.54 % w/w and 0.23 % w/w respectively. The Percentage of alcohol extractive was 0.99 % w/w and ether soluble extractive value was 0.061 but the percentage of water extractive was found to be significantly higher i.e. 1.32 %. The pH of 10 % w/v aqueous solution is 6.32. (Table 1)

For the detection of functional groups, various chemical tests were performed with aqueous and alcohol extract of the sample. Functional groups like alkaloid, tannin, triterpenoids (Steroid), flavonoid, and phenols were present in the sample. (Table 2)

Detection of HPTLC plate after spraying with Vanillin-Sulphuric acid reagent, 16 spots were detected at both 366 nm and 254 nm in *L. rugosa* leaf methanol extract. (Figure 1) (Table 3)

CONCLUSION

Qualitative tests indicated the presence of different types of functional groups like alkaloid, tannin, triterpenoids (Steroid), flavonoid, and phenols. The obtained 16 spots, detected under both long and short UV, at different R_f may act as fingerprint for the identification of the drug. Results showed the presence of tannin and phenolic compounds in the leaves.

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Table 1: Physicochemical parameters of *Limnophila rugosa* Leaves

Sr. No	Physicochemical Parameters	*Limnophila rugosa* Leaf
1.	Loss on Drying at 105°C (% w/w)	3.4
2.	Ash value at 450°C (% w/w)	7.54
3.	Acid insoluble ash at 450°C (% w/w)	0.23
4.	Extractive Value	
	(i) Water soluble extractive (% w/w)	1.32
	(ii) Alcohol soluble extractive (% w/w)	0.99
	(iii) Ether Soluble extractive (% w/w)	0.061
5.	pH of 10% **w/v** aqueous solution	6.32

Table 2: Preliminary tests results

Sr. No	Qualitative tests	*L. rugosa* Leaf
1.	Test for alkaloids	
	1) Dragendorff's reagent	+ve
	2) Mayer's reagent	+ve
	3) Hager' reagent	+ve
	4) Wagner's reagent	+ve
2.	Test for tannins	+ve
3.	Test for triterpenes (steroids) Salkowski reaction	+ve
4.	Test for saponins	-ve
5.	Test for fixed oil	-ve
6.	Tests for cynogenic glycosides / sugars Molisch's test	-ve
7.	Test for flavonoids / Shinoda's test	+ve
8.	Test for carbohydrates	
	1) Molish's test	-ve
	2) Fehling's test	-ve
	3) Pentose sugar	-ve
	4) Hexose sugar	-ve
	5) Non reducing	-ve
9.	Test for phenols / neutral $FeCl_3$	+ve
10.	Test for amino acids	-ve
11.	Test for proteins	
	1) Conc. H_2SO_4	-ve
	2) $CuSO_4$	-ve
	3) $HgCl_2$	-ve
	4) Lead acetate	-ve
	5) Ammonium Sulphate	-ve
11.	Test for resin	-ve
12.	Test for gum	-ve

+ve Present; -ve Absent

Table 3: HPTLC results

Track	R_f Value Long UV	R_f Value Short UV
Limnophila rugosa leaf methanol extract	0.02, 0.12, 0.19, 0.25, 0.30, 0.33, 0.35, 0.38, 0.40, 0.49, 0.53, 0.66, 0.68, 0.75, 0.81, 0.89	0.02, 0.13, 0.19, 0.25, 0.31, 0.40, 0.44, 0.49, 0.54, 0.62, 0.68, 0.75, 0.78, 0.87, 0.90, 0.95

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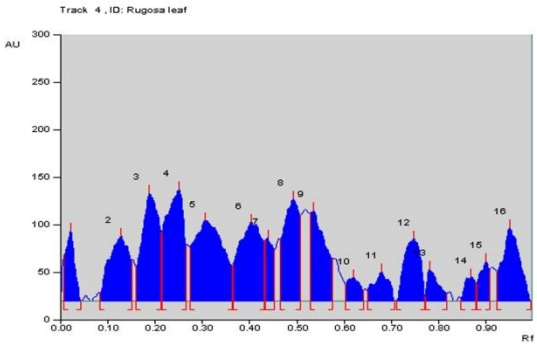
Figure 1: HPTLC of *Limnophila rugosa* leaf methanol extract

