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Pharmacological Study

Microbial evaluation of *Limnophila rugosa* Roth. (Merr) leaf

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Abstract

Background: *Limphonia rugosa* Roth. (Merr.), family-Scrophulariaceae is considered as a botanical source of classical Ayurvedic drug *Bhringaraja* by the traditional practitioners of Odisha and is being used for the management of various disorders. **Aim:** To study the antimicrobial activity of leaf of *L. rugosa*. **Materials and Methods:** Methanol extract of *L. rugosa* leaf (LRLM) has been studied, at various (5, 25, 50, 100, 250 µg/ml) dilutions, against medically important human pathogenic bacteria (two Gram-positive *Staphylococcus aureus, Streptococcus pyogenes* and two Gram-negative-*Escherichia coli, Pseudomonas aeruginosa*) and two fungal strains (*Aspergillus niger, A. clavatus, Candida albicans*) by using the agar disc diffusion method. A zone of inhibition of extract was compared with that of different standards such as ampicillin, ciprofloxacin, norfloxacin and chloramphenicol for antibacterial activity and nystatin and griseofulvin for antifungal activity. **Results:** The antibacterial and antifungal activities of the LRLM increased linear with the increase in concentration of extracts. When compared with standard drugs, the results revealed that, for bacterial activity *S. pyogenes* and *S. aureus* were more sensitive and in fungal activity *C. albicans* was more inhibited. The range of growth inhibition zone for all the sensitive bacteria was 11-20 mm and 13-19 mm for fungal strains. **Conclusion:** Methanolic extract of *L. rugosa* leaf is having antibacterial and antifungal activities.

Key words: Antifungal activity, *Bhringaraja*, Gandhamardan hills, *in vitro* antibacterial activity, *Limnophila rugosa* leaf, microbial load

Introduction

Limnophila rugosa Roth. (Merr.) of Scrophulariaceae family is an erect herbaceous, aromatic annual, 30-60 cm. high, found in aquatic situations and moist lands almost throughout India, ascending to 1800 m in the Himalayas. Locally known as *Bhringaraja*[1] [Figure 1], one of the famous drugs of Ayurveda, it is reported to be used in hair oil preparation.[2] The plant is claimed for its carminative and tonic action; and used for the management of diarrhea, dysentery, dyspepsia, in pestilent fever, elephantiasis and as a flavoring agent of food and perfuming.[3-5] Different types of functional groups such as alkaloid, tannin, triterpenoid (steroid), flavonoid, phenols and essential oil has been reported from *L. rugosa*.[4,6-9] The plant though highlighted for its use in many diseases, caused due to microbes, but has not been evaluated for its antimicrobial activities. Hence, the present study was designed to evaluate the antimicrobial properties of *Limnophila rugosa* leaf.

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Collection and authentication of plant material

L. rugosa Roth. (Merr.) (Scrophulariaceae), locally called as *Bhringaraja*, was collected from its natural habitat of Gandhamardan hill ranges, Bargarh, Odisha, India, in fully matured condition, with the help of local traditional healers. The correct identity and authenticity of the plant *L. rugosa* was done by studying its morphological characters and comparing them with the characters mentioned in various floras.[2,10-12] Plants were washed properly under running water to make it free from foreign matter such as sand, soil etc., Whole plant was dried under shade, powdered to 60# and few were preserved in solution of AAF (70% Ethyl alcohol: Glacial acetic acid: Formalin) in the ratio of (90:5:5).[13] Herbarium was also prepared and submitted to Pharmacognosy laboratory museum of Institute for Postgraduate Teaching and Research in Ayurveda, Jamnagar vide Herbarium no. 6003.

Materials and Methods

Preparation of plant extract

Powdered sample was extracted with methanol for 24 h (shaking for 18 h frequently and then kept aside as a

stand by for 6 h). Then extracts were filtered and methanol was added to prepare solutions with different concentrations 5, 25, 50, 100 and 250 μg/ml. The extract was coded as *Limnophila rugosa* leaf methanol (LRLM).

