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International Journal of Current Research
Vol. 4, Issue, 02, pp.093-096, February- 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PRELIMINARY SCREENING OF ENDOPHYTIC FUNGI FROM *TRIDAX PROCUMBENS*LINN. AND *ARGEMONE MEXICANA* LINN. FOR THEIR ANTIMICROBIAL ACTIVITY

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ARTICLE INFO

Article History:
Received 09th November, 2011
Received in revised form
04th December, 2011
Accepted 08th January, 2011
Published online 29th February, 2012

Key words:
Endophytic fungi,
Antimicrobial activity,
Tridaxprocumbens,
Argemonemexicana.

ABSTRACT

*Tridaxprocumbens*Linn.and*Argemonemexicana*Linn. are the two important medicinal plants used in the present study to determine the colonisation frequencyof endophytic bacteria, actinomycetes&fungi and antimicrobial activity of fungi. The endophytic fungi from leaves,stem and roots of both the plants were identified based on the morphology and characteristics of fungal spores. The crude extracts of fermentation broth of endophytic fungi were prepared in ethyl acetate, diethyl ether and water for testing against pathogenic microorganisms such as *Staphylococcus aureus, Pseudomonasaeruginosa, Escherichia coli* and *Candida albicans*by agar well diffusion method. The ethyl acetate and diethyl ether extracts of *Aspergillusflavus*were found to inhibit the growth of both Gram positive as well as Gram negative bacteria. The diethyl ether extracts of *Aspergillusfumigatus*showed inhibitory effects against *Candida albicans.*The data shows that endophytic*Aspergillusflavus*obtained from *Tridaxprocumbens*appears to show broad spectrum antimicrobial properties.

INTRODUCTION

Endophytic microorganisms are those that inhabit the internal part of plants, causing apparently no visible changes to their hosts. Endophytic organisms have received considerable attention after they were found to protect their host against insects, pest, pathogens even domestic herbivores (Weber, 1981). Almost all plant species (~400, 000) harbour endophytic organisms (Tan and Zou, 2001). Recent surveys of various host plants have demonstrated that fungal endophytes are ubiquitous in plant species (Kumar *et al*., 2004; Zhang *et al*., 2006; Huang *et al*., 2008; Oses *et al*., 2006). *Argemonemexicana*Linn.is a prickly, glabrous, branching annual herb with yellow juice and showy yellow flowers. It is used as a medicinal plant in several countries. In Mexico, the seeds are considered as an antidote to snake venom. In India, the smokes of the seeds are used to relieve toothache. *Tridaxprocumbens*is known for its wound healing activities. The extracts of these plants are well known for their medicinal properties among local natives. Previous studies have shown that both the plants showed antioxidant and hepatoprotective activity (Wagh and Shinde, 2010), antiurolithiatic (Sailaja *et al*., 2011), anti-hyperglycemic(Pareek *et al*., 2009), anti-inflammatory and analgesic properties (Das *et al*., 2009; Prabhu *et al*., 2011, Anarthe and Chaudhari 2011) and anticancer activities (Chang *et al*., 2003; Vishnu *et al*., 2011). The studies on endophyticmicroorganisms with respect to antimicrobial activity of these plantsare not well reported. Considering the significance of these plants, the present study was aimed to determine the colonization frequency of endophytic microorganisms, identify the endophytic fungi, and to evaluate the antimicrobial activity of crude extracts of endophytic fungi obtained from*Tridaxprocumbens*Linn. and*Argemone Mexicana* Linn.

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RESULTS AND DISCUSSION

The extracts from many types of local plants are used in traditional manner for treatments of various ailments (Beulah *et al*., 2004; Rajasekaraetal., 2006). Studies have demonstrated that crude extracts from culture broth of endophytic microorganisms displayed antibacterial, antifungal, antiviral, anti-inflammatory and anti-tumor activity (Silva *et al*., 2007). The endophytic flora, both in number & types varies and depends on the host and geographical position. Therefore, the use of endophytic fungifor cultivation opens up new possibilities for biotechnological applications.

