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ANTI-FUNGAL ACTIVITY OF SOME MEDICINAL PLANTS ON DIFFERENT PATHOGENIC FUNGI

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Abstract

The antifungal activity of different medicinal and locally available plants extracts (leaves, fruit, seeds) which are usually found in the surrounding of fields or in the fields on some fungi were tested in lab conditions. Six different plants were selected for testing these plants were *Acacia nilotica* (Lamk.) Willd., *Azadirachta indica* (A.) Juss., *Crotalaria juncea* L. *Eucalyptus camaldulensis* Dehnh., *Ocimum basilicum* L., and *Prosopis juliflora* (Sw.) Dc. These plants showed antifungal activity against the *Aspergillus flavus, A. niger, Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani.* These plants crude extracts of leaves showed inhibition activity against the fungi and suppressed the myclial growth. Over all selected plants exhibited moderate type of inhibition against these above mentioned pathogens. Among these plants, *Azadirachta indica, Ocimum basilicum* and *Crotalaria juncea* showed the most effective results against the *Aspergillus, Fusarium* and *Rhizoctonia* sp. of fungal pathogens. Whereas, *Acacia nilotica, Eucalyptus camaldulensis* and *Prosopis juliflora* showed least potential of inhibition against all above mentioned fungal pathogens. It is investigated in present studies that *Azadirachta indica, Ocimum basilicum* and *Crotalaria juncea* can be utilized against the management of fungal diseases particularly *Aspergillus flavus, A. niger, Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani.*

Key words: Antifungal activity, Medicinal plants, Fungi, Inhibition %, Plants extract.

Introduction

The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition (Hussain *et al*., 2009). Plants with possible antimicrobial activity should be tested against some microbes to confirm the activity (Shinwari *et al*., 2009). The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world (Vuuren & Naido, 2010; Bhengraj *et al.*, 2008; Walter *et al*., 2011). Medicinal plants represent a rich source of antimicrobial agents (Mahesh & Satish, 2008; Adnan *et al*., 2010). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al.*, 2008; Gilani *et al*., 2010; Hussain *et al.*, 2012). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides (Varma & Dubey, 1999; Hussain *et al.*, 2014).

Satish *et al*. (2007) reported that aqueous extract of *Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopis juliflora, Punica granatum* and *Sygigium cumini* have been recorded significant antifungal activity against one or the other *Aspergillus* species tested.

Gujar & Talwankar (2012) reported that six plants *Azadirachta indica, Aloe vera, Ocimum sanctum, Ocimum basilicum, Lantana camara* and *Asparagus* used for the testing of antifungal activities and these plants showed the antifungal activity against the *Aspergillus niger, Aspergillus flavus, Rhizoctonia solani* and *Rhizoctonia bataticola*.

Aspergillus niger as a saprophyte in soil causes black mould of onion, garlic and shallot, stem rot, root stalk rot and boll rot of cotton, spoilage of dates, crown rot of groundnut are the most serious plant disease caused by *A. niger* (Bobbarala *et al.,* 2009). The use of Neem cake and extract as a soil treatment measure have produced good result against various soil borne fungi like *Pythium aphanidermatum, Rhizoctonia solani* (Khan *et al.,* 1974) and *Fusarium oxysporum* (Kannaiyan & Prasad, 1981). Antifungal activity of selected medicinal plant diffusates against *Alternaria solani, Aspergillus niger, Rhizoctonia solani* and *M. phaseolina* (Bobbarala *et al.,* 2009). *Rhizoctonia solani* an important destructive soil-borne pathogen has detrimental effects on agricultural and horticultural crops by pre-emergence and post-emergence damping off, root rot and stem canker (Farr *et al.,* 1989).

Materials and Methods

Isolation of fungi: Infected Chilli plants samples like roots and fruits were collected from field of Department of Botany, Federal Urdu University of Arts, Science & Technology, Karachi to isolate the pathogen infected portions of roots and fruit samples were surface sterilized (70% ethanol and 0.1% mercuric chloride) and cultured on Potato Dextrose Agar (PDA). Plates were incubated for six days at 28±2°C.