Test microorganisms and growth media

The microorganisms and growth media were selected following standard guidelines.[14-18]

Selection of microorganisms

Staphylococcus aureus (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) and fungal strains *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323), *Candida albicans* (MTCC 227) were chosen based on their clinical and pharmacological importance. The bacterial strains, obtained from Institute of Microbial Technology, Chandigarh, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 h at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, Gujarat, India) respectively following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar plates at 37°C (The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Antimicrobial activity

Determination of zone of inhibition method

In vitro antimicrobial activity testing was carried out by using Agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of zone of influence, pure Gram-positive, Gram-negative and fungal strain antibiotics were taken as a standard for comparison of the results. All the extracts were screened for their antibacterial and antifungal activities against the *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes* and the fungi *C. albicans*, *A. niger*, and *A. clavatus*. The sets of five dilutions (5, 25, 50, 100 and 250 μg/ml) of LRL extract and standard drugs were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (108 cfu) and allowed to stay at 37°C for 3 h. Control experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin and norfloxacin for antibacterial activity; nystatin and griseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18-24 h of in incubation at 37°C for bacteria and 48-96 h for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks and values <8 mm were considered as not active against microorganisms.

Results and Discussion

Microbial load

The observations on the microbial load of LRLM showed that the tasted samples, when collected from their natural sources, are either free or within prescribed limit of the microbes [Table 1].

Antimicrobial activity

The result of antimicrobial activity of LRLM extract studied in different concentrations (5, 25, 50, 100 and 250 μg/ml) are presented in Table 2 and 3 and antibacterial and antifungal potential of standard drugs presented in Table 4.

The antibacterial and antifungal activities of the LRLM increased linearly with the increase in concentration of extracts (μg/ml). When compared with standard drugs, the results revealed that in the extracts for bacterial activity, *S. pyogenes and S. aureus* were more sensitive when compared to *E. coli* and *P. aeruginosa*, and for fungal activity *C. albicans* was more inhibited as compare to *A. niger* and *A. clavatus*. The growth inhibition zone measured ranged from 11-20 mm for all the sensitive bacteria and ranged from 13 to 19 mm for fungal strains [Tables 2-4].

Table 1: Microbial load report of LRLM

Test parameter	LRL	Limit
Total microbial count	40 cfu/g	100 cfu/g
Total bacterial count	30 cfu/g	
Total fungal count	10 cfu/g	
Pathogens		
E. coli	Absent	Should be absent per 10 g
Salmonella spp.	Absent	
P. aeruginosa	Absent	
S. aureus	Absent	

LRLM: *Limnophila rugosa* leaf methanol extract, *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa*

Table 2: Antibacterial activity of LRLM, ampicillin and chloramphenicol against gram +ve and gram –ve organisms

Sample	Standard drugs														
LRLM extract						Ampicillin					Chloramphenicol				
Test organism	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Gram –ve															
E. coli MTCC 443	-	12	15	16	17	14	15	16	19	20	14	17	23	23	23
P. aeruginosa MTCC 424	-	13	14	15	17	14	15	15	18	20	14	17	18	19	21
Gram +ve															
S. aureus MTCC 96	-	13	16	17	19	10	13	14	16	18	12	14	19	20	21
S. pyrogenes MTCC 442	-	12	13	16	18	11	14	16	18	19	10	13	19	20	20

LRLM: *Limnophila rugosa* leaf methanol extract, *E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, S. pyrogenes: Streptococcus pyogenes*

Table 3: Antibacterial activity of LRLM, ciprofloxacin and norfloxacin against gram +ve and gram –ve organisms

Sample	Standard drugs														
LRLM extract						Ciprofloxacin					Norfloxacin				
Test organism	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Gram –ve															
E. coli MTCC 443	-	12	15	16	17	20	23	28	28	28	22	25	26	27	29
P. aeruginosa MTCC 424	-	13	14	15	17	20	23	24	26	27	18	19	21	23	23
Gram +ve															
S. aureus MTCC 96	-	13	16	17	19	17	19	21	22	22	19	22	25	26	28
S. pyrogenes MTCC 442	-	12	13	16	18	16	19	21	21	22	18	19	20	21	21

