Sindh Univ. Res. Jour. (Sci. Ser.) Vol: 40 (2) 79-84 (2008)

Sindh Univ. Res. Jour.

SURJ

ELEMENTAL INVESTIGATION AND ANTI MICROBIAL ACTIVITY OF MEDICINAL PLANT *NERIUM INDICUM* MILL. AGAINST DERMATOPHYTIC FUNGI

A. J. Pirzada, W. Shaikh, A. M. Jakhar, S. I. Shah* and M. P. Pirzada**

Institute of Botany, University of Sindh. Jamshoro, Pakistan

(Received 12th February 2008 and Revised 25th August 2008)

Abstract

Five extracts such as ethanol, methanol, ethylacetate, chloroform and aqueous were obtained from the medicinal plant *Nerium indicum*. These extracts were tested against the Dermatophytic fungi, *Aspergillus niger, Aspergillus flavus, Paecilomyces varioti, Microsporum gypseum, and Trichophyton rubrum*. All crude extracts were found to be effective against test organism. Methanol, ethanol and aqueous extracts appeared to be most effective antifungal agents as compared to chloroform and ethylacetate extract. More over in present study nine elements, Al, Ca, Cu, Fe, Mg, Mn, P,S and Zn have been determined from the medicinal plant, *Nerium indicum*. by using atomic absorption spectrophotometry and U.V spectrophotometry. The medicinal plant *Nerium indicum*. contains considerable amount of elements which have therapeutic effects in skin diseases.

Keywords: The medicinal plant *Nerium indicum* the Dermatophytic fungi, *Microsporum gypseum, and Trichophyton rubrum*

1. Introduction

Nerium indicum, commonly known as Kanur is a traditional medicine which is utilized in many part of Pakistan for the treatment of various fungal skin diseases like *Tinea capitis, Tinea pedis, Tinea manum, and Tinea corporis* etc. The docection is recommended to reduce swellings and oil prepared from the root bark is cure for skin diseases for scaly nature and leprosy. The flower has a bitter sharp taste, good for inflammation, chronic pain in the muscles and the joints, in lumbago, headache and scabies. (Baquar 1989, Kirtikar and Basu 1935).

Metals and its elements as well as their compounds have been used since ancient times for therapeutic as well as cosmetic effects on skin. Aluminum acetate solution is used as skin disinfectant and cleansing agents. Copper sulphate is probably still used in some countries as mild astringent and antiseptic preparation to treat vitiligenous skin. In leprosy seven elements are found to have therapeutic effect such as the use of (Zn) tape for the treatment of leprosy wounds and lotion of zinc solution have a soothing and cooling effects. Calcium, Manganese, Magnesium are used in the formation of collagen, connective tissue and maintains electrical potential of nerve and muscles membrane for the transmission of impulses across junction. Phosphorus and sulpher are used for the treatment of scabies and leprosy. All living cells require iron for growth, replication, respiration and DNA synthesis. It can promote resistance against many diseases included skin disease. (Sahito *et al.*, 2003, soderberg *et al.*, 1982 underwood 1981).

Skin disease is one of the main problem of Sindh province which is usually caused by fungi. The present report describe the antifungal potential of different solvent extract of *N. indicum* against fungi causing skin diseases.

2. Materials and Methods

Plant Material:-

Plant material the *N. indicum*. leaves and shoots were collected from different area of Kohistan region District Dadu and reference

*Seed Certification Officer, Federal Seed Certification and Registration, Department Govt. of Pakistan, Bahawalpur

**Govt. Zubaida Girls College, Hyderabad, Sindh

samples were identified through literature Flora of Pakistan (Ilyas and Ali 1989). The collected plant material were washed with distilled water and placed in shade at room temperature for two weeks. One kg of dried plant material was dip in one litre (1 L) of ethanol solvent in bottle for 10 days for cold percolation. The extract was filtered and concentrated under reduced pressure below 40°C using rotary evaporator. The residue completely was dried as the syrupy liquid form. From the residue five different extracts such as ethanol, ethylacetate, chloroform, methanol and aqueous extract were prepared using separating funnel. The extracts were left at room temperature. The solvent were completely evaporated so the organic compounds remain in the dry form. These extracts so obtained were mixed with the sterilize water (1g/5ml) and each extracts sample was applied for antifungal activity

3. Collection of Dermatophytes

The dermatophytic fungi namely *Aspergillus niger*, *Aspergillus flavus*, *Paecilomyces varioti*, *Microsporeum gypseum*, *Trichophyton rubrum* were scraped from the different body parts of skin of patient departments of Liaquat University Hospital Jamshoro and Hyderabad.