Identification of fungi: Isolated fungi were identified by using of 10x and 40x magnifications on the microscope and identify hyphae, sporangia, sporangiophores, conidia, conidiophores and some other morphological characters including growth pattern, colony texture and growth rate of the colonies on PDA (Promputtha *et al*., 2005). Standard manuals or refernces including (Ellis, 1971; 1976; Barnett & Hunter, 1972; Nelson *et al.,* 1983; Domsch *et al.,* 1987; Singh *et al.,* 1991; Sutton, 1980) were also used for the confirmation of various species.

Collection of plant material: Six important medicinal plants were selected from local flora which has antimicrobial and antifungal activity in accordance with available literature (Table 1). Plant were collected and washed with tap water as well as surface sterilized with 1% sodium hypochlorite. These plants were oven dried at 40±2°C and their different parts were grinded to form powder extract.

Screening bioassay preparation of plant extract: Screening bioassay preparation of plant extract was conducted in lab condition. For this purpose, 16g of plant extract (powder) were macerated with 100 ml of distill water. The exudation of biochemicals was kept overnight and biomass was filtered by using of standard Whatman No. 1 filter paper. Filtered extract were sterilized. Poison food technique of plant extract against different fungi including *Aspergillus flavus, A. niger, Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani* were determined in lab conditions. Plant extract at 2.2ml of each stock solution (16% concentration) was added in PDA pour sterilized Petri plates. The disc of 5 mm diameter of different fungi 6 days old culture were placed at the centre of Petri plates. Three replicates were kept for each treatment and incubated at 28±2°C. After five days inoculation, radial growth of mycelium were measured and compared with the results of control. The following formula of percent inhibition was applied for each fungus in treatment.

$$\text{Percent inhibition} = \frac{Y - Z}{Y} \times 100$$

where Y = Mycelial growth of pathogen alone (control)
Z = Mycelial growth of pathogen along with antagonist

Statistical analysis: Data on inhibition of mycelia growth of different pathogenic fungi were subjected to one-way analysis of variance (ANOVA). The follow-up of ANOVA included Fisher's least significant test (LSD) at P=0.05 and Duncan's multiple range test.

Result and Discussion

The results as presented in Table 2 shows that that plant extracts were effective in significantly reducing the growth of mycelia as compared with control plates (Table 2). However, all plants show antifungal activity against *Aspergillus* spp. Maximum inhibition percentage was recorded in *Azadirachta indica, Eucalyptus camaldulensis* and *Crotalaria juncea* with 95%, 90% and 87%, respectively suppressed the mycelia growth of *Aspergillus* spp.

Almost all plants show antifungal activity against *Fusarium solani*. Maximum inhibition percentage was recorded in *Ocimum basilicum* and *Crotalaria juncea* with 96.30% and 86.30%, respectively aggressively suppressed the mycelia growth of *F. solani*. Whereas, in *Macrophomina phaseolina* all plant extracts shows significant antifungal activity. But *Azadirachta indica* and *Acacia nilotica* were the dominant plant species with inhibition % of 93.70% and 89.60% than other species.

Table 1. Uses or ailments treated of selected medicinal plants.

