Indian Journal of Science and Technology Vol. 5 No. 9 (Sep. 2012) ISSN: 0974- 6846

Study on the antimicrobial activity of *Aspergillus* sp isolated from *Justicia adathoda*

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Abstract

A number of new bioactive compounds from endophytes have been recognised as potential sources of antimicrobial substances. In the present study, the leaf samples of *Justicia adathoda* was screened for endophytic fungi. The isolated fungi was identified as *Aspergillus* sp. based on morphological features. Fungal metabolites of the mycelia and the culture broth were extracted with ethyl acetate. The antimicrobial activity of the mycelia extracts and crude culture broth were evaluated by agar well diffusion method against few representative strains. Crude mycelial extract inhibited all the strains significantly, with a mean strongest zone of inhibition of 12.0±1mm against E.coli. Crude culture broth inhibited the strains of *Pseudomonas* and *Klebsiella* alone and had a mean stronger zone of inhibition of 13±1mm and 14±1mm than crude mycelia extract. Further FTIR analysis indicated the nature of the antimicrobial compound.

Keywords: Endophytic fungi, Justicia adathoda, Agar well diffusion, Aspergillus sp.

Introduction

Endophytic microorganisms are mutualistic symbionts of plants which colonise the healthy tissues of plants without causing any overt negative effects. The most frequently isolated endophytes are the fungi (Strobel & Daisy, 2003). An estimate of about a million undescribed species of endophytic fungi occurs within the plant aerial tissues (Jianglin Zhao *et al.,* 2010; Wu-Yang Huang *et al.,* 2007). During their long co-evolution with their host plant, endophytes have adapted themselves to their microenvironment by genetic variation, which has led to the ability to synthesize some phytochemicals originally associated with the host plants (Nithya & Muthumary, 2009;2011).

Endophytes are viewed as novel sources of secondary metabolites recently. Though there are many publications on the secondary metabolite production by endophytes, very little information is available on the mechanism of symbiosis and significance of the products (Joong-Hyeop *et al.*, 2003). Some of the compounds are proven to be useful for novel drug discovery, as it solves the problem of the slow growth of plants and environmental damage (Xiang Lin *et al.,* 2007). Endophytes could be utilized for their fermentation and biotechnology capabilities as an alternative mode of production of the bioactive components (Arunachalam *et al.,* 2010). Distinctly from plants, endophytes can be cultured quickly and the biomass can be accumulated by large scale fermentation. Ultimately endophytic fungi have emerged as an alternative source for the production of new antimicrobial agent (Lei Guo *et al.*, 2008).

Endophytes of ethanomedicinal plants could be a promising source of antimicrobial substances (Maksum Radji *et al.*, 2011). In the present investigation we screened the leaves of *Justicia adathoda* for endophytic fungi and determined its antimicrobial activity. *Justicia adathoda* (also known as *Adathoda beddomei*) is an endemic species of India occurring in the Travancore hills of South Western Ghats, Valparai (South Arcot), Akkamalai (Coimbatore Dist.) and Mahendragiri (Kanniyakumari Dist.). It is a well known medicinal plant which has been used as a effective drug for asthma and cough (Seema Sharma *et al.*, 2010).

Materials and methods

Isolation of Endophytes

The fresh healthy leaves of *Justicia adathoda* were obtained from Siddha Institute, Chennai. The leaves were cleaned under running tap water and air dried. Surface sterilization was carried out by sequential washings in 70% ethanol for 1 min, 5% sodium hypochlorite solution for 5 min and sterile distilled water for 1min twice. The surface sterilized leaves were cut in to small pieces using a sterile blade and transferred to sterile Potato dextrose agar (PDA) plate supplemented with chloramphenicol (50µg/ml). The plates were incubated at 27ºc for 7-14 days (Jalgoanwala *et al.,* 2010). The endophytic fungi grown on plates were transferred to sterile PDA slants to maintain culture purity. The fungi were identified by colony morphology and LPCB mount.

Extraction of bioactive compound

The fungal endophytes were recultivated on potato dextrose broth by placing agar block of actively growing pure culture in 250ml Erlenmeyer flask containing 100ml of the medium. The flasks were incubated at 27°c for 7 days with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out and filtered through sterile mesh cloth to yield the crude culture filtrate (CCF).

Fungal metabolite in the mycelial mat was extracted by solvent extraction procedure with ethyl acetate as organic solvent (Suthep Wiyakrutta *et al.*, 2004). The mycelia mat was soaked in ethyl acetate for 2hrs and ground in a mortar pestle. The solvent was evaporated in a Soxhalate Apparattus. The resultant crude extract (CME) dissolved in DMSO for further analysis.

