Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2013, 4(1), 27-33

Evaluation of antimicrobial activity of *Limnophila Heterophylla* (roxb.) Benth. (Scrophulariaceae) whole plant

Research article

Padiya RH[1]*, Patel ED[1], Acharya RN[2]

1. Ph.D. Scholar, Dept. of Dravyaguna, 2. Associate Professor, Dept. of Dravyaguna, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat – 361 008.

Abstract

Antibacterial and antifungal activity of the *Limnophila heterophylla* (Roxb.) Benth. whole plant methanol extract against four pathogenic bacterial strains two Gram positive (*B. subtilis* & *S. aureus*), two Gram negative (*E. coli and K. pneumonia*) and two fungal strains (*S. flavus* & *C. albicans*) in different concentrations (5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml, 250µg/ml), were evaluated following standard procedure. A zone of inhibition of extract was compared with that of different standards like Streptomycin, Cefpodoxime and Gentamycin for antibacterial activity, Amphotericin B, Fluconazole and Clotrimazole for antifungal activity. The extract showed remarkable inhibition of antibacterial and antifungal activities comparable with that of standard against the organisms tested.

Keywords: Antifungal activity, Gandhamardan hills, In vitro antibacterial activity, *Limnophila heterophylla* whole plant, Microbial load

Introduction:

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. In the past few decades, the search for new anti-infection agents has occupied many research groups in the field of Ethnopharmacology (1). Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (2). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (3). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (4). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (5). India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. The Botanical Survey of India has accounted the availability of 220 plant species of medicinal significance in the

*Corresponding Author:
Padiya Riddhish,
Ph. D. scholar,
Department of Dravyaguna,
IPGT&RA,
Gujarat Ayurved University
Jamnagar.
E.mail : riddhish.padiya@gmail.com

Gandhamardan hills, Odisha. Local people, however, claim that there are more than 500 species of important medicinal plants in this Gandhamardan hills range (6). From this, hill ranges we found ethnic people use *Limnophila heterophylla* (Scrophulariaceae) in hair oil preparation (7). Nevadensin (5,7-dihydroxy-6,8,4'-trimethoxyflavone), natural flavonoid, present in *Limnophila heterophylla* is reported for antibacterial activity against some test organisms (8).

Materials and Method

Collection of plant materials

The plant, commonly known by tribal people as 'Ambakasia' is growing in different parts of Odisha (9). It was identified as *Limnophila heterophylla* (Roxb.) Benth., belonging to family Scrophulariaceae, by studying the morphological characters of its various parts and comparing them with the various characters mentioned in various floras and herbarium(10-13). The plants were shaken to remove adherent soil, dirt etc. and washed with water, wherever required and herbarium specimen was prepared (Herbarium No. 6064) and was stored in Pharmacognosy department, for further documentation.

Sample preparation:

For the analysis, *Limnophila heterophylla* (Roxb.) Benth. whole plant was shade dried and was coarsely powdered to 60# and kept in airtight glass jar bottle for future use.

Extract preparation:

1g of *Limnophila heterophylla* whole plant powder was extracted with methanol by sonicating it for 10 min and then keeping it overnight. Next day after filtration, methanol evaporated, then by taking weight of residue, 5 different concentrations 5 µg/ml, 25 µg/ml, 50 µg/ml, 100µg/ml, 250µg/ml of the sample, were prepared. These are used for determination of antimicrobial activity and coded as LHWM.

Determination of microbial load for plant material

Microbial load of the sample was done by total viable aerobic count method (14-15).

To 500 mg, accurately weighed sample, 1-2 drops of Tween80 and a homogeneous suspension was prepared by slowly adding 5 ml of sterile buffered sodium chloride peptone (SBSCP) solution of pH 7.0. This suspension was diluted 10^{-1} onwards as required in sterile dilution blanks (SBSCP). One ml each from these aliquots was added to sterile melted and cooled top agar (Soyabean casein digest agar, for fungal count Potato dextrose agar medium used) tubes. These tubes were poured to sterile petridish and allowed to solidify. These plates were incubated at 30-35^{0}C for 48 hours. The numbers of colonies were counted and the results were expressed in Cfu / g.

