

Original Article

Studies on the elemental composition and anti fungal activity of medicinal plant *Lippia nodiflora* L against skin fungi

A.J. Pirzada*, P. Iqbal**, W. Shaikh*, T.G. Kazi†, K.U. Ghani‡

* Department of Botany, University of Sindh, Hyderabad

** Department of Dermatology, Liaquat University Hospital, Hyderabad.

† Center of Excellence in Analytic Chemistry, University of Sindh, Hyderabad

‡ Faculty of Eastern Medicine, Hamdard University, Karachi.

Abstract *Background* *Lippia nodiflora* L, an indigenous herb has many medicinal uses.

Objective Present work was carried out to study the antifungal activity of crude extracts of *L. nodiflora* L. against the human pathogenic fungi.

Materials and methods Crude extracts from the leaves and shoots of plant were prepared in different solvents including ethanol, methanol, ethyl acetate, chloroform and water and tested for antifungal activity against *Aspergillus niger*, *A. flavus*, *Paecilomyces varioti*, *Microsporum gypseum*, and *Trichophyton rubrum*. Concentration of various elements e.g. Al, Ca, Cu, Fe, Mg, Mn, P, S and Zn were determined by using atomic absorption spectrophotometer and UV spectrophotometer.

Results All crude extracts including ethanol, methanol, ethyl acetate, chloroform and aqueous extracts showed high activity against test organisms. Ethanol and aqueous extracts appeared to be the most effective antifungal agents as compared to methanol, chloroform and ethyl acetate. Moreover, the plant *L. nodiflora* L has high concentration of various essential elements.

Conclusion The medicinal plant *Lippia nodiflora* L contains considerable amount of elements which are important component of many formulation, used in skin care.

Key words

Lippia nodiflora L, antifungal activity

Introduction

Lippia nodiflora L (*Verbenaceae*) is a widely creeping perennial herb, rooting at nodes leaves opposite, spatulate, subsessile, 2-3.5 cm long 1-2 cm broad

toothed towards the rounded apex; peduncles 2.5-7.5 cm long with 1-3 cm long flowering heads; flowers white or pinkish about 3 mm long; fruit about 1.6 mm long globose, oblong, glabrous, splitting into 2 one seeded pyrenes.¹⁻⁴

Address for correspondence

Dr. AJ Pirzada,
Department of Botany,
University of Sindh,
Hyderabad, Sindh, Pakistan

L. nodiflora L, commonly known as Booken, is a traditional medicine in many parts of Pakistan and used for the treatment

of various dermatomycoses like tinea capitis, tinea pedis, tinea manuum, tinea corporis etc. The plant is acrid, cooling, aphrodisiac, astringent to the bowels, stomach, vulnerary, antihelminthic, useful in diseases of the heart, blood pressure, the eye sore, improver of taste, ulcer, wounds, burning sensation, asthma, bronchitis, increased thirst, loss of consciousness (Ayurveda). The plant is hot and dry, diuretic, useful in fever and in urinary concretions (Yunani).^{3,5-9}

It is also well established that elements play a vital role in the health and disease of human body. The amount of minerals and trace elements in plants is so small that their presence and importance were almost ignored for a long time. Experimental evidence has proved that they play a key role in nutritive, catalytic and physiological balancing function.¹⁰

In the current study, we aimed to evaluate the antifungal activity of *L. nodiflora* L against human pathogenic fungi isolated from superficial dermatomycoses.

Materials and Methods

Plant material

The *L. nodiflora* L leaves and shoots were collected from different areas of Kohistan Region, District Dadu, and reference samples were identified through literature on flora of Pakistan.⁶ The collected plant material was washed with distilled water and placed in shade at room temperature for two weeks. One kg of dried plant material was dipped in five liters of ethanol solvent in bottle for 20 days for cold percolation. The extract was then filtered and concentrated

under reduced pressure below 40°C using rotary evaporator. The residue was completely dried and then converted into powdered form. From the residue, five different extracts using ethanol, ethyl acetate, chloroform, methanol and distilled water were prepared using separating funnel. The extracts were left at room temperature. The solvent portion was completely evaporated and organic compounds remaining in dry form were mixed with sterilized water (1 g:5 ml), respectively. Each extract was tested for antifungal activity.

