Studies on Bioassay Directed Antifungal Activity of Medicinal Plants *Calotropis procera, Skimmia laureola, Peltophorum pterocarpum* and two pure Natural compounds ulopterol and 4-methoxy-1-methyl-3-(2'S-hydroxy-3'-ene butyl)-2-quinolone

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Summary: Our investigations on the antifungal activity of the crude ethanolic extracts of different plants including *Calotropis procera, Skimmia laureola* and *Peltophorum pterocarpum,* have led to the determination of good results of these activities. We report the evaluation of antifungal activity of crude extracts of *Calotropis procera, Skimmia laureola* and *P. pterocarpum*. In addition, we also report the antifungal activity of ulopterol (1) and 4-methoxy-1-methyl-3-(2'*S*-hydroxy-3'-ene butyl)-2-quinolone (2) isolated from *Skimmia laureola* [1]. These plants were selected because of their reported medicinal importance in indigenous system of medicines from ancient times and are abundantly available in various parts of Pakistan [1,2].

Introduction

Calotropis procera (Ait) R. Br. (Asclepiadaceae), a xerophytic shrub widely distribu-ted in the tropics of Asia and Africa is commonly known as "Akra" in India and Pakistan. In the traditional Indian medicinal system, different parts of the plant have been advocated for a variety of diseases and have also been considered as an antidote for snake poisoning [2]. It has been reputed in the Indian traditional medicine for a variety of ailments including leprosy, ulcer and piles. Different parts of the plant posses antimicrobial, anti-inflammatory, analgesic [3] and anticancer [4] activities.

Skimmia laureola is found in the Northern areas of Pakistan and is used in the indigenous system of medicine for the treatment of various ailments. The soot obtained from the burning of leaves is inhaled for treatment of body pain, fever and influenza [5].

Peltophorum pterocarpum is an ornamental tree grown in homes and gardens in India and Pakistan [6], bearing fragrant yellow flowers, reported to posses antibacterial, anti-inflammatory [7] and fungitoxic activity [8].

Results and Discussion

The crude extract of Calotropis procera exhibited antifungal activity [9] against Trichophyton longiformis, Candida albicans, Aspergillus flavus, Microsporium canis, Fusarium solani and Fusarium moniliformis. The minimum inhibitory concentration (MIC) of crude extract against these fungi was used as 400 µg/ml. The growth of Trichophyton longiformis, Candida albicans and Fusarium moniliformis was inhibited in 100% by the crude extract at a concentration of 400 µg/ml, while standard fungicide Miconazole and Ketocanazole totally inhibited the growth at a concentration of 70 µg, 110.8 µg and 110.8 µg respectively. The growth of Microsporium canis was inhibited in 90% by the crude extract at a concentration of 400 µg/ml, while standard fungicide Miconazole totally inhibited the growth at the concentration of 98.4 µg/ml. These results are summarized in Table-1.

The crude ethanolic extract of *S. laureola* showed good activity against animal pathogen *Microsporium canis* and plant pathogen *Fusarium solani* var. *lycopersici* (Tomato) at a concentration of 400 µg/ml. Minimum inhibitory concentration (MIC) of the crude extract against *Microsporium canis* was used as 400 µg/ml. The growth of *Microsporium canis* was inhibited in 67.7% by the crude extract at a concentration of 400 µg/ml, while standard fungicide Miconazole and Ketoconazole totally inhibited the growth of *Microsporium canis* at a concentration of 72.10 and 62.25 µg/ml, respectively. The crude extract of *S. laureola* was found to be active against *Fusarium solani* var. *lycopersici* at a concentration of

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Table-1: Antifungal activity of crude extract of *C. procera*.

Fungi	Sample	Control	% Inhibition	Standard Drugs	% Inhibition	MIC µg/ml
Trichophyton longiformis	0	90	100	Miconazole Ketoconazole	100	70
Candida albicans	0	100	100	Miconazole Ketoconazole	100	110.8
Aspergillus flavus	100	100	0	Amphotericin B	100	70
Microsporium canis	10	100	90	Miconazole Ketoconazole	100	98.4
Fusarium solani	95	95	0	Miconazole	100	73.25
Fusarium moniliformis	0	100	100	Miconazole	100	110.8

Concentration of crude extract = 400 µg/ml.

Table-2 : Antifungal activity of crude extract of *Skimmia laureola*.

Fungi	Crude extract of *S. laureola*	% Inhibition	Miconazole	Ketoconazole	Benlate	% Inhibition
Microsporum canis	400 µg/ml	67.7	72.10	62.25 µg/ml	-	100
Fusarium solani	400 µg/ml	57.7	-	-	73.25 µg/ml	100

Table-3 : Antifungal activity of ulopterol (1).

Fungi	Compound (1)	% Inhibition	Miconazole	Ketoconazole	% Inhibition
Drechslera rostrata	200 µg/ml	50.8	25 µg/ml	25 µg/ml	100
Curvularia lunata	200 µg/ml	49.5	25 µg/ml	25 µg/ml	100

Table-4: Antifungal activity of 4-methoxy-1-methyl-3(2'*S*-hydroxy-3'-ene butyl)-2-quinolone (2).

Fungi	Compound (2)	% Inhibition	Miconazole	Ketoconazole	%Inhibition
Microsporium canis	200 µg/ml	68.7	72.10 µg/ml	62.25 µg/ml	100
Pseudoallescheria boydii	200 µg/ml	56.8	38.75 µg/ml	29.50 µg/ml	100

400 µg/ml, while standard fungicide Benlate completely inhibited the growth of *Fusarium solani* var. *lycopersici* at a concentration of 73.25 µg/ml. *Fusarium solani* var. *lycopersici* causes root rot, stem cankers associated with wounds, damping-off seedlings, destruction of spawn in beds of cultivated mushrooms and pea crop. The antifungal results are summarized in Table-2.

