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Full Length Research Paper

Efficacy of *Avicennia marina* (Forsk.) Vierh. leaves extracts against some atmospheric fungi

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Crude ethanolic extract of *Avicennia marina* leaves was tested against seven allergenic fungi viz., *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Cladosporium herbarum, Penicillium notatum* and *Saccharomyces cerevisiae* using five different solvents: dimethyl sulphoxide (DMSO), distilled water (DW), chloroform, ethanol and acetone at 2000, 4000 and 6000 ppm doses. Dose dependent tendency in the increase or decrease in the growth of fungi was observed. Two synthetic drugs miconazole and amphotericin-B were used as positive control. Miconazole was 100% effective against *A. alternata, C. herbatum, P. notatum* and *S. cerevisiae* with the concentrations of 95.00 ± 1.62, 78.00 ± 4.99, 100.00 ± 0.69 and 110.00 ± 2.33 (µg/ml of SDA medium), respectively. Amphotericin-B completely controlled the growth of *A. flavus, A. fumigatus* and *A. niger* in the concentration of 24.00 ± 17.00, 30.00 ± 15.66 and 18.00 ± 18.34 (µg/ml of SDA medium), respectively. Distilled water and DMSO were considered to be the most effective solvents preventing 83.00 ± 4.73% growth of *A. niger*, 80.33 ± 5.60% *A. flavus*, 78.58 ± 3.18% *A. alternata*, 72.91 ± 7.96% *P. notatum,* 65.25 ± 3.55% *C. herbarum*, 63.25 ± 4.52% *A. fumigatus* and 48.5 ± 7.89% *S. cerevisiae*. Statistically, the results were compared with negative control and found to be highly significant (p<0.01).

Key words: Allergenic fungi, inhibition, dose dependent, growth control, synthetic drugs.

INTRODUCTION

In developing countries, more than 5 billion people live in extreme poverty and a large number among them are suffering and trying to get safe water and basic medicines (WHO, 1995). In these peculiar environments, the population has no other alternative than to rely on traditional medical practitioners and medicinal plants for primary health care (WHO, 1995; Sood et al., 2007) and more than 80% of the rural population depends on plants (Okoro et al., 2010). Today, there is no doubt that complimentary medicines have wide acceptability and are attractting attention from scientific community world-wide (Newal et al., 1986). Therefore, the use of herbal drugs is gaining importance day by day (Adegoke et al., 2010; Maiyo et al., 2010).

Among the other human health problems, allergy and hypersensitivity are increasing day by day which are directly related to the growing populations of immune compromised individuals (Ben-Ami et al., 2010). It results from changes in medical practice such as use of intensive chemotherapy and immunosuppressive drugs. Human immunodeficiency virus (HIV) and other disease causing immune suppressives have also contributed to these problems (Portillo et al., 2001). One of the main causal agents of skin and respiratory allergy are fungi (Burr et al., 1999; Bush and Portny, 2001; Eggleston and Bush, 2001). The fungi commonly causing allergenic problems are the species of Penicillium, Aspergillus, Chaetomium,, Ulocldium, Stachybotrys and Cladosporium (Gravesen et al., 1999).

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Out of 80% asthmatic patients of a population, the 1991). The increase and prevalence of allergic diseases are increasing day by day (Pope et al., 1993; Gravesen et al., 1999). For the treatment of these fungal problems, synthetic antifungal drugs are used. These drugs relieve the patients for the time being but in the long run, adversely affect the health of the patients. The use of several antifungal drugs available at present is restricted by the production of resistant strains. Besides this, the antifungal drugs are also limited due to poor solubility, their toxicity and low potency. Somchit et al. (2002, 2004) were of opinion that antimycotic therapy causes hepatotoxicity and induce liver, kidney and gastrointestinal toxicities.