Collection of fungi

Different fungi, namely *Aspergillus niger*, *A. flavus*, *Paecilomyces varioti*, *Microsporum gypseum*, *Trichophyton rubrum* were collected from patients of superficial dermatomycoses at the out-patient Department of Dermatology, Liaquat University Hospital, Hyderabad, by scraping different parts of body.

Preparation of culture for fungi

Sabouraud's glucose agar was used to culture the fungi. Following composition was used for this purpose: peptone 10g, glucose 20g, agar 20g and distilled water 1000 ml with pH 5.4. All the contents were mixed and dissolved in distilled water. The solution was then autoclaved at 120°C, 15 lb/inch² pressure for 20 minutes.

Treatment of different solvent extract layers

The human skin pathogens were treated with different extracts and results were taken after 72 hours at 30°C.¹¹

Methodology for determination of elements

Experimental

A suitable dissolution method for biological sample to yield homogenous solution is a crucial first step in atomic absorption spectrophotometer and UV technique. The decomposition of organic matter must be completed to avoid interference by organic residue. For this purpose, samples were digested with nitric acid and 30% hydrogen peroxide solution.

* Appropriate working standard solution of aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), zinc (Zn) were prepared from stock standard solution (1000 ppm) in 2N nitric acid.

* Calibration curves were drawn for each elements using atomic absorption spectrophotometer (Hitachi model 180-50®).

* The calibration curves obtained for concentration absorbance data were statistically analyzed using fitting of state line by least square method. All elements were determined in medicinal plant under investigation.

* A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements.

Percentage recovery test

The efficiency of extraction method was checked by standard addition method. The sample was spiked with known standards and digested with nitric acid and hydrogen peroxide mixture.

- The matrix of standard and sample solution was the same.

- The percentage recovery test for different elements by digestion method adopted was 98.5-99% in range.

Results

All crude extracts had significant antifungal activities against most of the fungi, but the activity of inhibition varied for the fungi with respect to the type of plant extract (**Table 1**).

Ethanol extract

The maximum inhibition activity was observed against test fungi, *T. rubrum*, *A. niger*, *P. varioti*, *A. flavus*, *M. gypseum* (100%) each.

Methanol extract

The maximum inhibition activity was observed against *A. flavus*, *P. varioti* and *A. niger* as 100%, 99%, 96%, respectively, while moderate inhibition activity against *T. rubrum* (82%) and minimum inhibition activity against *M. gypseum* (75%) were seen.

Chloroform extract

The maximum inhibition activity was observed against *P. varioti* (87%) while moderate inhibition activity was recorded against *A. flavus*, *A. niger* and *M. gypseum* in 79%, 78%, 77%, respectively and minimum inhibition activity against *T. rubrum* (61%) were observed.

Ethyl acetate extract

The maximum inhibition activity was observed against *T. rubrum*, *P. varioti*, (95.7% and 87%, respectively), with moderate inhibition activity against *A. niger*

Table 1 Antifungal activity of different extracts of *L. nodiflora* L against test organisms.

Test extract	<i>Aspergillus niger</i>	<i>A. flavus</i>	<i>P. varioti</i>	<i>Microsporium gypseum</i>	<i>Trichophyton rubrum</i>
<i>Ethanol</i>					
Controlled reading at 30°C after 72 hours (cm)	18	10.5	30.74	12.9	5.75
Inhibited reading at 30°C after 72 hours (cm)	0.0	0.0	0.0	0.0	0.0
Inhibited (%)	100	100	100	100	100
<i>Methanol</i>					
Controlled reading at 30°C after 72 hours (cm)	18	10.5	30.34	12.9	5.75
Inhibited reading at 30°C after 72 hours (cm)	0.64	0.0	0.25	3.34	1.0
Inhibited (%)	96	100	99	75	82
<i>Chloroform</i>					
Controlled reading at 30°C after 72 hours (cm)	18	10.5	30.34	12.9	5.75
Inhibited reading at 30°C after 72 hours (cm)	4.0	2.25	4.0	3.0	2.25
Inhibited (%)	78	79	87	77	61
<i>Ethyl acetate</i>					
Controlled reading at 30°C after 72 hours (cm)	18	10.5	30.74	12.9	5.75
Inhibited reading at 30°C after 72 hours (cm)	4.0	9.0	4.0	5.0	2.5
Inhibited (%)	78	31	87	61	55.7
<i>Aqueous</i>					
Controlled reading at 30°C after 72 hours (cm)	18	10.5	30.74	12.9	5.75
Inhibited reading at 30°C after 72 hours (cm)	0.20	0.0	1.7	2.25	0.25
Inhibited (%)	99	100	94.5	82.6	95.7

Table 2 Quantity of different elements in *L. nodiflora* L.

