ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

SCREENING OF ENDOPHYTIC FUNGI FROM A MEDICINAL PLANT, *JUSTICIA ADATHODA* L. FOR ANTIBACTERIAL ACTIVITY

K. Sowparthani [1]*, K. Rajagopal [2]
*[1]Department of Microbiology, Apollo Arts and Science College, Chennai.
[2]Department of Biotechnology, VELS University, Pallavaram, Chennai.
Email: sowpavenkat@gmail.com

Received on 09-07-2011 *Accepted on 24-07-2011*

Abstract

Justicia adathoda (Linn) is predominantly one of the herbs in waste lands throughout the plains of India. The leaves are used in traditional medicine. In the present investigation, the isolation and identification of endophytic fungi of *Justicia adathoda* was carried out. Ethyl acetate extract of endophytic fungi *Curvalaria tuberculata* were investigated for their antibacterial activity by using disc diffusion method. The ethyl acetate extract of *Curvalaria tuberculata* showed significant antibacterial activity against some Gram positive (*Staphylococcus aureus, Streptococcus pneumoniae*) and Gram negative bacteria (*Salmonella typhi, E. coli*) of pathogenic bacterial strains.

Keywords: Antibacterial activity, *Curvalaria tuberculata,* Endophytic fungi, *Justicia adathoda,* Natural product.

Introduction

The endophytic fungi are of biotechnological importance as new pharmaceutical compounds, secondary metabolites, agents of biological control and other useful characteristics could be found by further exploration of endophytes. There is a general call for new antibiotics, chemotherapeutic agents and agrochemicals that are highly effective, posse's low toxicity, and will have a minor environmental impact.

Endophytic fungi live asymptomatically within the living tissue of the host plant and form mutulastic

symbiosis. There could be more than one type of endophytic fungi found within one plant. Endophytic fungi are relatively unexplored producers of metabolites useful to pharmaceutical and agricultural industries [1]. Different endophytic fungal species are found in different parts of a plant; this represents an adaption mechanism of endophyte against micro ecology and physiology of host plant. Various studies demonstrated that endophytic fungi produce secondary metabolites such as enzyme and growth hormone which are useful for treatment of various diseases. These bioactive compounds demonstrated potent anti-bacterial, anti-arthritis, anti-cancer activity as well as immunosuppressive activity [2].

The temporal pattern of endophyte infection in the leaves of *Plumeria rubra*, a tropical deciduous tree, was studied by sampling the leaves of an individual tree for a period of one year. Endophytes could be isolated from the leaves throughout the study period. Older leaves were more densely colonized than the younger leaves. Hypomycetes dominated the endophyte assemblage of the younger leaves, while the older leaves harboured more coelomycetes. This study indicated that there is temporal variation of endophyte assemblages in leaves of some tropical plant hosts [3].

Endophytic fungi may also produce metabolites with thermo protective role. For example, plants in some volcanic areas in USA were found colonized by an endophytic fungus *Curvularia* sp. [4]. Whereas the plants grown from surface-sterilized seeds in sterile soil that had been inoculated with *Curvularia s*p. survied constant soil temperature of 50°C, the non- symbiotic plant died.

Antibacterial resistance especially among gram-negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search for alternative drugs of natural anti-bacterials [5]. In Malaysia, extract from many types of local plants are used in traditional manner for treatments of various ailments [6]. The question is whether they are produce by the plant itself or as a consequence of a mutualistic relationship with beneficial organisms in their tissue. Many reports showed that in a plant-microbe relationship, endophytes contribute substances that possess various types of bioactivity, such as antibacterial and antifungal. In this study screening endophytic fungi isolated from a medicinal plant *Justicia adathoda* L.

for its antibacterial activity against human pathogenic bacteria.

Materials and Methods

Collection of leaf samples

The *Justicia adathoda* leaves samples were collected from Anna Herbal Garden, Tamilnadu. The samples were transported in closed sterile polythene bags and processed within 24 hours collection.