Colonisation frequency (CF) of endophytic microorganisms:

The colonisation of plant tissues by endophytic fungi occurs in a manner similar to those of plant pathogens and mycorrhizae (Lumyong *et al*., 2004). Colonisation comprises a sequence of steps involving host recognition by the fungus, spore germination, penetration of the epidermis, and tissue colonisation.In the current study, the colonisation frequency of endophytic microorganisms (bacteria, actinomycetes and fungi) was observed in all the parts (leaves, stem and root) of *Tridaxprocumbens*and *Argemonemexicana*. Endophytic bacteria showed 100% CF in all parts of *Argemonemexicana.*

However, endophyticactinomycetes colonised leaves and stem of *Argemone Mexicana* to a greater extent as compared to that of *Tridaxprocumbens*. The CF of endophytic fungi was found to be more in leaves and stem of *Argemonemexicana* followed by *Tridaxprocumbens*root segments (Figure 1). The observations in our study are in conformity with the findings reported in *Withaniasomnifera*(L.) Dunal(Rezwana *et al*., 2010).

Occurrence of endophytic fungi

A total of 10 endophytic fungi from *Tridaxprocumbens*and *Argemonemexicana*were identified. The endophytic fungal communityof *Tridaxprocumbens*include*Aspergillusniger*, *A. flavus* and *Mucor spp.* Moreover, the endophytic fungi such as *A.fumigatus*, *A. parasiticus*, *A.nidulens* and four different unidentified species of Aspergillus were found in *Argemonemexicana*.Some of these Ascomycota forms have been previously reported as endophytes (Blodgett *et al*., 2000; Suryanarayana*et al*., 1998, 2002) except *Mucor spp.* (Zygomycota).Among the endophytic fungal population, *Aspergillus species* was the most dominant endophyte in *Argemonemexicana*. Previously, this fungus was reported as an endophyte in other plant species like *Tripterygiumwilfordii* (Siva and Kevin 2004), *Calotropis gigantean* (Srimathi *et al*., 2011), *Calotropisprocera* (Rezwana *et al*., 2007), *Withaniasomnifera* (Rezwana *et al*., 2010), *Azadirachtaindica* (Tenguria and Firoz, 2011) and *Meliaazadirachta* (Kaushal *et al*., 2010).Previous studies reported distinct endophyte community compositions in differenthost plants suggesting host preference (Cannon and Simmons, 2002; Cohen, 2006).

Antimicrobial activity

Theethyl acetate, diethyl ether and water extracts of 10 different endophytic fungi were tested against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*and one fungus, *Candida albicans.*It is observed from our data that six endophytic fungi displayed antimicrobial activity against at least one test microorganism. Further, it was observed that ethyl acetate and diethyl extracts of *Aspergillusflavus*were found to be effective against Gram positive and Gram negative pathogenic bacteria (Figure 2).The extracts of *A. niger, A. parasiticus*and *A. fumigatus*displayed antifungal activities against *Candida albicans*(Table-1). In contrast, the aqueous extracts were found to be ineffective. Similar results have been obtained by Haque *et al*. (2005) in that ethyl acetate extract of the fungal strain 2L-5 was found active against *Bacillus cereus* and *Staphylococcusaureus.*Antimicrobial activities of endophytic fungi from other plants have also been reported by several groups (Rodrigues *et al*., 2000; Ghadin *et al*., 2008; Xu *et al*., 2008; Hazalin *et al*., 2009; Li and Guo, 2009). Guimaraes *et al*. (2008) screened extracts from 39 endophytic fungi isolated from *Viguieraarenaria*and *Tithoniadiversifolia*, and reported 5.1% active extracts against *S. aureus*and 25.6% active extracts against *E.coli*. An extract of *Streptomyces* sp. (SUK 06) isolated from the stem of a Malaysian plant was found to be as effective as oxacillin against *B. subtilis*(Ghadin *et al*., 2008). In conclusion, our study has shown that the colonization frequency of endophytic microorganisms was more in *Aregemonemexicana* as compared to that of *Tridaxprocumbens*. The antimicrobial activity of fungal endophytesindicated that the ethyl acetate and diethyl ether extracts of *Aspergillusflavus*from *Tridaxprocumbens*showed broad spectrum antimicrobial activity.

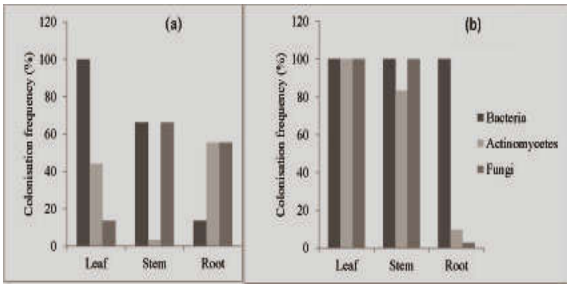

Figure 1: Colonisation frequency (%) of endophytic bacteria, actinomycetes and fungi in different parts (leaves, stem and root) of medicinal plant; (a): *Tridaxprocumbens*Linn.and (b): *Argemonemexicana* Linn.