Medicinal plants are used as alternative medicine which are known for their healing, disease cure and antimicrobial qualities for a long time (Gedif and Hahn, 2003; Mukherjee and Wahile, 2006; Velazquez et al., 2006; Cravotto et al., 2010). The mangrove (*Avicennia marina* Forsk.) Vierh locally known as Teemar, belongs to family Avicenniaceae/Verbenaceae. It is used to treat the skin and respiratory problems. It is a high salinity tolerant plant and is found on the entire coast line of Sind and Balochistan. The plant is 2 to 5 m tall with 10 to 20 cm long pneumatophores. *A. marina* is locally known as astringent and cicatrizing agent to skin diseases. The available data shows the use of *A. marina* as astringent for healing the pustular skin and leprotic skin lesions (Kiritikar and Basu, 1933; Chopra, 1958; Julia, 1965). Previously, *A. marina* juice was used as abortifacient and the roots of *Arctostaphylos tomentosa* were used as aphrodiasiac (Nadkarni and Nadkarni, 1976). The *A. marina* is extraordinary tolerant to pest attack and diseases. Its bitter aromatic juice is used as an abortive in Tropical Africa and Asia (Lewis and Elvin-Lweis, 1977).The reported natural products in mangroves are tannins, alkaloids, polyphenols, proteins, aminoacids and other compounds (Combs and Anderson, 1949; Julia, 1965). Tariq et al. (2007) reported the nematicidal property of *A. marina*. It releases some compounds toxic to nematodes like phenols, tannins, azadirachtin and ricinine. *A. marina* has anticancerous property and flavor-noids like compound 1 of aglycone, luteolin 7-O-methyl ether (Sharaf et al., 2000). The flavonoids are most active type of phenols which have ability to act as antioxidant in biological system (Rice-Evans, 1998). Orhans et al. (2009) reported the antibacterial, antifungal and antiviral activities of flavonoids. Flavonoids restrict the mito-chondrial enzyme system which is important in cyto-toxicity and certain other biological effects and may be target for anticancer chemotherapy (Gosalves et al., 1976).

We demonstrated the aforesaid remedial potential of *A. marina*, against seven allergenic fungi of Pakistan.

MATERIALS AND METHODS

Plant material and extraction

The leaves of *A. marina* with other parts of plant were collected from Sands pit, Karachi. A voucher specimen No. 68660 was deposited at Karachi University Herbarium. The collected leaves were washed thoroughly and dried at room temperature. The leaves were pulverized into powder form and soaked in ethanol. After one week, the material was filtered with filter paper and kept in sterile bottles at 4°C in refrigerator.

Test organisms, solvents and medium

The test organisms used in this study were *Alternaria alternata* (Fr) keissler, *Aspergillus flavus* Link., *Aspergillus fumigatus* Fres, *Aspergillus niger* Von Tiegh, *Cladosporium herbarum* Pers Link ex S.F.Gray, *Penicillium notatum* Westling and *Sacchromyces cerevisiae* Meyer. The test fungi were directly recorded from the environment of Karachi University Campus (Horne, 1935; Turner, 1966) and identified on the basis of morphological and microscopic features by the second author of this paper. The solvents used in this technique were dimethyl sulphoxide (DMSO), distilled water (DW), chloroform, ethanol and acetone. The medium used in this technique was Sabourad dextrose agar (Oxoid, UK).

Technique

For antifungal bioassay, poisoned food technique (Grover and Moore, 1962) with some modifications was used. The fungi were incubated for seven days and linear growth of the fungi was measured so as to determine the inhibition.

Positive and negative controls

For positive control, two standard/reference antifungal drugs were used, miconazole and amphotericin-B. Miconazole was used against *A. alternata, C. herbarum*, *P. notatum* and *S. cerevisiae* at the concetrations of 95 + 1.62, 78 + 4.99, 100 + 0.69 and 110 + 2.33 (ug/ml of SDA medium). Amphotericin-B was used against *A. flavus, A. fumigatus* and *A. niger* in the concentrations of 24 ± 17.00, 30 ±15.66 and 18 + 18.34 (ug/ml of SDA medium), respectively. Percent growth inhibition for each was recorded in millimeter.

Similarly, the growth of test fungi was determined in all five solvents, viz., DMSO, DW, chloroform, ethanol and acetone. For negative control, 0.1 ml of each aforementioned solvents (DW, DMSO, chloroform, ethanol and acetone) was pipetted in test tube and 5 mm diameter of fungal inoculum was placed. Growth of each fungus was recorded in millimeter (Attaaurrehman et al., 2001).

Calculation of inhibition

Percent inhibition in the growth of each fungus due to different extracts in different solvents was calculated according to the following formula:

(C-T/C) x100

Where, C is the linear growth in control tubes (mm) and T is the linear growth in treatments (experimental tubes).

Statistical analysis

All the data were presented in the form of ±SEM (standard error means, n = 3). In order to show significant difference found in the antifungal activities of extracts at 2000, 4000 and 6000 ppm doses, one way analysis of variance was carried out by using SPSS V.11,

Table 1. Antifungal bioassay of *A. marina* (Forsk.) Vierh leaves extracts.