4. Preparation of fungal culture

Sabouraud glucose-agar media, following composition were used for this purpose, Peptone 10g, glucose 20g, Agar 20g, distil water 1000ml with 5.4 pH. All the contents were mixed and dissolved in distilled water. The solution were autoclaved at 120° C, 15 lb/sq inch pressure for 20 minutes.

Treatment of different solvent extract layers:-
The human skin pathogen were treated with different extracts and result were taken after 72 hours at 30°C. The percentage of mycelial inhibition was calculated as follows; Usmanghani and Shaneel (1986), Ali Shtayeh and Suheil (1999).

% Mycilial inhibition = [(dc-dl)/dc]x100

dc=colony diameter in control, dl= colony diameter in treatment

Methodology for Elements determination.

[illegible]

5. Results

All the crude extracts has significant anti fungal activity 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 *A. Flavus* and *T rubrum* 100 % each while moderate inhibition activity against *T. varioti*, *A. niger* and *M. Gypseum* was 96.37%, 90% and 88% respectively. (Table-1).

Methanol extract: The maximum inhibition activity was observed against *T. varioti*, *T rubrum* and *A.niger* 99.1%, 84% and 79% respectively while moderate inhibition activity against *A. flavus*, 71.41% and minimum inhibition activity against *M.gypseum* was reduced 50.1%. (Table-1).

Chloroform extract: The maximum inhibition activity was found against *M. gypseum, and P.varioti* 60% and 54.55% each. While moderate inhibition activity against, *A. niger* 50.%, and minimum inhibition activity against *A. flavus and T.rubrum* 42.85% , 40% was recorded. (Table-1)

Ethylacetate extract: The maximum inhibition activity was noticed against, *P. varioti and M. gypseum* 54.55% and 50 % respectively While moderate inhibition activity against *A. flavus and A.niger* 34.29% and 30.% and minimum inhibition activity against *T. rubrum* 28.% was measured. (Table-1).

Aqueous extract: The maximum inhibition activity was observed against *A.niger T.rubrum, and P varioti* 87.5%, 87.28% and 80% respectively While moderate inhibition activity against *M.gypseum* 50% and minimum inhibition activity against *A.flavus* 48.58 % was determined. **(Table-1).**

Elements:-The considerable amount of various elements such as: Aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), Magnesium (Mg), Manganese (Mn), Phosphorus (P), Sulpher (S), Zinc (Zn) have been determined from the medicinal plant *Nerium indicum* **(Table-2)** These elements are biologically very much important for the treatment of different skin diseases.

Table-1. Antifungal activity of different solvent extract of *N. indicum* Mill against test organisms.

Test extract	*A . niger*	*A flavus*	*P. varioti*	*M. gypseum*	*T. rubrum*
Ethanol					
Controlled reading at 30°C after 72 hours (mm)	40	35	55	30	25
Inhibited reading at 30°C after 72 hours (mm)	04	00	02	06	00
Inhibited (%).	90	100	96.37	80	100
Methanol					
Controlled reading at 30°C after 72 hours (mm)	40	35	55	30	25
Inhibited reading at 30°C after 72 hours (mm)	06	10	06	15	04
Inhibited (%).	75	71.41	89.10	50	84
Chloroform					
Controlled reading at 30°C after 72 hours (mm)	40	35	55	30	25
Inhibited reading at 30°C after 72 hours (mm)	20	20	25	12	15
Inhibited (%).	50	42.86	54.54	60	40
Ethyl acetate					
Controlled reading at 30°C after 72 hours (mm)	40	35	55	30	25
Inhibited reading at 30°C after 72 hours (mm)	28	23	25	15	18
Inhibited (%).	30	34.29	54.55	50	28
Aqueous					
Controlled reading at 30°C after 72 hours (mm)	40	35	55	30	25
Inhibited reading at 30°C after 72 hours (mm)	05	18	07	15	05
Inhibited (%)	87.50	48.58	87.28	50	80