Experimental procedures

Healthy leaves, roots and stems of *Tridaxprocumbens*Linn. and *Argemonemexicana* Linn.were collected from Swami RamanandTeerthMarathwada University Campus, Nanded, Maharashtra, India.The colonization frequency of endophytic microorganisms was determined from surface sterilized plant segment according to the methods previously described with slight modification(Petrini*et al*., 1986;Schulz *et al*.,1993).The surface sterilized segments were placed in separate petridish containing potato dextrose agar supplemented with penicillin (100 µg/ml) and streptomycin (100 µg/ml) for fungi, Starch casein agar supplemented with nystatin (50 µg/ml) and cycloheximide (50 µg/ml) for actinomycetes and Nutrient agar supplemented with nystatin (50 µg/ml) for bacteria. The plates were sealed, and incubated for 5 days (bacteria), 3 weeks (actinomycetes) and 5 weeks (fungi) at 30 ºC. The colonization frequency was determined as reported earlier (Suryanarayanan*et al*.,2003). Subsequently, endophytic fungi were isolated and pure cultures were maintained on potato dextrose agar slants at 4°C.

Table 1: Antimicrobial activity of endophyticfungal extracts

No.	Name of Endophytic fungi	Zone of inhibition (mm)							
		Ethyl acetate fungal extract				Diethyl ether fungal extract			
		Ec	Sa	Pa	Ca	Ec	Sa	Pa	Ca
1.	*Aspergillusflavus*	6	5	7	-	6	6	7	-
2.	*A. niger*	-	-	-	1	-	-	-	2
3.	*A. parasiticus*	4	4	5	2	1	1	1	2
4.	*A. fumigatus*	-	-	2	2	-	-	1	3
5.	*Aspergillus spp.*1	2	-	1	-	3	-	2	-
6.	*Aspergillus spp.*2	-	-	-	3	-	-	-	1

Where, - is no zone of inhibition; Ea: *Escherichia coli*, Sa:*Staphylococcusaureus* , Pa: *Pseudomonas aeruginosa,* and Ca: *Candida albicans.*

Experimental procedures

Endophytic fungi were identified according to their macro and microscopic structures (Mukadam, 1997). Fermentation and extraction was carried out as per the procedures described previously (Sutjaritvorakul *et al*., 2011).The crude extracts were prepared in ethyl acetate, diethyl ether and water and the contents, evaporated and dissolved in dimethyl sulphoxide. These extracts were tested for antimicrobial activity by agar well diffusion method (Nithya and Muthumary, 2011). The ethyl acetate, diethyl ether, dimethyl sulphoxide and sterile

potato dextrose broth were used as controls.The zone of inhibition was measured in mm.

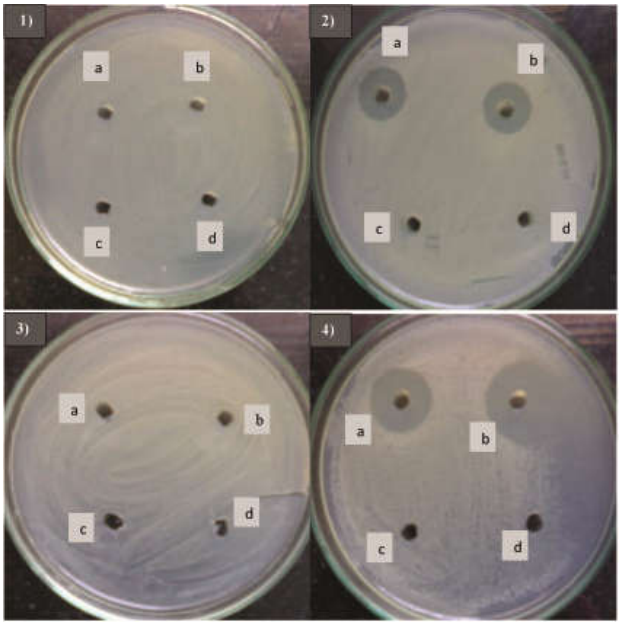

Figure 2: Antibacterial activity of endophytic fungus *Aspergillusflavus*: 1&3- Controls for *Escherichia coli*& *Pseudomonas aeruginosa*respectively; a, b, c and d denotes ethyl acetate, diethyl ether, dimethyl sulphoxide and sterile potato dextrose broth respectively. 2& 4-experimental for *Escherichia coli* and *Pseudomonas aeruginosa*; a, b, c and d denotes ethyl acetate, diethyl ether, aqueous extracts and sterile potato dextrose broth respectively.

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