Test bacterial strains

The pathogenic bacterial strains such as *Salmonella typhi, E. coli, Staphylococcus aureus* and *Streptococcus pnumoniae* were received from Department of Botany, Madras University.

Surface sterilization

The leaves were thoroughly washed in running tap water. Approximately 0.5 cm^2 on leaf segments were cut from the healthy leaves. Then the leaf segments were surface sterilized by immersion in 70% ethanol for five seconds, followed by treatment in 4% sodium hypochloride for 90 seconds and finally rinsed in sterile distilled water for 10 seconds [7].

Isolation of endophytic fungi

The surface sterilized leaf segments were evenly spaced in Petri dishes containing Potato Dextrose Agar (PDA) medium amended with 10 mg of chloromphenicol. The Petri dishes incubated at 26 ± 1ºC in a light chamber [8] and monitored every day for the growth of endophytic fungal colonies from leaf segments. The hyphal tips, which grew out from leaf segments were isolated and brought into pure culture. The isolated endophytic fungi were identified down to species level using standard manuals and monographs [9, 10].

Data Analysis

The colonization frequency of each endophyte species was calculated by the method of Hata and Futai [11].

$$CF\% = \frac{\text{The number of colonized segments}}{\text{Total number of segments observed.}} \text{ x } 100$$

Extraction of bioactive compounds

The selected endophytic fungi (*Curvalaria tuberculata*) were grown in Czapek's broth for 48 hrs and incubated for 21 days at 120 rpm. The extract was separated using filtration procedure (Whatman No 1). Ethyl acetate was added in culture filtrate and the compounds were separated using separating flask and concentrated in rotary vacuum evaporator. The dry semi solid residue was redissolved in ethylacetate for further use [12].

Antibacterial activity

The concentrate crude extract (*C. tuberculata*) was then impregnated (80 µl/disc) on to sterile Whatman 6 mm diameter disc and the antibacterial activity was assayed against *Salmonella typhi, E. coli, Staphylococcus aureus* and *Streptococcus pnumoniae* following the disc diffusion assay [13,14]. The assay was carried out in triplicate. Control plates with solvents were also maintained separately. The zone of inhibition was measured from the edge of the disc to the clear zone in millimeter.

Column chromatography purification

The active *C. tuberculata* extracts were then column fractionated using normal phase silica gel chromatography employing a step gradient (hexane 100%; hexane 75%: ethyl acetate 25%; hexane 50%: ethyl acetate 50%; hexane 25%: ethyl acetate 75%; ethyl acetate 100). The fractions were concentrated and used for antibacterial assay was carried out to find out the active fraction [12].