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Fig.1. FTIR spectrum of CCF

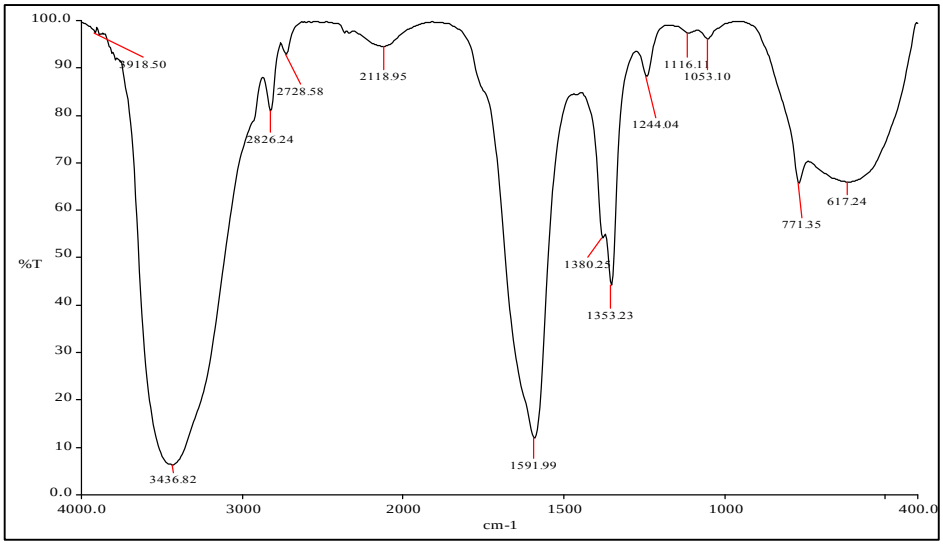
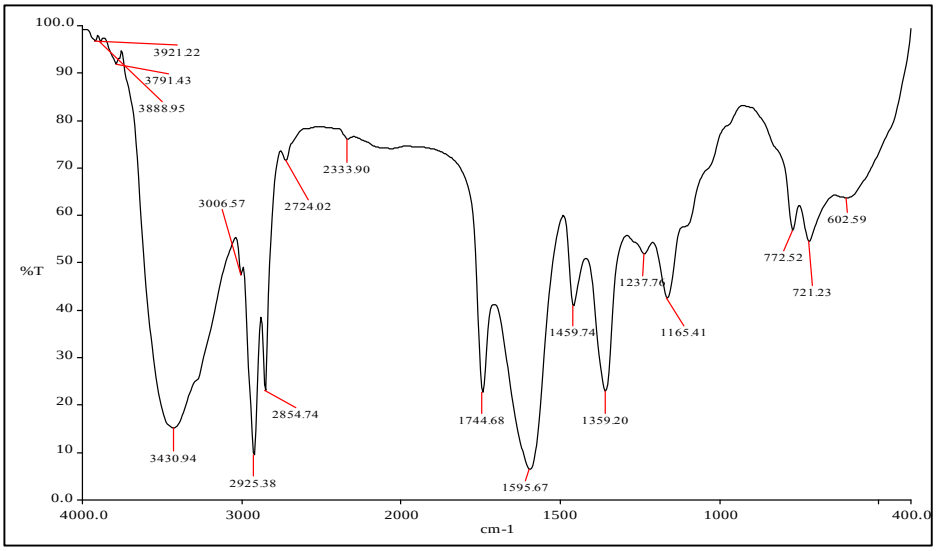


Fig.2. FTIR spectrum of CME



Determination of antimicrobial activity

The antimicrobial activity of the extracts was evaluated by agar well diffusion method. (Maksum Radji *et al* 2011). The test cultures used are: *Candida albicans ATCC 24433, Escherichia Coli ATCC 35218, Staphylococcus aureus ATCC25923, Klebsiella pneumoniae ATCC* 700603, *Pseudomonas aeruginosa ATCC27853.*

A volume of 50 ml of nutrient broth was prepared and dispensed into boiling tubes (10ml each) and they were sterilized. In each of sterilized nutrient broth tubes a loopful of test cultures were inoculated and kept in a shaker overnight at 37°c for growth. A volume of 500ml of nutrient agar was prepared and sterilized.1 ml of test culture solution was added into the each 100ml of agar solution.