E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, S. pyrogenes: Streptococcus pyogenes, LRLM: Limnophila rugosa leaf methanol extract

Table 4: Antifungal activity of LRLM, griseofulvin and nystatin against fungal strains organisms

Sample	Standard drugs														
LRLM extract						Griseofulvin					Nystatin				
Test organism	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
A. niger MTCC 282	-	13	14	17	19	19	23	25	25	28	18	19	24	29	29
A. clavatus MTCC 132	-	14	15	19	21										
C. albicans MTCC 227	-	12	14	17	18	18	21	22	22	24	18	21	24	25	26

LRLM: *Limnophila rugosa* leaf methanol extract, *A. niger: Aspergillus niger, A. clavatus: Aspergillus clavatus, C. albicans: Candida albicans*

Figure 1: Photograph of *Bhringaraja (Limnophila rugosa)*

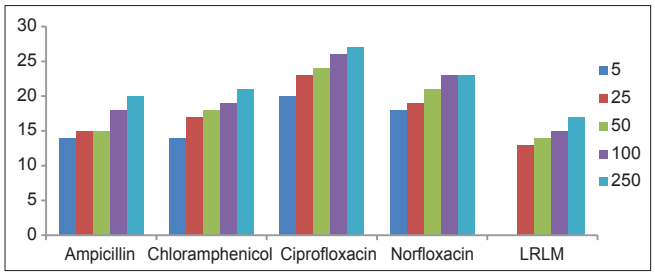

Figure 3: Effect of standard drugs and test drug against *Pseudomonas aeruginosa*

The inhibitory effect of LRLM showed at (25, 50, 100, 250 µg/ml) were (12, 15, 16, 17) against *E. coli* MTCC 443, (13, 14, 15, 17) against *P. aeruginosa* MTCC 424, (13, 16, 17, 19) against *S. aureus* MTCC 96, (12, 13, 16, 18) against *S. pyrogenes* MTCC 442, (13, 14, 17, 19) against *A. niger* MTCC 282 and (12, 14, 17, 18) against *C. albicans* MTCC 227 [Figures 2-7].

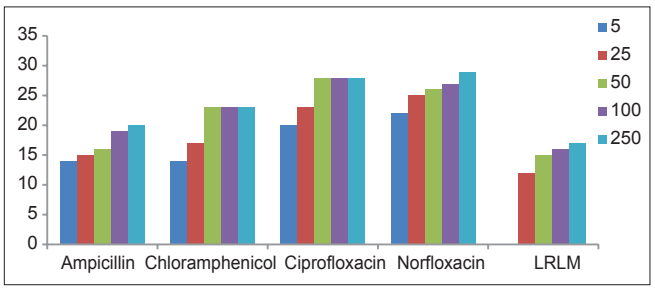

Figure 2: Effect of standard drugs and test drug against *Escherichia coli*

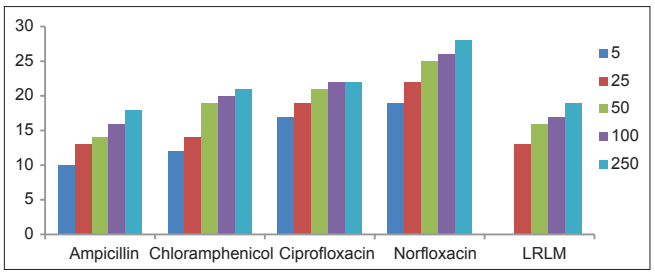

Figure 4: Effect of standard drugs and test drug against *Staphylococcus aureus*

The results showed that the extracts of all samples were found to be more effective against all the microbes tested, which may be due to the reported phyto constituents present in the plant.