Dose (ppm)	Solvent	*A. alternata*	*A. flavus*	*A. fumigatus*	*A. niger*	*C. herbarum*	*P. notatum*	*S. cerevisiae*
	DMSO	56.75±5.75**	61.66±10.75*	43.00±3.89**	44.08±6.74 $^{n-s}$	52.00±3.55**	61.33±5.63*	12.75±4.86**
	DW	64.83±3.1***	64.50±6.60*	47.75±4.52***	44.08±6.74**	51.41±3.40 $^{n-s}$	58.00±7.96***	25.16±7.89*
2000	Chloroform	47.41±3.15**	44.00±6.5***	12.76±5.74*	12.16±5.24 $^{n-s}$	27.26±4.46**	47.90±6.99***	10.65±5.07^{n-s}
	Ethanol	48.24±6.17***	56.84±5.33***	41.25±10.24***	6.66±13.42**	41.66±5.04***	57.77±5.05***	24.24±5.82*
	Acetone	42.41±4.62***	33.58± 8.24	23.75±4.45***	28.66±3.12***	9.77±5.05***	31.25±2.60 **	7.74±4.29***
	DMSO	64.50±5.75***	70.00±10.75***	51.00±3.89***	74.33±4.73^{n-s}	53.00±3.55**	61.33±3.75**	20.16±4.86***
	DW	74.50±3.10**	75.75±6.60***	55.50±4.52***	47.75±6.74***	56.34±3.40***	66.50±7.96 $^{n-s}$	39.00±2.89***
4000	Chloroform	59.50±3.15***	51.00±6.50**	29.91±5.74 $^{n-s}$	50.50±3.06***	36.95±4.6***	49.47±6.99***	13.89±4.73***
	Ethanol	61.66±6.17*	67.01±5.33***	48.50±10.24***	58.94±13.42***	46.42±5.04***	61.01±5.05***	34.37±5.82*
	Acetone	55.25±4.62***	66.50±4.45***	34.25±4.45*	48.00±4.73***	18.00±5.05***	34.17±2.60***	17.64±4.29***
	DMSO	69.08±5.75**	75.00±10.75***	63.25±4.52 **	83.00±4.73***	65.25±3.55***	70.50±5.63**	41.86±4.86*
	DW	78.58±3.19***	80.33±5.60***	63.20± 4.50***	59.08±6.74**	64.50± 3.40**	72.91± 7.89*** .	50.00±7.89**
6000	Chloroform	63.00±3.15***	59.00±6.50*	61.63±5.74 $^{n-s}$	59.08±3.06*	50.43±4.46*	60.00±6.99**	33.00±5.07*
	Ethanol	66.38±6.17**	78.86± 11.13***	56.00± 10.24**	61.40±13.42*	58.33± 5.04***	71.66±5.05*	39.68±5.82**
	Acetone	57.42±4.62*	68.67±3.67**	35.50± 4.45**	61.66± 3.12*	20.68±5.05**	46.59± 2.60*	22.74±4.29 $^{n-s}$
Positive control	**Drugs (µg/ml)**	**Miconazole**	**Amphotericin-B**	**Amphotericin-B**	**Amphotericin-B**	**Miconazole**	**Miconazole**	**Miconazole**
		95.00±1.26	24.00±17.00	30.00±15.66	18.00±18.34	78.00±4.99	100.00±0.68	110.00± 2.33

Data represents the means of difference in the growth inhibition of fungi at three different ppm doses ± SEM (n = 3) ,p*<0.05, less significant, p**<0.01, more significant and p***<0.001, most significant. DMSO, Dimethyl sulphoxide; DW, distilled water.

soft ware package.

RESULTS AND DISCUSSION

The antifungal activity of *A. marina* leaves extract in five different solvents: DMSO, DW, chloroform, ethanol and acetone at three doses: 2000, 4000 and 6000 ppm is given in Table 1. The inhibition results using DW as a solvent at 6000 ppm were 80.33 ± 5.60% in *A. flavus* followed by *A. alternata* (78.58 ± 3.10%), *P. notatum* (72.91 ± 7.89%), *C. herbarum* (64.50 ± 3.40%), *A. fumigatus* (63.20 ± 4.50%), *A. niger* (59.08 ± 6.74%) and *S. cerevisiae* 50.00 ± 7.89%). Similarly, the inhibition results in the case of DMSO solvent were in the descending order of *A. niger* (83.00 ± 4.73%), *A. flavus* (75.00 ± 10.75%), *P. notatum* (70.50 ± 5.63%), *C. herbarum* (65.25 ± 3.55%), *A. fumigatus* (63.25 ± 4.52%) and *S. cerevisiae* (41.86 ± 4.86%). It was observed that inhibition in the growth of *S. cerevisiae* in the cases of all solvents is comparatively low. The maximum growth inhibition was found in DW (50.00 ± 7.89% and minimum in acetone (22.74 ± 4.29%). As a whole, the dose dependent tendency in the increase or decrease in the growth inhibition of fungal allergens was found. At 6000 ppm dose, the inhibition was prominently high as compared to 4000 and 2000 ppm doses. On an overall basis, DW was found to be the most effective solvent followed by DMSO, chloroform, ethanol and acetone (Table 1)