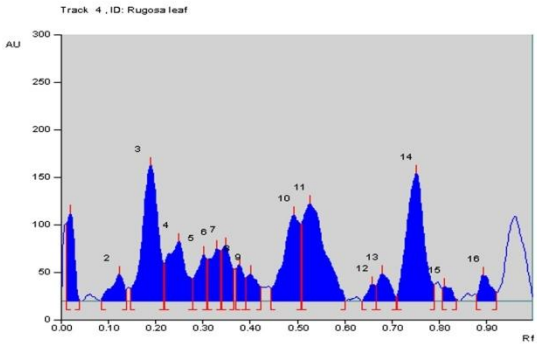
254nm

366nm

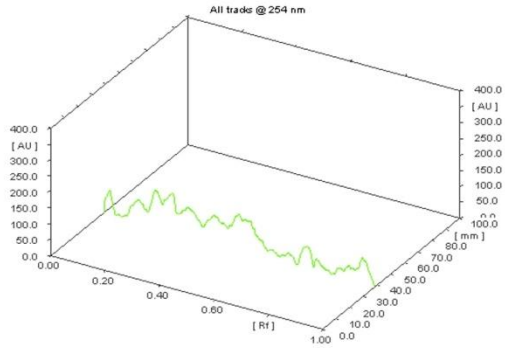
After spray



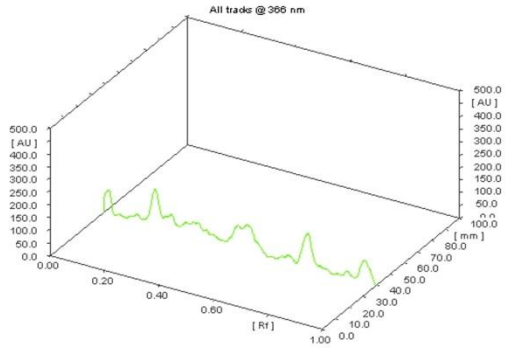
Densitogram under 254nm



Densitogram under 366nm



3D graph under 254nm



3D graph under 366nm

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REFERENCES

1. Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology 1999; 37**:**80-91.
2. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4**:**685-688.
3. Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. Phytotherapy Research 2005; 19**:**633-42.
4. Gupta MP, Solis PN, Calderon AI, Guionneau-Sinclair F, Correa M, Galdames C, Guerra C, Espinosa A, Alvenda GI, Robles G, Ocampo R. Medical ethnobotany of the Teribes of Bocas del Toro, Panama. Journal of Ethnopharmacology 2005; 96:389-401.
5. Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complementary and Alternative Medicine 2006; 6**:**2. [http://www.biomedcentral.com/1472-6882/6/2]
6. Anonymous, The wealth of India (Raw Materials). Vol-4: J-Q. Reprinted, CSIR, NewDelhi; 2003. p.40.
7. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 1st ed. New Delhi: CSIR; 1956.p.55.
8. Misra OP. Botanical identity of Sugandhabala. J. Res. Ind. Med. Yoga Homoeo 1978; 13:110-114.
9. Goutam Brahmachari. Limnophila (Scrophulariaceae): Chemical and Pharmaceutical Aspects. The Open Natural Products Journal 2008; 1:34-43.
10. Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L. Antibacterial screening of some Peruvian medicinal plants used in Callería District. Journal of Ethnopharmacology 2005; 99**:**309-312.
11. Erdogrul OT. Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biology 2002; 40:269-273.
12. http://www.rprcbbsr.com/herbarium/species?id=380 [Accessed on: 25th September, 2012]
13. Saxena HO, Brahman M. The flora of Orissa, Vol. III. 1st ed. Bhubaneswar: Regional Research Laboratory, Orissa Forest Development Corporation Ltd.; 1995. p.1237.
14. Hooker JD. The flora of British India, Vol. IV. 1st ed. Dehradun: Bishen Singh Mahendra Pal Singh; 1885. p.394-395.
15. Haines HH. The Botany of Bihar and Orissa, Part III-IV. 2nd ed. Dehradun: Bishen Singh Mahendra Pal Singh; 1988. p.659.
16. Wallis TE. Analytical microscopy. 3rd ed. London: J. & A. Churchill Ltd.; 1965. p.123-132; 210-215.
17. Anonymous. The Ayurvedic Pharmacopeia of India, Part-I, Vol. – I. 1st ed. New Delhi: Govt. of India, Ministry of Health and Family welfare, Department of I.S.M. & H; 1999.p.143.
18. Khandelwal KR. Practical Pharmacognosy – Techniques and Experiments. 9th ed.: Nirali Prakashan, Pune. 2002. p.24-29, 149-153.
19. Anonymous. Planner chromatography, Modern Thin Layer Chromatography. Switzerland: 1999. p.2-16.

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