Name of the plants	Family	Local name	Parts used	Uses / Ailments treated
Acacia nilotica (Lamk.) Willd.	Mimosaceae	Bubar	Bark, Roots, Fruit, Leaves	It is used against hepatitis, ulcer and infertility of women. It provides help in the control of diarrhea, cough and dysentery
Azadirachta indica (A.) Juss.	Meliaceae	Nim	Whole plant	Most parts of the tree are medicinal. The extract of leaf is recommended for the purification of blood. It is also used for the treatment of bronchial asthma, mouth blister, toothache, bone pain, hair dandruff, as a control of nematodes in plants while flowers are used against the eye infection and diabetes.
Crotalaria juncea L.	Fabaceae	Bhang	Leaves	It contains valuable source of proteins and utilized to prevent blood poisoning, burns, child birth, cough, malaria, anti-lice and as a beverage in hot season.
Eucalyptus camaldulensis Dehnh.	Myrtaceae	Safeedo	Leaves	The leaves of eucalyptus are medicinally very important. It is used as an antiseptic, anesthetic and used for the remedies of colds, diarrhea, sore throat, cough and toothache.
Ocimum basilicum L.	Lamiaceae	Nazboo	Leaves, Seeds	It is the main source of Vitamin A, C, calcium and phosphorus. It does also provide the strength to cardiovascular system through its smell. It possesses high concentration of carotenoids.
Prosopis juliflora (Sw.) Dc.	Fabaceae	Devey	Fruits, Leaves	The velvet mesquite is used as antibacterial agent in alcoholic extracts. It is used in the treatment of colds, diarrhea, flu and head cold.

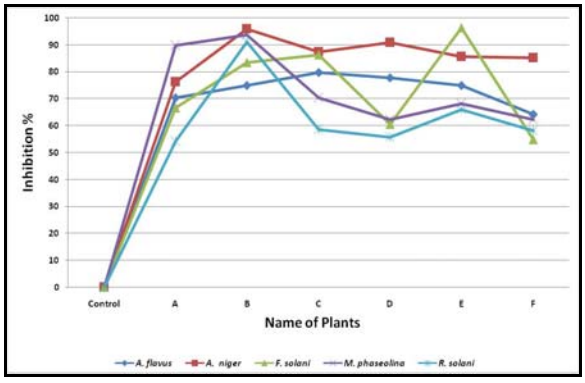
Table 2. Radial mycelia growth (mm) and percent mycelia inhibition of different fungi with plant extracts.

Plant name	*A. flavus*	Inhibition %	*A. niger*	Inhibition %	*F. solani*	Inhibition %	*M. phaseolina*	Inhibition %	*R. solani*	Inhibition %
Control	9±0	0	9±0	0	9±0	0	9±0	0	9±0	0
Acacia nilotica	2.67±1.33	70.37	2.13±0.22	76.30	3±0.32	66.67	0.93±0.50	89.63	4.13±0.26	54.07
Azadirachta indica	2.27±0.09	74.81	0.37±0.37	95.93	1.50±0.32	83.33	0.57±0.57	93.70	0.80±0.42	91.11
Crotalaria juncea	1.83±0.03	79.63	1.13±0.03	87.41	1.23±0.03	86.30	2.67±0.23	70.37	3.73±0.33	58.52
Eucalyptus camaldulensis	2±0.49	77.78	0.83±0.43	90.74	3.57±0.23	60.37	3.40±0.40	62.22	4±0.15	55.56
Ocimum basilicum	2.27±0.55	74.81	1.30±0.15	85.56	0.33±0.33	96.30	2.87±0.39	68.15	3.07±0.20	65.93
Prosopis juliflora	3.23±0.48	64.07	1.33±0.07	85.19	4.07±0.23	54.81	3.40±0.17	62.22	3.77±0.33	58.15

Table 3. F-ratios derived from ANOVA for antifungal effect of different plants on pathogenic fungi based on analysis of colony diameters.

Source	F-ratio	P-value	$LSD_{0.05}$
Treatments	246.91	0.0000***	0.47
Pathogenic fungi (5 spp.)	20.17	0.0000***	0.397
Treatment × Pathogenic fungi	5.48	0.0000***	

F= F-ratio was obtained from ANOVA tables, **LSD**=Least significant difference at P=0.05



A= *Acacia nilotica*, **B=** *Azadirachta indica*, **C=** *Crotalaria juncea*, **D=** *Eucalyptus camaldulensis*, **E=** *Ocimum basilicum* and **F=** *Prosopis juliflora.*

Fig. 1. Show inhibition % of different plant's extract against five pathogenic fungi

All six plants show better antifungal activities against *Rhizoctonia solani* and suppressed the mycelia growth. But maximum inhibition percentage was noted in *Azadirachta indica* and *Ocimum basilicum* with 91.11% and 65.93% respectively than other plant species (Table 2).