Compound (1) exhibited *in vitro* antifungal activity against *Drechslera rostrata* and *Curvularia lunata* with minimum inhibitory concentration of 200 µg/ml. Compound (1) also exhibited weak activity against *Culvularia lunata*. The fungicides Miconazole and Ketoconazole were used as standards. The results of antifungal assay results are summarized in Table-3.

Compound 2 exhibited some *in vitro* antifungal activity against *Microsporium canis* and *Pseudoallescheria boydii*. *Microsporium canis* causes infection of hair and skin in dogs and cats, while *Pseudoallescheria boydii* causes infection of skin, subcutaneous tissue, nasalsinuses and mycetoma. The minimum inhibitory concentration (MIC) of the compound 2, against *Microsporium canis* and *Pseudoallescheria boydii* was 200 µg/ml. The growth of *Microsporium canis* and *Pseudoallescheria boydii* was strongly inhibited (68.7 and 56.8%) by the compound (2), while standard fungicide Miconazole and Ketoconazole totally inhibited the growth of *Microsporium canis* and *Pseudoallescheria boydii* at 72.10 µg/ml, 62.25 µg/ml and 38.75 µg/ml, 29.50 µg/ml concentration respectively [1,10]. The results of antifungal assay are summarized in Table-4.

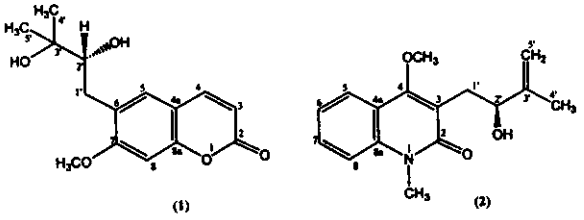
The crude extract of P. pterocarpum exhibited activity against a number of fungi. The in vitro antifungal activity was tested against Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporium canis, Fusarium solani and Candida glaberata. Minimum inhibitory concentration of crude extract against Microsporium canis was used as 400 µg/ml. The growth of Microsporium canis was inhibited in 65% by the crude extract at a concentration of 400 µg/ml, while standard fungicide Miconazole totally inhibited the growth of Microsporium canis at a concentration of 98.4 µg/ml. The crude extract of P. pterocarpum was found to be active against Trichophyton longifusus and Aspergillus flavus at a concentration of 400 µg/ml, while standard fungicide Miconazole totally inhibited the growth of Trichophyton longifusus and Aspergillus flavus at a concentration of 70 µg/ml and 20 µg/ml respectively. The crude extract of P. pterocarpum exhibited no activity against Candida

Table-5: Antifungal activity of crude extract of *P. pterocarpum*

Fungi	Sample	Control	% Inhibition	Standard drugs	MIC (μg/ml)
Trichophyton longifusus	70	95	26.3	Miconazole	70
Candida albicans	90	90	0	Miconazole	110.8
Aspergillus flavus	90	95	15.7	Amphotericin B	20
Microsporium canis	45	100	65	Miconazole	98.4
Fusarium solani	100	100	0	Miconazole	73.25
Candida glaberata	95	95	0	Miconazole	110.8

Concentration of sample = 400 μg/ml.

albicans, Fusarium solani and Candida glaberata. These results are summarized in Table-5.



(1) (2)

Experimental

Calotropis procera, Skimmia laureola and *P. pterocarpum* were collected from the suburban areas of Karachi, Pakistan. The fresh dried plants of *C. procera* (1.0 kg), *S. laureola* (60 kg) and *P. pterocarpum* (1.0 kg) were ground and soaked in ethanol for 2 weeks and then filtered. The filtrate was concentrated under reduced pressure at 40°C to a gummy mass of *C. procera* (142.91 g), *S. laureola* (821.93 g) and *P. pterocarpum* (175.31 g). ulopterol (**1**) and 4-methoxy-1-methyl-3-(2'S-hydroxy-3'-ene butyl)-2-quinolone (**2**) were isolated from alkaloidal fraction of *Skimmia laureola* by using extraction and column chromatography methods [1]. Antifungal screening has been performed on the crude extracts of *C. procera, S. laureola, P. pterocarpum* and two natural compounds **1** and **2**. Sabouraud agar was used as antifungal control. Micaonazole and Ketoconazole were used as standard drug.

Antifungal Bioassay

All antifungal assay employed a standard Agar Tube Dilution Method [11]. The test fungi were maintained on Sabouraud's agar slants. A 4 mm diameter piece of fungal inoculum removed from 7 days old culture of fungi was transformed in solid media. The test sample of crude extract was dissolved in sterile DMSO to obtain 200 μg/ml concentration. Sabouraud agar was prepared by mixing 32.5 gram Sabouraud dextrose agar, 4% glucose agar and 7.5 gram of agar-agar in 500 ml distilled water. Tubes were allowed to cool to 50°C and non-solidified Sabouraud agar media were inoculated with 200 ml of compound pipetted from the stock solution. This gave a final concentration of 200 μg/ml of media. The tubes were then allowed to solidify in a slanted position at room temperature and the tubes were incubated at 27-29°C for 7-10 days and the visible growth observed. Minimum inhibitory concentrations (MIC) were expressed in μg/ml.

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