$$\text{Cfu / g} = \frac{\text{Number of average colonies}}{\text{Dilution x Volume plated}}$$

Antimicrobial activity of plant materials (16)

Culture conditions: The antimicrobial efficacy of these plant materials tested on 6 different strains, 2 Gram positive bacteria namely *Bacillus subtilis* (NCIM 2063) & *Staphylococcus aureus* (NCIM 2079), 2 Gram negative bacteria namely *Escherichia coli* NCIM 2065 and *Klebsiella pneumoniae* (NCIM 2719). Two Fungal strains namely *Aspergillus flavus* (NCIM 1028) & *Candida albicans* (NCIM 3471). All cultures were obtained from NCL, Pune. 24 hour old cultures of all these organisms were inoculated in sterile broths and incubated till 0.5 Mcfarland standard turbidity obtained, and then used for assay.

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2013, 4(1), 27-33

Antimicrobial assay (17)

Sterile soybean casein digest agar (25 ml/plate) used for antibacterial activity and sterile sabouraud agar (25ml per plate) used for antifungal activity. Medium obtained from Himedia laboratories. Sterile 20 ml medium poured in sterile plates aseptically and let them solidified. Then inoculate 0.5 ml of culture in 5 ml sterile melted and cooled medium and poured them on solidified agar plates aseptically. After solidification made well with the help of cup borer and inoculate 0.3 ml of each sample in the well and for antibiotic discs there is no need to make wells and directly place disc on agar surface aseptically. For diffusion purpose plates were placed in refrigerator for 20-25 minutes. Then Incubate plates at 37^0 C for 24 hrs except sabouraud agar plates and plates containing *K. pneumoniae* organism, they incubated at 30^0 C for 24-48 hrs. After incubation zone of inhibition was measured with Himedia antibiotic zone scale- c.

Pathogen study (18)

Same extracts were used as for antimicrobial activity assay, these extracts were transferred to specialized mediums given below and incubated at their optimum temperature for growth, then after incubation plates were observed and results were concluded.

Selective differential mediums according pathogens:

- *Pseudomonas aeruginosa* – Citrimide agar
- *Salmonella typhi* – TSI agar slant, XLD agar
- *Escherichia coli* – EMB agar
- *Staphylococcus aureus* – Mannitol salt agar

Results and Discussion

The observations on the microbial load of *Limnophila heterophylla* (Roxb.) Benth. whole plant showed that the tasted samples, when collected from their natural sources, are either free or within prescribed limit of the microbes (19). (Table 1)

Table 1 : Microbial load report of *Limnophila heterophylla* whole plant

Parameter	**Sample**	**Permissible Microbial contamination limits(19)**
	***L. heterophylla* whole plant**	
Description	Greyish coloured powder	
Total Viable Aerobic Count (Cfu/g)		
a) Bacterial count	7.3×10^3	10^5/g
b) Fungal count	10	10^3/g
Pathogens (per gram)		
a) *S. aureus*	Absent	Absent
b) *E. coli*	Absent	Absent
c) *P. aeruginosa*	Absent	Absent
d) *S. typhi*	Absent	Absent

Antimicrobial activity:

The antimicrobial activity of methanol extracts of *Limnophila heterophylla* whole plant was studied in different concentrations (5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml,

250µg/ml) against four pathogenic bacterial strains (two Gram positive *B.subtilis* NCIM 2063 & *S.aureus* NCIM 2079; two Gram negative (*E. coli* NCIM 2065, *K. pneumoniae* NCIM 2719) and two fungal strains (*S. Flavus* NCIM 1028 and *C.albicans* NCIM 3471). Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition(ZOI).