Two synthetic drugs: miconazole and amphotericin-B were used as positive control (Table 1). Miconazole was effective against *A. alternata, C. herbatum, P. notatum* and *S. cerevisiae* in the concentrations of 95.00 ± 1.26, 78.00 ± 4.99, 100.00 ± 0.68 and 110.00 ± 2.33 (µg/ml of

SDA medium), respectively. Amphotericin-B was very effective against *A.*

flavus, A. niger, A. fumigatus and *A. niger* in the concentration of 24.00 ± 17.00, 30.00 ± 15.66 and 18.00 ± 18.34 (µg/ml of SDA medium), respectively. Statistically, the data was compared with negative control (±SEM, n = 3) and found to be highly significant ($p<0.01$). Odebode and Che (2001) reported the DW as an effective solvent during the studies on fungal rot of *Citrus sinesis.* Similarly, very significant efficacy of DMSO as an organic solvent was discussed by different workers (Attaurrehman et al., 2001; Tsuzuki and Kitamura, 2001). During this study, *A. marina* exhibited good antifungal activity. Inhibition in the growth of two fungi was excellent according to the inhibition scale adopted during the present work (83.00% in *A. niger* and 80.33% in *A. flavus*). Similarly, the other results are also significant. The results coincide with the results of Gunnar et al. (1991) and Owoseni et al. (2010) who observed a range of efficacy of different plant extracts with respect to the inhibition effects on microorganisms in their studies, separately. Previously, the significant antibacterial activity of *A. marina* was tested by Abeysinghe et al. (2006). Likewise, significant antifouling activity against *Pseudomonas vulgare* was observed by Chen et al. (2008). Bhosale et al. (1999) screened seventeen floral and faunal organisms for their antifungal activity, out of seventeen species, twelve exhibited significant activity

against all the three species of food spoilage strains of *Aspergillus.* Similarly, the cytotoxic activity of mangrove plants was observed by Cyrus et al. (2008). The presence of these plants in high salinity habitat might have caused the toxic microbial compounds with increased resistance and tolerance in these plants. Mangroves might be tolerant to waterborne pollutants as a result of some physiological process that preclude uptake of salts (Snedaker and Brown, 1981).

The reported natural products are tannins, alkaloids, polyphenols, proteins, aminoacids and other compounds (Combs and Anderson, 1949; Julia, 1965). *A. marina* has nematicidal property and releases some compounds toxic to nematodes like phenols, tannins, azadirachtin and ricinine (Tariq et al., 2007). *A. marina* has anticancerous property and flavonoids like compound 1 of aglycone, luteolin 7-O-methyl ether (Sharaf et al., 2000). Okoro et al. (2010) stated that polyphenols are well documented to have microbicidal activities against bacteria and fungi. Ghafar et al. (2010) studied the high antioxidant activity of flavonoids and other phenolic compounds in citrus plants. The flavonoids are most active type of phenols which have ability to act as antioxidant in biological system (Rice-Evans, 1998). Orhans et al. (2009) stated the antibacterial, antifungal and antiviral roles of flavonoid. Flavonoids restrict the mitochondrial enzyme system which is important in cytotoxicity and certain other biological effects and may be target for anticancer chemotherapy (Gosalves et al., 1976). They are strong antifungal candidates that play very important role in the health and disease of a man. Various agents that exhibit antitumor activity interfere directly with nucleic acid metabolism and could impact on their mutagenic and carcinogenic effects (Pratt, 1979). Gosalves et al. (1976) stated that flavonoids restrict the mitochondrial enzyme system which is important in cytotoxicity and other biological effects and may be target for anticancer chemotherapy. Zandi et al. (2009) used the *A. marina* against herpes simplex virus type 1 and a strain of polio virus. Manilal et al. (2009) reported the high biopotential of mangrove plants.

Bohm (1980), Niemann et al. (1987) Siddiqui and Ahmad (1999) were of the opinion that plants subjected to stress conditions produce elevated level of phenolic compounds in order to compensate for the effect of particular stress. Curir et al. (2003) reported the effective use of fungitoxic phenols from carnation *Dianthus caryophyllus* against *Fusarium oxysporum*. Among antifungal agents, phenols have received particular attention because of their importance in secondary metabolites and ubiquitous presence in plant tissues. Niemann et al. (1987) reported that the biosynthesis of fungitoxic phenols may be elicited by fungal presence. Fungal toxins may be present as a constituent of plant tissues.

Conclusion

The leave extracts of the medicinal plant, *A. marina* were used against some atmospheric fungal allergens using five different solvents at 2000, 4000 and 6000 ppm dose levels. Significant antifungal potential was found in DW and DMSO extracts followed by chloroform, ethanol and acetone. The presence of phenols and flavonoids in the *A. marina* made this plant to be very potent and effective against the fungi. Dose dependent tendency was found during the study and the maximum inhibition was found at 6000 ppm dose.

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