Table-2. Quantity of different elements in *N. indicum* Mill

Name of Elements	Formula	Amount mg/ kg
Aluminum	Al	5.91-6.42
Calcium	Ca	20956.97-22811.57
Copper	Cu	8.26 - 9.69
Iron	Fe	73.49-127.84
Magnesium	Mg	6601.89 - 6863.49
Manganese	Mn	81.00 - 94.3
Phosphorus	P	65.24 - 76.59
Sulphur	S	314.59 - 326.12
Zinc	Zn	31.26 - 44.52

6. Discussion

In the present study crude extracts of the plant material obtained in polar and less polar organic solvent were tested against fungi causing skin diseases. All the crude extracts had significance antifungal activity on most of the fungi. Ethanol, Methanol and Aqueous extract had maximum inhibition activity 48.58% - 100% against test pethogens as compared to Chloroform and Ethylacetate extract. The extracts exhibited a very active inhibition activity 40–60% against test dermatophytes. While Ethylacetate extract had weak inhibition activity (28 % -54.55% against test organism. Similar work have been done by Anjum and Khan (2003), where chloroform extract of *Cuscuta reflexa* Roxb. displayed minimum inhibition zone against *A.niger*. Adekunel *et al.*, (2002), reported that water extract of *Chasmanthera dependens* Hochest did not inhibit the growth of *A.niger* and *A.flavus*. Sakharkar *et al.*, (1999), cited that crude extract of *Cassia alata* linn had shown significant anti fungal activity against *A.niger*. However Ficker *et al.*, (2003) claimed that the crude extract of *Juglans cinerea* L. were found to be very active inhibitory agent against *M.gypseum*. Thebo and Abro (2000) described leaves extract of *Azadirachta indica* A.Juss were very active against *M.gypseum* and *T.rubrum*. While Natarjan *et al.*, (2003) provided evidence that ethonal extract of *Azadirachta indica* A. Juss exerted highly inhibitory activity against *T.rubrum*. Usmanghani and Shameel (1986) communicated that methanolic extract of some marine algal species were found very active to inhibit *A.niger*. While (Shaikh *et al.*, 1990), observed that crude extract of *S. marginatum* were very active against *T-rubrum*, but activity was devod of against *A.niger* and *A. flavus*. Pirzada *et al.*, (2007) reported that crude extract of *Rhazya stricta* have strong anti fungal activity against *A. flavus*, *A. niger*, *M. gypseum*, *P. varioti* and *T. rubrum*.

In present study this was the first attempt to study the anti fungal activity of the medicinal plant *N. indicum* against the dermatophytic Fungi such as *A.niger*, *A.flavus*, *P.varioti*, *M. gypseum*, *T.rubrum* causing different skin diseases. In this study all the solvent extract were very active to inhibit test organism except Ethylacetate which was weak to inhibit *T.rubrum*. In the continuation of this study nine elements (Al), Calcium (Ca),Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Phosphorous (P), Sulphar (S) and Zinc (Z) have been analized in variable range from the medicinal plant *N. indicum*. However, calcium (Ca), Manganese (Mn) and Zinc (Zn) were found in sufficient amount in the range of (20956.97 – 22811,57) mg/kg, (81.00 – 94.31) mg/kg and (31.26 – 44.52) mg/kg respectively. All these elements play essential role for the treatment of skin disease. (Janjua 1990, Saily *et al.*, 1994). Copper, Aluminum, Zinc and their salts are used in skin infection as disinfectant and cleansing agents. Zinc is co-factor for many enzymes required for the healing damaged skin. Sahito *et al.*, (2003). This current experiment provides information about mineral contents present in *N. indicum* and scientific justification for the utilization of crude extract of *N.indicum* for the treatment of different skin disease such as Tinea capitis, Tinea carporis, Tinea manum, Tinea pedis etc to researchers and to the person dealing with herbal extracts.

