

ANTIFUNGAL ACTIVITY OF *DODONAEA VISCOSA* JACQ EXTRACT ON PATHOGENIC FUNGI ISOLATED FROM SUPERFICIAL SKIN INFECTION

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ABSTRACT

Antimicrobial activity of solvent extracts of leaves and shoot of *Dodonaea viscosa* Jacq have been determined against fungi, *Aspergillus niger*, *Aspergillus flavus*, *Paecilomyces varioti*, *Microsporum gypseum*, and *Trichophyton rubrum* causing skin diseases. All crude extracts were found to be effective against tested fungi. However chloroform has strong inhibition activity against fungi as compared to ethanol, methanol, ethylacetate and aqueous extracts. More over in present study some basic elements have been analyzed, Al, Ca, Cu, Fe, Mg, Mn, P, S and Zn from the medicinal plant *Dodonaea viscosa* Jacq. by using atomic absorption spectrophotometry and UV spectrophotometry. The medicinal plant *Dodonaea viscosa* Jacq contains considerable amount of elements which have therapeutic effects in skin diseases.

Keywords: *Dodonaea viscosa*, antifungal activity, elements detection.

INTRODUCTION

The schematic search of higher plants for antifungal activity has shown that some plants extracts have the ability to retard fungal growth or completely inhibit the fungus. Interest in new safer and more effective antifungal agents has grown with the increasing incidents of fungal resistance especially in immunosuppressive situation (Adedotum *et al.*, 2002). It is estimated that approximately fifty six percent of lower income world population use herbal medicine and supplementation for their primary health care (Planta *et al.*, 2000). Skin disease diarrhea, diabetes, malaria, respiratory infection, fungal and bacterial infection are the common health problem in rural communities in Pakistan.

Dodonaea viscosa Jacq is a traditional medicine which is utilized in folklores medicine in Pakistan for the treatment of various fungal skin diseases like *Tinea capitis*, *Tinea pedis*, *Tinea manum*, and *Tinea corporis* etc. The powdered leaves of *D-viscosa* applied over a wound in burns and scalds, to possess febrifuge properties and useful for different skin diseases (Baquar, 1989; Kirtikar & Basu, 1935; Shahani & Memon, 1988).

Dodonaea viscosa inhibited the adherence of *Candida albicans* to oral epithelial cells, which is the initial step of colonization in the infection process and this plant has a therapeutic potential at subinhibitory concentration (Patel *et al.*, 2009). The crude ethanol extract and *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and aqueous fractions of *Dodonaea viscosa* were analyzed for antibacterial potential against four Gram positive bacteria: *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, and three Gram negative bacteria:

Escherichia coli, *Salmonella typhi*, *Pseudomonas aeruginosa*. Screening showed inhibition against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Khurram *et al.*, 2009).

Dodonaea viscosa (Sapindaceae), a medicinal plant commonly used for skin diseases in Ethiopia was subjected to a systematic dermatotoxicity study. To this effect, the dermatotoxicity of an 80% methanol extract of the leaf was investigated in animals following standard procedures for irritation, sensitization, acute toxicity and repeated toxicity tests. *D. viscosa* is not associated with any toxicologically relevant effects and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts (Kefale *et al.*, 2009).

Investigation of the aerial parts of *Dodonaea viscosa* led to the isolation of a new ent-labdane 1 [ent- 15, 16-epoxy-9 α H-labda-13(16), 14-diene-3 β , 8 α -diol] and a novel p-coumaric acid ester 4 of 1 -L-myoinositol { 1-L-1-O-methyl-2-acetyl-3-p-coumaroyl-myoinositol}, along with other known compounds (Rachel *et al.*, 1991).

Dodonaea viscosa Jacq is a popular medicinal plant. Its leaves are used as anti-inflammatory, anti-ulcer, antibacterial and antifungal agents and in the treatment of fractures (Sama *et al.*, 2008).

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, copper sulphate and lotion of zinc solution are used for skin disinfectant, cleansing agents, antiseptic, soothing and cooling effective. Calcium, magnesium manganese are used in the formation of the collagen and connective tissue. Phosphorus and sulphur are used for the treatment

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of scabies and leprosy. Sahito *et al* (2003) Soder Berge *et al* (1982) Underwood (1981) Skin disease is one of the main problems of Sindh province which is usually caused by fungi. The present report describes the antifungal potential of different solvent extract and elemental study *Dodonaea viscosa* Jacq against fungi causing skin diseases.

MATERIALS AND METHODS

Plant material

Plant material the *Dodonaea viscosa* Jacq leaves and shoots were collected from different area of Kohistan region District Dadu and reference sample were identified through literature Flora of Pakistan (Nasir & Ali, 1990). 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 dipped in five liter (L) of ethanol solvent in bottle for 20 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 extract was left at room temperature. The solvent were completely evaporated so that organic compounds remain in the dry form. These

extracts so obtained were mixed with the sterilized water (1 g: 5 ml) and each extracts sample was applied for antifungal activity.