Table 2: Antibacterial activity of methanol extracts of *Limnophila heterophylla* whole plant, Gentamycin, Cefpodoxime and Streptomycin against Gram +ve and Gram -ve organisms

Sample	Concentration	Zone of inhibition (mm)			
		Gram positive strain		Gram negative strain	
		B.subtilis (NCIM 2063)	*S.aureus* (NCIM 2079)	*E.coli* (NCIM 2065)	*K.pneumoniae* (NCIM 2719)
L. heterophylla whole plant	5 µg/ml	13	Nil	14	13
	25 µg/ml	14	Nil	16	14
	50 µg/ml	15	12	18	15
	100 µg/ml	16	13	20	19
	250 µg/ml	18	14	22	21
Methanol (Control)	-	Nil	Nil	Nil	Nil
Gentamycin	10 µg	28	25	22	24
Cefpodoxime	10 µg	22	23	21	19
Streptomycin	10 µg	27	17	17	24

Table 3: Antifungal activity: Methanol extracts of the sample were used for study

Sample	Concentration	Zone of inhibition (mm)	
		5 *flavus* (NCIM 1028)	*C. albicans* (NCIM 3471)
L. heterophylla whole plant	5 µg/ml	Nil	14
	25 µg/ml	12	15
	50 µg/ml	13	16
	100 µg/ml	13	17
	250 µg/ml	14	19
Methanol (Control)	-	Nil	11
Amphotericin B 50 µg	10 µg	14	19
Fluconazole 30 µg	10 µg	11	28
Clotrimazole 10 µg	10 µg	24	30

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2013, 4(1), 27-33

Figure 1: Effect of *L. heterophylla* whole plant methanol extract against gram +ve and gram -ve strains

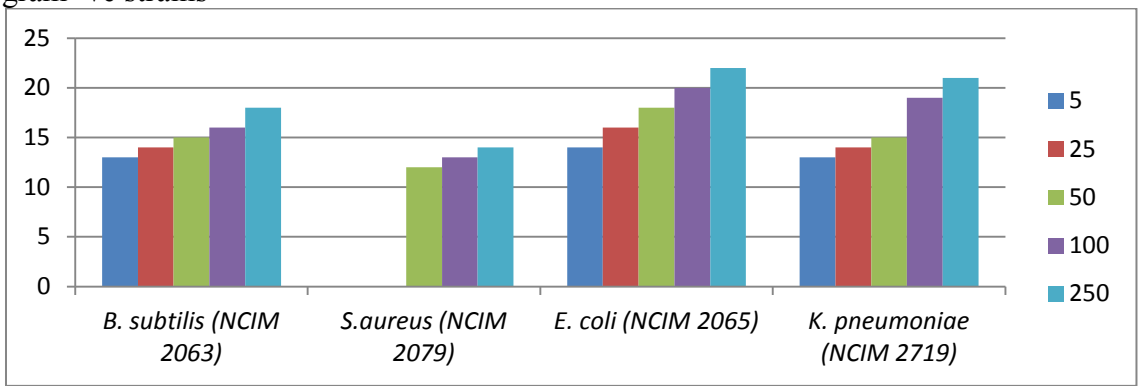


Figure 2: Effect of standard drug against gram +ve and gram -ve strains

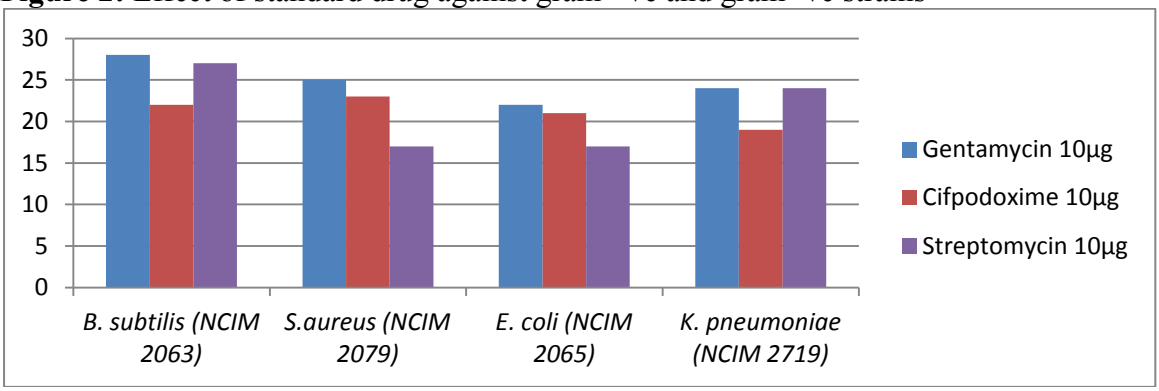


Figure 3: Effect of *L. heterophylla* whole plant methanol extract against two fungal strains