Conclusion

The present study justified the claimed ethnic uses of *L. rugosa* in the preparation of hair oil, to treat various infectious disease caused by the microbes. However, further studies are needed, on different extract and concentrations,

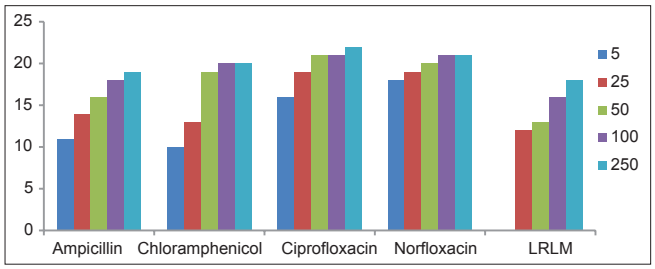

Figure 5: Effect of standard drugs and test drug against *Streptococcus pyrogenes*

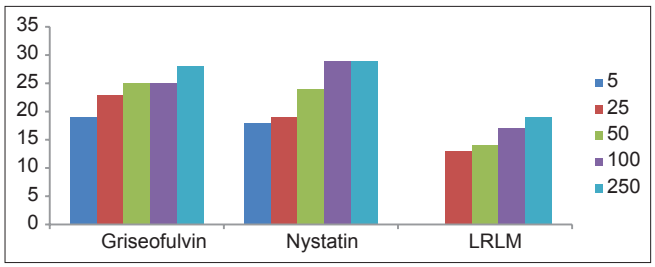

Figure 6: Effect of standard drugs and test drug against *Aspergillus niger*

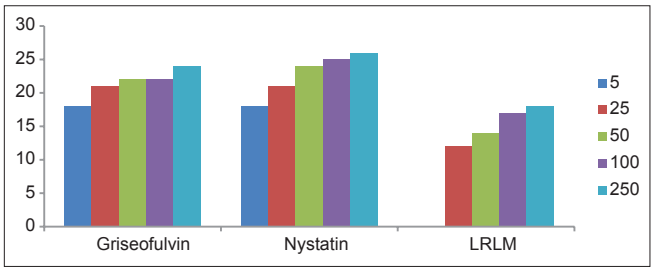

Figure 7: Effect of standard drugs and test drug against *Candida albicans*

to better evaluate the potential effectiveness of the crude extracts as antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural anti-microbial drugs.

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हिन्दी सारांश

लिम्नोफिला रूगोसा पत्र का माईक्रोबियल परीक्षण

रबिनारायण आचार्य, रिद्धीश एच. पडीया, ईशा डी. पटेल, हरीशा सी.आर., विनय जे. शुक्ला

लिम्नोफिला रूगोसा रोथ.(मेर्र.) (स्क्रोफुलारीएसी) पत्ते के मिथॅनॉलिक सत्व को निकाल कर चिकित्सकीय दृष्टी से महत्वपूर्ण मानव रोगजनक जीवाणू (दो ग्राम पॉजिटिव – एस. औरिअस, एस.पायरोजेन और दो ग्राम नेगेटिव – ई.कोलाई, पी.एरूजिनोसा) और तीन कवक उपभेदों (ए. नाइजर, ए. क्लेवेट्स, सी. अल्बिकन्स) के खिलाफ, (५, २५, ५०, १००, २५० माइक्रोग्राम/मी.ली.) के विभिन्न मात्रा में घोलकर डिस्क प्रसार विधि का उपयोग करके अध्ययन किया गया । जीवाणू उपभेदों के क्षेत्र रोधी गतिविधि के लिए एम्पीसिलीन, सिप्रोफ्लोक्सासिन, नोरफ्लोक्सासिन, क्लोरॅम्फेनिकोल; कवक उपभेदों के लिए ग्रेसिओफुल्विन और निस्टॅटिन जैसे विभिन्न मानकों के साथ तुलना की गयी । परिणाम से पता चलता है कि, लिम्नोफिला रूगोसा पत्ते के मिथॅनॉलिक सत्व मे जीवाणू तथा कवक विरोधी क्षमता है ।