Results and Discussion

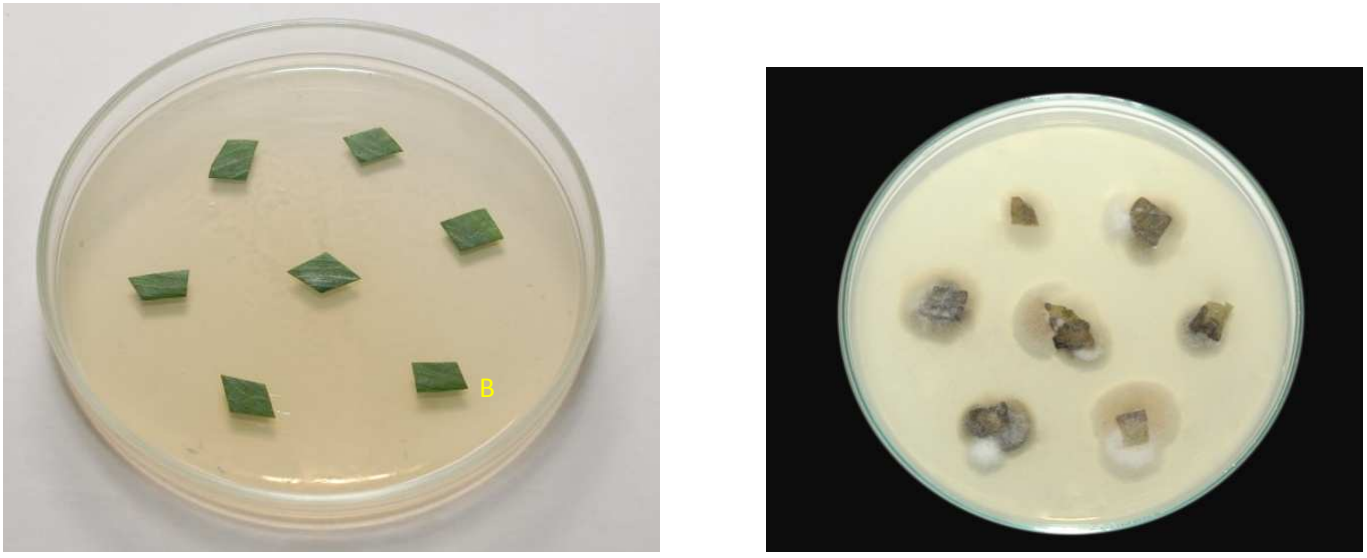
Plants have long provided mankind with a source of medicinal agents, with natural products once serving as source of all drugs [15]. Though synthetic chemical also have long been used as active agents in reducing the incidence of plants, animals and humans diseases, they are costly, have potentially harmful effect on the environment and may induce pathogen resistance. Thus, biological controls or the use of microorganisms or their secretions to prevent diseases offer an attractive alternative or supplement to disease management without the negative impact of chemical control.

The surface sterilized leaf segments were plated on PDA medium (Figure 2A) amended with

chloramphenicol and incubated at 30±1°C for 2 weeks. The fungi grow out from tissues were brought into pure culture and identified (Figure 2B). *Aspergillus* sp., *Alternaria* sp. *and Curvalaria* sp. occur as phylloplane fungi but are capable of penetrating superficial layers of leaf; when they do so they survive the vigorous surface sterilization steps during isolation and grow out as colonies in plates [16] suggesting that phylloplane fungi too resort to an endophytic mode of life to overcome drastic environmental conditions such as compelion and desiccation [17].

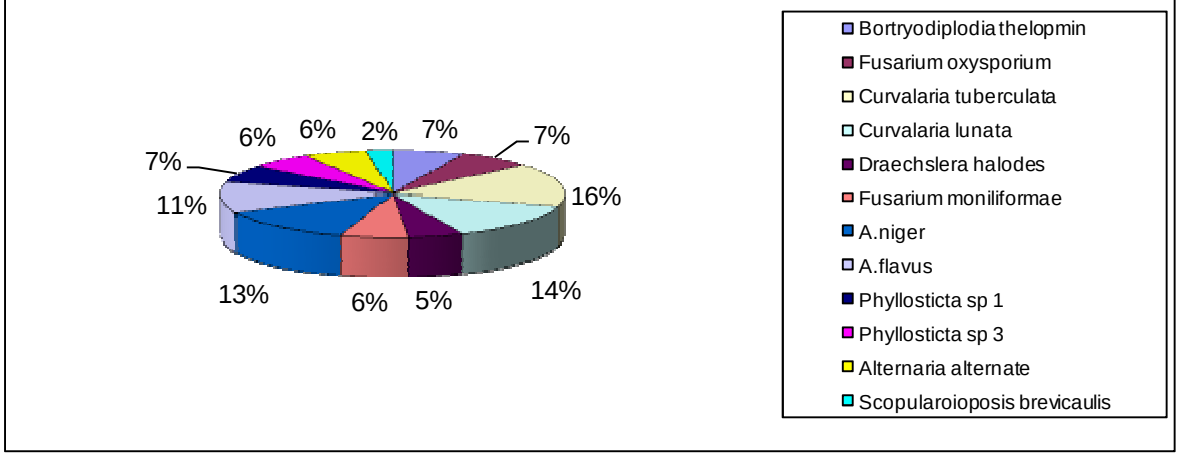
A total of 12 fungal endophytes were obtained from *Justicia adathoda* plant. Out of 12 fungal endophytes, 9 hypomycetes and 3 coelomycetes were isolated as endophytes from the leaves of *Justicia adathoda. Curvalaria tuberculata* is dominant in *Justicia adathoda* plant studied. The more number of endophytes could be recovered from the midrib region than the lamina. The occurrence and distribution of endophytic fungi from leaves of *Justicia adathoda* results are presented in table (Figure 2). Among the hypomycetes and Coelomycetes the *C.tuberculata* showed maximum colonization frequency 7.7% (Figure 1). *Pestalotiotsis* sp. is dominant in *Acalypha indica* plant studied. *Aspergillus* genus and *Pestalotiopsis* occurs as endophyte in a wide range of plant species [18].