References

Ali Shtayeh, M.S., and I. S. A. Ghdeib (1999) Antifungal activity of plants extracts against dermatophytes, *Mycoses* (**42**): 665-672.

Adekunle, A. A., and S.O. Okoli, (2002) Antifungal activity of crude extracts of *Alfia barteri* Oliver (Apocynaceae) and *Chasmanthera dependens* Hochst (Menispermaceae). *Hamd. Med.* XLV, (**3**):52-56.

Anjum N., and Z. Khan, (2003) Antimicrobial activity of the crude extract of *Cuscuta reflexa* Rox b. *Pak. J. Bot.* 35 (**5**): 999-1007.

Baquar S.R., (1989) Medicinal and Poisonous Plants of Pak. Printas, Karachi. .

Ficker C.E., J.T. Arnason, P.S. Vindas, (2003) Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts. *Mycoses* (**46**): 29-37.

Janjua. K.M., (1990) Trace elements in *Allium cepa* (Onion) and their therapeutic importance. *Hamd. Med.* XXXIII, (**2**): 87-90.

Kirtikar. K. R., and B.D. Basu, (1935) Indian Medicinal Plants 1-IV Lalit Mohan Basu, Allahabad, India. 426- 428.

Nasir. E., and S. I. Ali, (1990) Flora of Pakistan, Department of Botany, University of Karachi.

Natarjan. V., P.V. Venugopal, and T. Memon, (2003) effect of *Azadirachta indica* (neem) on the growth pattern of darmatophytes. Ind. *J. Med. Microbiol* (**21**): 2,1-4.

Pirzada, A.J., W. Shaikh, and T.G. Kazi, (2007) Isolation of essential elements and inhibition activity of medicinal plant *Rhazya stricta* Dcne. Against dermatophytic fungi. *Pak. J. Agri., Agril. Engg., Vet. Sci.*, **23** (**1**): 34-38.

Sahito, S.R., M.A. Memon, T.G. Kazi, and G. H. Kazi, (2003) Evaluation of mineral contents in medicinal plant *Azadirachta indica* (neem). *J. Chem. Soc.Pak.* (**25**): (2), 139-143.

Shaikh, W., M. Shameel, A. Hayee-Memon, K. Usmanghani, S. Bano and V.U. Ahmed (1990) Isolation and characterization of chemical constituents of *Stoechospermum marginatum* (Dictyotales, Phaeophyta) and their antimicrobial activity. *Pak. J. Pharm. Sci.* 3 (**2**): 1-9.

Saily, A., R. Sahu, B. Gupta and S.M. Sondhi. (1994) Analysis of mineral elements of medicinal plants used for the treatment of asthma, syphilis, diarrhea, skin diseases and rheumatism, *Hamd. Med.* XXXVII (**4**): 18-22.

Sakharkar, P.R., and A.T. Patil, (1999) Antifungal activity of *Cassia alata*. *Hamd. Med.* XLI, (**3**): 20-22.

Soderberg, T., and G. Halimans, (1982) Treatment of leprosy with adhesive Zinc tapes. Lep. Rev., (**53**): (4) 271.

Thebo, N. K., and H. Abro, (2000) Antifungal activity of *Azadirachta indica* against human pathogenic fungi *Sindh Univ. Res. J. (Sci. Ser.)* 32 (**2**): 35-42.

Underwood. E., (1981) Trace metal in human and animal health *J. Hum.nutr.* 53, 37.

Usmanghani, K., and M. Shameel, (1986) Studies on the antimicrobial activity of certain sea weads from Karachi coast. In: R. Ahmad and A. San Pietro, (eds.): Prospects for Biosaline Res. Proc. US-Pak Biosal. Res. Workshop: Karachi. 519- 526.

CamScanner