Collection of dermatophytes

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

Preparation of fungal culture

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

Treatment of different solvent extracts layers

The human skin pathogen was treated with different extracts and results were taken after 72 hours at 30°C. The percentage of mycelial inhibition was calculated as follows (Usmanghani & Shameel, 1986; Ali Shtayeh and Suheil, 1999).

$$\% \text{ Mycelia inhibition} = [(dc-d1)/dc] \times 100$$

dc = colony diameter in control,
d1 colony diameter in treatment

Table 1: Antifungal activity of different solvent extract of *Dodonaea viscosa* Jacq

Control reading at 30°C after 72 hrs (mm)	<i>Aspergillus niger</i> 40	<i>Aspergillus flavus</i> 35	<i>Paecilomyces varioti</i> 55	<i>Microsporeum gypseum</i> 30	<i>Tricophyton rubrum</i> 25
Text extract					
Ethanol					
Inhibited reading at 30°C after 72 hrs (mm)	15	12	10	08	05
Inhibited (%)	62.50	65.72	81.82	73.34	80
Methanol					
Inhibited reading at 30°C after 72 hrs (mm)	15	15	10	14	05
Inhibited (%)	62.50	57.15	81.82	53.34	80
Chloroform					
Inhibited reading at 30°C after 72 hrs (mm)	20	10	05	08	05
Inhibited (%)	50	71.41	90.91	73.34	80
Ethyl acetate					
Inhibited reading at 30°C after 72 hrs (mm)	20	15	20	08	05
Inhibited (%)	50	57.15	63.64	73.34	80
Aqueous					
Inhibited reading at 30°C after 72 hrs (mm)	10	15	10	12	06
Inhibited (%)	75	57.15	81.82	60	80

Methodology for elements determination

A suitable dissolution method for biological sample to yield homogenous solution is a crucial first step to determine in atomic absorption spectrophotometer and U.V. techniques. The decomposition of organic matter must be completed to avoid interference by organic residue. Sample digested with nitric acid: 30% hydrogen peroxide determination of mineral elements. Appropriate working standard solution of aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), sulphur (S), zinc (Zn) were prepared from stock standard solution (1000 ppm), in 2N nitric acid. Calibration curves were drawn for each element using atomic absorption spectrophotometer Hitachi model 180-50 and UV-Spectrophotometer. The calibration curves obtained for concentration VS. absorbance data were statistically analyzed using fitting of state line by least square method. A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements. 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 same. The percentage recovery test for different elements by digestion method adopted was 98.5-99% in range.

RESULTS

All the crude extracts have 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 *P. variety*, *T. rubrum* and *M. gypseum* 81.82%, 80% and 73.34% respectively while moderate inhibition activity against *A.flavus* 65.72% and minimum inhibition activity against *A.niger* 62.5% was recorded.

Ethyl acetate extract

The maximum inhibition activity was observed against *T. rubrum*, *M. gypseum* and *P. varioti* 80%, 73.34 and 63.64% while moderate inhibition activity against *A.flavus* 57.15 and minimum inhibition activity against *A.niger* 50% was measured.

Chloroform extract

The maximum inhibition activity against *P.varioti* *T. rubrum* and *M. gypseum* 90.91%, 80% and 73.34% respectively while moderate inhibition activity against *A.flavus* 71.41% and minimum inhibition activity against *A.niger* 50% was noticed.

Methanol extract

The maximum inhibition activity was observed against *P.varioti* and *T.rubrum* 81.82 and 80% while moderate inhibition activity against *A.niger* and *A.flavus* 62.5% and 57.15% respectively and minimum inhibition activity against *M.gypseum* 53.34% was detected out.

Aqueous Extract

The maximum inhibition activity was observed against *P.varioti*, *T.rubrum* and *A.niger* 81.82%, 80% and 75% while moderate inhibition activity against and *M.gypseum* 60% and minimum inhibition activity against *A.flavus* 57.15% was measured.

Elements

The considerable amount of various elements such as: aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), sulphur (S), zinc (Zn) have been determined from the medicinal plant *Dodonaea viscosa* Jacq (table 2). These elements are biologically very much important for the treatment of different skin diseases.

Table 2: Quantity of different elements in *Dodonaea viscosa* Jacq

Name of elements	Formula	Amount Mg/Kg
Aluminum	Al	6.93-7.44
Calcium	Ca	11683.98-12054.90
Copper	Cu	6.48-9.69
Iron	Fe	83.85-120.08
Magnesium	Mg	2711.53-2965.67
Manganese	Mn	11.42-14.25
Phosphorous	P	167.37-224.11
Sulphur	S	213.66-222.31
Zinc	Zn	55.30-59.45

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 have significant antifungal activity on most of the fungi, but chloroform extract had maximum inhibition activity 50-90.91% as compared to ethanol, methanol, ethylacetate and aqueous extracts have active inhibition activity in the range of 50-81.82% against test dermatophytes. Although many scientist Anjum & Khan (2003), Adedotum *et al* (2002) Sakharkar *et al* (1999), Ficker *et al* (2003), Thebo and Abro (2000) Natarjan (2003), Usmanghani & Shameel (1986), Skaikh *et al* (1990) Rukhsana (2006) Bajwa *et al* (2006) Pirzaida *et al* (2007), has been screening the antifungal activity of medicinal plants against dermatophytes. In this study to investigate the antifungal activity of medicinal plant *Dodonaea viscosa* against dermatophytic fungi such as

Aspergillus flavus, *A. niger*, *Microsporeum. gypseum*, *Paecilomyces. varioti*, *Trichoptron. rubrum* caused different skin diseases like Tinea. Capitus, T. pedis, Tmanum and T.corporis.

Further some basic elements aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorous (P), sulphur (S) and zinc (Z) have been analyzed in variable range from the medicinal plant *Dodonaea viscosa* Jacq but the maximum amount of the sulphur and zinc and present in the range of 213.66-222.31, 55.30-59.45Mg/Kg respectively. All these elements play essential role for the treatment of skin disease. Janjua (1990), Saily *et al* (1994).

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