The result of all almost all plants indicating the differential activities of the plant extracts on the mycelium growth of *A. niger* and *F. solani*. Because several of these extracts have been shown very strong inhibition against the mycelia growth of *A. niger* and *F. solani* fungi (Table 2 and Fig. 1).

Table 3 shows the results of ANOVA for antifungal effect of different plants on pathogenic fungi. Mostly plants showed highly significant differences and inhibited the growth of pathogenic fungi including *A. flavus, A. niger, F. solani, M. phaseolina* and *R. solani*. All five pathogenic species were inhibited by plants extracted material.

Medicinal plants represent a rich source of antimicrobial agents (Mahesh & Satish, 2008). Many of the plant materials are used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al.,* 2008). Considering the need for an alternative eco-friendly approach to control the phytopathogens, it was believed to be worthwhile to screen the antifungal effects of locally available flora (Bhardwaj, 2012). Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Okigbo & Ogbonnaya, 2006; Shariff *et al.,* 2006; Bouamama *et al.,* 2006; Ergene *et al.,* 2006; Kiran & Raveesha, 2006; Mohana & Raveesha, 2006).

The antimicrobial activities of plant studied have also been found registered in various literature i.e. *Acacia* (Satish *et al.,* 2007; Bhardwaj, 2012), *Azadirachta* (Suhag *et al.,* 2003; Gujar & Talwankar, 2012), *Crotalaria* (Chouhan & Singh, 2010), *Eucalyptus* (Satish *et al.,* 2007), *Ocimum basilicum* (Piyo *et al*., 2009; Gujar & Talwankar, 2012), *Prosopis juliflora* (Satish *et al.,* 2007). Effectiveness of neem extract and oil as a fungicide has earlier been reported by several workers (Ilyas *et al.,* 1997; Dubey & Kumar, 2003; Dubey *et al.,* 2009) found almost similar effect of *Azadirachta* on growth of *M. phaseolina* and some other fungi.

The result of this study indicating that differential activities of plant extracts on the mycelium growth of different fungi because many of these extracts have shown significant and aggressively inhibition observed against the mycelium of growth of test fungi. But *Azadirachta indica* and *Ocimum basilicum* found maximum inhibition (%) and aggressively suppressed the mycelia growth of all pathogenic fungi including *Aspergillus flavus, A. niger, Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani.* The current results demonstrate that selected medicinal plant extracts effective suppressed the radial mycelia growth of all species and these findings confirm the result of Satish *et al.* (2007); Bobbarala *et al.* (2009) and Gujar & Talwankar (2012).

Conclusion

The study has shown that medicinal plants namely *Acacia nilotica, Azadirachta indica, Crotalaria juncea, Eucalyptus camaldulensis, Ocimum basilicum* and *Prosopis juliflora* are very effective and suitable for inhibiting the mycelia growth of *Aspergillus flavus, A. niger, Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani.* These plants could be utilized to field

trials to access their effectiveness in field condition. It will be help in the formulation of ecofriendly control measures, which is cheap and can be recommended for the farmers as reported by Sultana *et al*. (2011) and Saqib *et al*. (2011).

It is concluded in all results that five pathogenic fungi *A. flavus, A. niger, F. solani, M. phaseolina* and *R. solani*, were testes against the extract of six selected plants which have antifungal activities against pathogenic fungi. In present study, it is showed that all plants extract were found maximum affected against the activity of *A. niger* and *oxysporum* and aggressively inhibited the growth of both these species (Fig. 1).

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