Figure-2: Endophytic fungus from *Justicia adathoda* leaf samples.



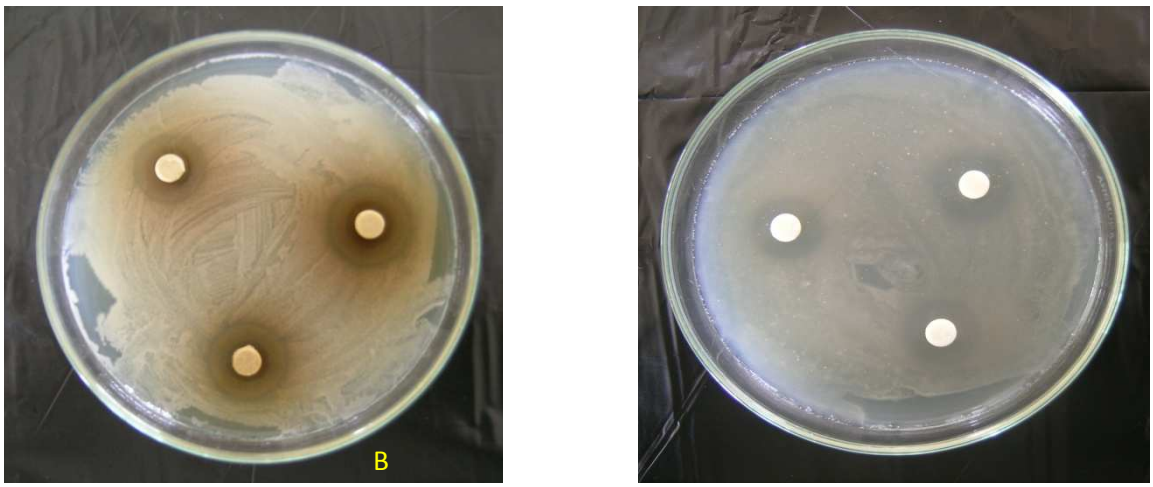
A. At the time of inoculation; **B- After incubation.**

Figure 1: Occurrence of endophytic fungi in lamina and midrib of *Justicia adathoda*



Gunignardia sp. demonstrated a non selective antibacterial activity against Gram- positive (*S. aureus*) and Gram- negative (*E. coli*) bacteria. The secondary metabolites produced by species of *Guignardia* with such antibiotical activity, on the other hand several phytotoxins have been described to be produced in vitro by *Phyllosticta* sp. its anamorph genus [19]. In this study *Curvalaria tuberculata* demonstrated a selective antibacterial activity against Gram positive bacteria (*Staphylococcus aureus, Streptococcus pneumoniae*) and Gram negative bacteria (*Salmonella typhi, E.coli*). The *Curvalaria tuberculata* crude extracts inhibit the bacteria, 38 µg/ml inhibited Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*), and 80µg/ml inhibited Gram negative bacteria (*Salmonella typhi* and *E.coli*). The *E.coli* showed higher antibacterial activity on ethyl acetate crude extracts of *Curvalaria tuberculata* against column purified compound (Figure 3 & 4). The column purified different fraction of *Curvalaria tuberculata* showed Antibacterial activity. The *Escherichia coli* showed maximum activity (5.6±0.80) in (75% Hexane: 25% Ethyl acetate) than *Salmonella typhi* (3.0±0.12), *Staphylococcus aureus* (2.8±0.14) and *Streptococcus pneumoniae* (3.8±0.36) (Table 1). The Preliminary screening of endophytic fungi from medicinal plants for antimicrobial against gram positive bacteria, gram negative bacteria, yeast and fungi and anti-tumour activity was also carried out by Radu *et al.*, [20].