The agar solutions with culture were poured in to the sterile Petri dishes and allowed to solidify. Wells was punched using a puncher on the plates. A volume of 50 µl of the CCF and CME were loaded to each of the well. The test was carried out in triplicates. The negative control plate was loaded with DMSO on the well. Simultaneously a positive control was maintained with streptomycin. After 24 hrs incubation at 37°c, the plates were observed for zone of inhibition and measured. The mean of the inhibition zones and the standard deviation was calculated statistically.

Spectral analysis

CCF and CME were further characterised by FTIR analysis in Perkin Elmer IR Spectrophotometer.

Result and discussion

Studies indicated the frequently isolated endophytic filamentous fungi, which were studied for antimicrobial activity belonged to Ascomycetes group (Maruette dos *et al.*, 2009). In this study we have isolated endophytic fungi from Justicia a*dhathoda* and identified to be *Aspergillus sp.* by colony morphology and characterisation by LPCB mount.

Ethyl acetate extract of the mycelial mat (CME) and the culture broth (CCF) were evaluated for their antimicrobial activity against the test organisms *Candida albicans ATCC 24433, Escherichia coli ATCC 35218, Staphylococcus aureus ATCC25923, Pseudomonas aeruginosa ATCC27853, Klebsiella pneumoniae* ATCC 700603.

Table 1 shows the zone of inhibition obtained for each of the test cultures indicating the antimicrobial activity. The mean and standard deviation was calculated for the triplicate values. Among the two samples tested, CME inhibited all the strains significantly. This result attributes the inhibition by intracellular compounds of the extract which are extracted by ethyl acetate. The strongest zone of inhibition was observed against *E.coli*. No zone of inhibition was observed with three strains tested with CCF. Comparatively, the strains of *Pseudomonas sp.* and *Klebsiella sp.* were susceptible to the components in CCF than CME. This indicates the susceptibility of these strains to the extracellular compounds of the endophyte found in the crude fermented broth. These results comply with the earlier findings in the difference of inhibition by plant and fungal extracts against Gram positive and

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Gram negative organisms (Supaluk *et al.,* 2008). The

Table 1. Values are mean inhibition zone (mm) ± S.D of three replicates

Test Organism	Zone of inhibition (mm)	
	CCF	CME
Candida albicans	Nil	10.33±1.52
Escherichia Coli	Nil	12.0±1
Staphylococcus aureus	Nil	9±1
Pseudomonas aeruginosa	13±1	8.6±1.5
Klebsiella pneumonia	15±1	11±1

fermented extract of endophytic *Phoma sp.* showed variation in the antimicrobial activity, with strong activity against Gram positive than Gram negative organisms.

The differential susceptibility was attributed to the culture conditions, extraction procedure and the test strain used for antimicrobial analysis (Hoffman *et al.,* 2008). Our results indicate the broad antimicrobial spectrum and strong toxicity of the bioactive components of CME. Conclusively, the antimicrobial activity of endophytic *Aspergillus sp.* is due to the intracellular bioactive components rather than the crude cultured broth.

Tan & Zou (2001) have reviewed the diversity of metabolites isolated from endophytic fungi; like polyketide, isoprenoids, aminoacid derivatives. Schulz *et al.* (2002) have reviewed and identified diverse structural groups e.g, steroids, xanthones, phenols, quinines terpenoids. The FTIR spectra of CCF and CME are given in Fig.1 and 2 respectively. The spectrum indicates presence of long aliphatic chains and simple, unsaturated aromatic rings in CCF, whereas CME is characterised by presence of aromatic groups. The multiple functionality reflects either the complex structure or indicates the nature of the sample as a mixture (John Coates, 2000). The results urge the need to separate and further purify the compounds. Due to the diversity and complexity of the natural mixtures of bioactive compounds in crude fungal culture, the compounds present and their structure cannot be elucidated in a single study. Further investigation is needed to discover the unidentified bioactive constituents in the endophytic fungal isolates.

Conclusion

The endophytic *Aspergillus sp.* isolated from *Justicia adathoda* in this study displayed antimicrobial activity against *Candida albicans* and few bacteria. It would be of interest to test it against a range of human pathogens. Further study is in progress on the bioguided fractionation which would identify the individual components and lead to isolation of the active principle.