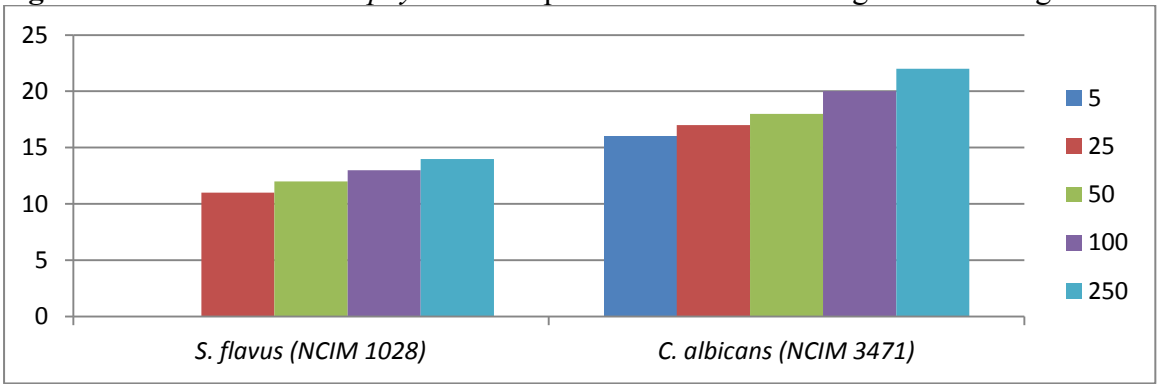
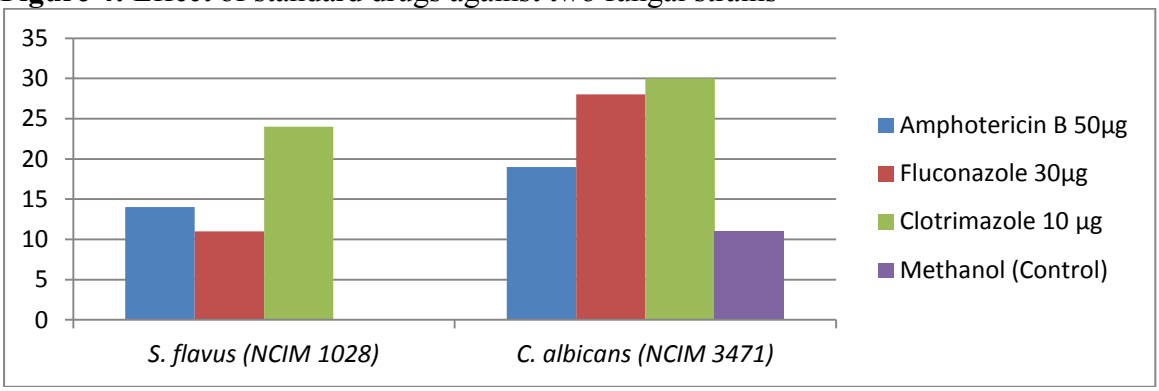


Figure 4: Effect of standard drugs against two fungal strains



The result shows that the extracts of all samples were found to be more effective against all the microbes tested.

The antibacterial and antifungal activity of the LHWM increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, *E. coli* and *B. subtilis* were more sensitive as compared to *K. pneumoniae and S. aureus*, and for fungal activity. *C. albicans* showed good result as compare to *S. flavus* but *S. flavus* is more sensitive. The growth inhibition zone measured ranged from 12-22 mm for all the sensitive bacteria, and ranged from12-19 mm for fungal strains.

The inhibitory effect of LHWM showed at (5, 25, 50, 100, 250µg/ml) were (14, 16, 18, 20, 22mm) against *E.coli* NCIM 2065, (13, 14, 15, 19, 21mm) against *K. pneumoniae*, (00, 00, 12, 13, 14mm) against *S.aureus* NCIM 2079, (13, 14, 15, 16, 18mm) against *B.subtilis* NCIM 2063, (00, 12, 13, 13, 14mm) against *S. flavus* NCIM 1028, (14, 15, 16, 17, 19mm) against *C.albicans* NCIM 3471.