Name of elements	Amount (mg/kg)
Aluminum	3.88-4.39
Calcium	32455.49-33939.17
Copper	18.59-19.66
Iron	91.61-122.67
Magnesium	18429.39-18644.43
Manganese	21.57-22.40
Phosphorus	2834.09-2913.53
Sulphur	807.67-830.73
Zinc	52.81-57.79

and *M. gypseum* (78% & 61%), respectively and minimum inhibition activity against *A. flavus* (31%) was observed.

Aqueous extract

The maximum inhibition activity was observed against *A. flavus* and *A. niger* (100%, 99%), respectively with moderate inhibition activity against *T. rubrum* and *P. varioti*, (95.7%, 94.5%), respectively. and minimum inhibition activity against *M. gypseum* (82.6%) was observed.

Considerable amount of essential elements i.e.: Al, Ca, Cu, Fe, Mg, Mn, P, S, Zn was found in the extracts from *L. nodiflora* L (Table 2).

Discussion

In the present study, crude extracts of the plant material obtained in polar and less polar organic solvents were tested against fungi causing skin diseases. All crude extracts had significant inhibition activity against most of the fungi. Ethanol extract had maximum inhibition activity (100%) against test organisms as compared to aqueous, chloroform, methanol and ethyl acetate extracts. The aqueous, methanol and chloroform extract had very active inhibition activity 82.6%-100%, 75%-100%, 61%-87%, respectively against test fungi, while ethyl acetate extract had active inhibition activity against *T. rubrum*, *P. varioti*, *A. niger* and *M. gypseum* (61%-95.7%) but very weak inhibition activity against *A. flavus* (31%).

Many previous studies affirm our findings.¹²⁻¹⁸ It was reported that the chloroform extract of *Cuscuta reflexa* Roxb had minimum inhibition zone against *A. niger*.¹² Water extract of *Chasmanthera dependens* did not inhibit the growth of *A. niger* and *A. flavus*.¹³ The crude extract of *Cassia alata* linn was significantly active against *A. niger*.¹⁴ The crude extract of *Juglans cinerea* L actively inhibited growth of *M. gypseum*.¹⁵ Leaves extract of *Azadirachta indica* A. Juss were very active against *M. gypseum* and *T. rubrum*.¹⁶ Ethanol extract of *Azadirachta indica* A Juss has high inhibition activity against *T. rubrum*.¹⁷ Methanolic extract of some marine algal species had very active inhibition activity against *A.niger*.¹¹ The

crude extract of *Solanum marginatum* actively inhibited against the *T. rubrum*, but no activity was found against *A. niger* and *A. flavus*.¹⁸

Present study is the first attempt to assess the inhibition activity of the medicinal plant *Lippia nodiflora* L against fungi such as *A. niger*, *A. flavus*, *P. varioti*, *M. gypseum* and *T. rubrum* causing different skin diseases. In this study all the solvent extracts very actively inhibited test organisms except ethyl acetate which showed a weak inhibitory activity against *A. flavus*.

Moreover, in the present study nine elements Al, Ca, Cu, Fe, Mg, Mn, P, S and Zn were analyzed, which have therapeutic role in numerous diseases. These minerals may have contributory role in the therapeutic efficacy of this plant. Deficiency of these elements in human body can create many diseases. Calcium is required in connective tissue, bone and teeth spurs. Copper sulphate is probably still used in some countries as mild astringent and antiseptic preparation to treat vitiliginous skin, wounds, alopecia and liver disorders. Magnesium is important for nerves and muscles. It is also used for the treatment of asthma, confusion and depression. Manganese is used in collagen deficiency, asthma and pancreatic atrophy. Phosphorus and sulphur are used for the treatment of scabies and leprosy. Zinc solutions have a soothing and cooling effect. It is used for the treatment of splitting of nails, wound healing, eczema, depression and diabetes. All living cells require iron for growth, replication, respiration and DNA synthesis besides promoting resistance against many diseases. It is used in anemia and memory

disorders. However, aluminum is a toxic element but is used as a skin disinfectant and a cleansing agents.^{2,10,19, 20,21}

This study provides some scientific justification for the utilization of crude extract of *L. nodiflora* L in the treatment of different skin diseases e.g. tinea capitis, tinea corporis, tinea manuum, tinea pedis etc.