References

1. Angela M Hoffman, Steven G Mayer, Gary Strobel *et al*. (2008) Purification, identification and activity of phomodione,a furandione from an endophytic *Phoma species*. *Phytochem*. 69,1049-1056.
2. Arunachalam C and Gayathri P (2010) Studies on bioprospecting of endophytic bacteria from the medicinal plant of *Andrographis paniculata* for their antimicrobial activity and antibiotic susceptibility pattern. *Int. J. Curr. Pharma. Res*. 2,(4), 63-68.
3. Barbara Schulz, Christine Boyle, Siegfried Draeger, Anne-Katrin Rommert and Karsten Krohn (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycol.Res*.106 (9), 996-1004.
4. Gary Strobel and Bryn Daisy (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Rev*. pp: 491-502.
5. Jalgaonwala RE, Mohite BV and Mahajan RT (2010) Evaluation of endophytes for their antimicrobial activity from indigenous medicinal plants belonging to North Maharashtra region India. *Int. J. Pharma. Biomed. Res*.1(5),136-141.
6. Jianglin Zhao, Yan Mou, Tijiang Shan, Yan Li, Ligang Zhou, Mingan Wang and Jingguo Wang (2010) Antimicrobial metabolites from the endophytic fungus *Pichia guilliermondii* isolated from *Paris polyphylla var. Yunnanensis. Molecules*. 15, 7961-7970.
7. John Coates (2000) Interpretation of infrared spectra, A practical approach in encyclopedia of analytical chemistry. Meyers RA (Ed.) John Wiley & Sons Ltd, Chichester. pp: 10815-10837.
8. Joong-Hyeop Park, Ji Hyup Park, Gyung Ja Choi, Seon-Woo Lee *et al*., (2003) Screening for antifungal endophytic fungi against six plant pathogenic fungi. *Mycobiol*. 31(3),179-182.
9. Lei Guo, Jin-zhong Wu,Ting Han, Khalid Rahman and Lu-ping Qin (2008) Chemical composition, antifungal and antitumor properties of ether extracts of *Scapania verrucosa Heeg*. and its endophytic fungus *Chaetomium fusiforme*. *Molecules*. 13, 2114-2125.
10. Maksum Radji, Ateik Sumiati, Renita Rachmayani and Berna Elya (2011) Isolation of fungal endophytes from *Garcinia mangostana* and their antibacterial activity. *Afr. J. Biotechnol*.10(1), 103-107.
11. Maurette dos Reis Vieira Fernandes, Tales Alexandre Costa e Silva, Ludwig Heinrich Pfenning *et al*, (2009) Biological activities of the fermentation extract of the endophytic fungus *Alternaria alternata* isolated from *Coffea Arabica* L. *Brazilian J. Pharma. Sci*. 45 (4), 677-685.
12. Nithya K and Muthumary J (2011) Bioactive metabolite produced by *Phomopsis sp*., an endophytic fungus in *Allamanda cathartica* Linn. *Recent Res. Sci.Technol*. 3(3), 44-48.
13. Nithya. K and Muthumary J (2009) Growth studies of *Colletotrichum gloeosporioides* (Penz.) Sacc. - a taxol producing endophytic fungus from *Plumeria acutifolia*. *Indian J.Sci.Technol*. 2(11),14-19. Domain site: http://www.indjst.org.
14. Seema Sharma, Dimple Sharma, Raghuvanshi RK, Sharma RA, Payal Chandrawant (2010) Cytological studies of *Adathoda* L species and *Barleria* L species. *The Bioscan*. 5(1) 67-70.

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15. Supaluk Prachayasittikul, Prasit Buraparuangsang, Apilak Worachartcheewan *et al.* (2008) Antimicrobial and Antioxidative activities of bioactive constituents from *Hydnophytum formicarum* Jack. *Molecules*.13, 904-921.
16. Suthep Wiyakrutta, Nongluksna Sriubolmas,Wattana Panphut,Nuntawan Thongon,Kannawat Danwisetkanjana, Nisri Ruangrungsi and Vithaya Meevootisom (2004) Endophytic fungi with antimicrobial, anticancer and antimalarial activities isolated from Thai medicinal plants. *World J. Microbiol. Biotechnol.* 20, 265-272.
17. Tan RX and Zou WX (2001) Endophytes: a rich source of functional metabolites. *Natural Products Rep*.18, 448-459.
18. Wu-Yuang Huang, Yi- Zhong Cai, Kevin D. Hyde, Harold Corke, Mei Sun (2007) Endophytic fungi from *Nerium oleander* L (Apocynaceae): main constituents and antioxidant activity. *World J. Microbiol. Biotechnol.* 23. 1253-1263.
19. Xiang Lin, Chunhua Lu Yaojian Huang, Zhonghui Zheng, Wenjin Su and Yemao Shen (2007) Endophytic fungi from a pharmaceutical plant, *Camptotheca acuminate*: isolation, identification and bioactivity. *World J. Microbiol. Biotechnol.* 23, 1037-1040.