Conclusion:

In the present work, the extracts with different concentration found sensitive against all the tested bacterial and fungal strains and justified the claimed ethnic uses of these plants. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. It may form the basis for selection of plant species for further investigation, in the potential discovery of new natural bioactive compounds.

Acknowledgement

We express our thankfulness to Mr. Hota BN, Rtd. DFO, Govt. of Odisha; Mr. Govind Baba, Traditional practitioner; Mr. Pareswar Sahu, Pharmacognosy expert; Mr. Malaya Das, Forest Range Officer, Govt. of Odisha and other traditional healer who helped us during drug collection at Gandhamardan hills, Bolangir and Bargarh, Odisha. Authors are thankful to Dr Subrata De, Dr. Pankaj Nariya and Ms. Rinkal Rana, RMD Research Centre, Waghaldhara, Gujarat, for providing facilities for antimicrobial studies.

Reference:

1. Recio M. C, R´ıos J. L, Villar A. A review of some antimicrobial compounds isolated from medicinal plants reported in the literature. Phytotherapy Research; 1978–88; 3; 117–125p.
2. Westh H, Zinn C. S, Rosdahl V. T et al. An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. Microb Drug Resist; 2004. (10); 169-176p.
3. Bandow J. E, Brotz H, Leichert LIO et al. Proteomic approach to

understanding antibiotic action. Antimicrob Agents Chemother; 2003. 47; 948-955p.
4. Rojas R, Bustamante B, Bauer J et al. Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol; 2003. 88: 199- 204p.
5. Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). Lebensm-Wiss u-Technol; 2004. 37: 263-268p.
6. http://himanshu-guru.blogspot.in/2011/08/miraculous-aromatic-plant-recognized-at.html.
7. Brahmam M, Saxena H. O. Ethnobotany of Gandhmardan Hills – Some Noteworthy Folk-Medicinal Uses. Ethnobotany, Vol.2; 1990. 71–79p.
8. Brahmachari G. Nevadensin: Isolation, chemistry and Bioactivity. Review article, International Journal of Green Pharmacy, October-December; 2010. 213-219p.
9. Saxena H. O, Brahman M. The flora of Orissa, vol. III, Regional Research Laboratory Bhubaneswar, Orissa.(India); Orissa Forest Development Corporation Ltd., Dec 1995. 1237p.
10. Hooker J. D. The flora of British India, Vol. IV. Dehradun, India, (London); Bishen Singh Mahendra Pal Singh, 1885. 394-395p.
11. Haines HH, The Botany of Bihar and Orissa, part III-IV. Dehradun (India); Bishen Singh Mahendrapal Singh, 1988. 659p.
12. RPRC Digital herbarium, Regional Plant Resource Centre, Bhubaneshwar. (http://www.rprcbbsr.com/herbarium/herbarium/images/id/443)
13. Saxena H. O, Brahman M. The flora of Orissa, vol. III, Regional Research Laboratory Bhubaneswar, Orissa. (India); Orissa Forest Development Corporation Ltd, 1995. 1237p.
14. Anonymous. Indian Pharmacopoeia. Delhi: Government of India, Ministry of Health and Family Welfare— Controller of Publications, 1996; 1(1) 37-43p.
15. Anonymous. Quality control methods for herbal materials, W.H.O Monograph for limitation of microbes, WHO Press, World Health Organization, 20-Avenue Appia, 1211 Geneva 27; Switzerland, 1998. 75p.
16. Anonymous. Indian Pharmacopoeia. Delhi: Government of India, Ministry of Health and Family Welfare— Controller of Publications, 1996; 1(1) 37-39p.
17. Dorman H. J. D, Deans S. G. Antimicrobial agents from plants. Antimicrobial activity of plant volatile oils, Journal of Applied Microbiology; Feb-2000.88(2), 308–316p.
18. Anonymous. Indian Pharmacopoeia. Delhi: Government of India, Ministry of Health and Family Welfare— Controller of Publications, 1996. 1(1); 43-49p.
19. Anonymous. The Ayurvedic Pharmacopoeia of India. Part-II, Volume-II, First edition, Ministry of Health and Family Welfare, Government of India, Department of Indian Systems of Medicine & Homoeopathy, 2008; 2(2):199p.

*****