References

1. Jafri SM, ed. *Flora of Karachi*. Karachi; Printas; 1969.
2. Janjua KM. Trace element in *Allium cepa* (Onion) and their therapeutic importance. *Hamdard* 1990; **XXXIII** (2): 87-90.
3. Kirtikar KR, Basu BD, eds. *Indian Medicinal Plants I-IV*. Allahabad: Lalit Mohan Basu; 1935.
4. Nasir E, Ali SI, eds. *Flora of Pakistan*. Karachi; Department of Botany, University of Karachi; 1970.
5. Ansari KA, Malik AR, Mahar AO. Survey of medicinal plants in District Khairpur. *Scientific Sindh Annual J Res* 1993; **1**: 19-26.
6. Baquar SR, ed. *Medicinal and Poisonous Plants of Pakistan*. Karachi: Printas; 1989.
7. Pirzada AJ, Abro H *Survey and uses of medicinal plants in Kohistan Region (District Dadu)*. Research Report. Hyderabad: Department of Botany, University of Sindh; 2004.
8. Shahani NM, Memon MI. *Survey and domestication of wild medicinal plants of Sindh, Pakistan*. Research Report. Islamabad: Agricultural Research Council Pakistan; 1988.
9. Sharma R, ed. *Medicinal plants of India: an encyclopedia*. Delhi: Daya Publishing House; 2003.
10. Sahito SR, Memon MA, Kazi TG, Kazi GH. 2003. Evaluation of mineral contents in medicinal plant *Azadirachta indica* (neem). *J Chem Soc Pak* 2003; **25**: 2-5.
11. Ghani KU, Shameel M, ed. *Studies on the antimicrobial activity of certain sea weeds from Karachi coast*. Karachi: Department of Botany University of Karachi Pakistan; 1986.
12. Anjum N, Khan Z. Antimicrobial activity of the crude extract of *Cuscuta reflexa* Roxb. *Pak J Bot* 2003; **35**: 999-1007.
13. Adekunle AA, Okoli SO. Antifungal activity of crude extracts of *Alfia barteri* oliver (Apocynaceae) and *Chasmanthera dependens* (Hochst Menispermaceae). *Hamdard* 2002; **XLV**: 52-6.
14. Sakharkar PR, Patil AT. Antifungal activity of *Cassia alata*. *Hamdard* 1999; **XLI**: 20-2.
15. Christine E, Ficker J, Arnason T, Vindas PS. Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts. *Mycoses* 2003; **46**: 29-37.
16. Thebo NK, Abro H. Antifungal activity of *Azadirachta indica* against human pathogenic fungi. *Sindh Univ Res J* 2000; **32**: 35-42.
17. Natarjan. V, Venugopal PV, Memon T. Effect of *Azadirachta indica* (neem) on the growth pattern of dermatophytes. *Indian J Med Microbiol* 2003; **21**: 2-5.
18. Shaikh W. Shameel M, Hayee-Memon A *et al.* 1990. Isolation and characterization of chemical constituents of *Stoechospermum marginatum* (Dictyotales, Phaeophyta) and their antimicrobial activity. *Pak J Pharm Sci* 1990; **3**: 1-9.
19. Saily A, Sahu R, Gupta B, Sondhi SM. 1994. Analysis of mineral elements of medicinal plants used for the treatment of asthma, syphilis, diarrhea, skin diseases and rheumatism. *Hamdard* 1994; **XXXVII**: 18-22.
20. Soderberg T, Halimans G. Treatment of leprosy with adhesive zinc tapes. *Lepr Rev* 1982; **53**: 271-3.
21. Underwood EJ. Trace metal in human and animal health. *J Hum Nutr* 1981; **53**: 37-48.