Figure-3: Crude and column purified compound antibacterial activity against *E.coli.*



Crude extract; **A- Column purified fraction (EA 20%: H 80%).**

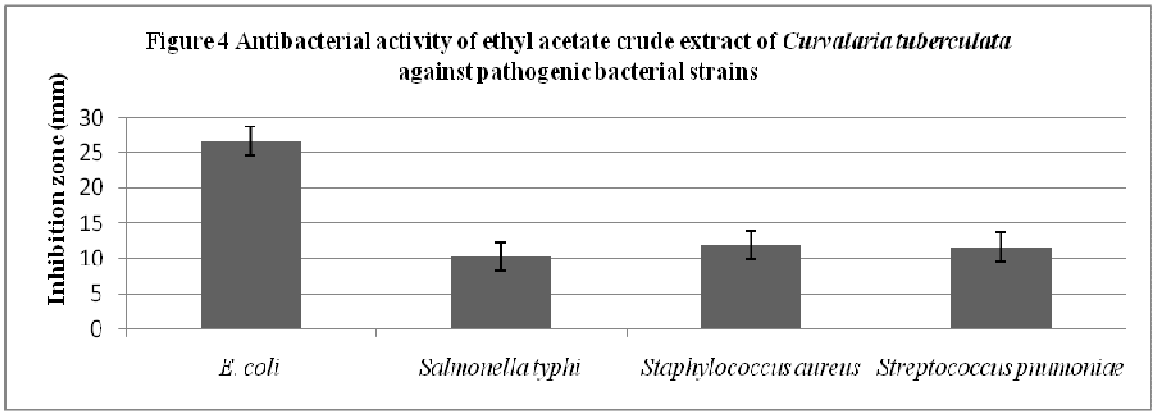


Table-1: Antibacterial activity of *Curvalaria tuberculata* column purified different fractions.

Si.No	Pathogenic bacterial strains	Zone of inhibition (mm) (mean ± SD) n = 5 Experiments				
		H 100%	H:EA 75%:25%	H:EA 50%:50%	H:EA 25%:75%	EA 100%
1	*Escherichia coli*	-	5.6±0.80	4.3±0.44	3.0±0.22	1.9±0.25
2	*Salmonella typhi*	-	3.0±0.12	1.8±0.16	3.4±0.64	1.3±0.19
3	*Staphylococcus aureus*	-	2.8±0.14	2.1±0.74	1.3±0.14	T
4	*Streptococcus pneumoniae*	-	3.8±0.36	1.5±0.12	T	3.4±0.14

EA- Ethyl Acetate; H- Hexane; T- Trace.

Conclusion

In the current study, the endophytic fungi isolated from the leaves of *J. adathoda* showed promising antibacterial activity against the gram positive and gram negative bacteria. Endophytic fungi are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural and industrial areas. Hence, the isolation of endophytic fungi from medicinal plants for any bioactive compound may facilitate the product discovery process.

Acknowledgement

The authors acknowledge with thanks the facilities provided by the Department of Botany, Madras University to carry out this work.

References

1. Petrini, O., Sieber, T.N., Toti, L. and Vivet, O., 1992, *Natural Toxins*, Vol1, pp 185-196.
2. Caroll, C.G., 1988. *Ecology*, Vol 69, pp 2-9.
3. Suryanarayanan, T.S. and Thennarasan, S., 2004, *Fungal diversity*, Vol 15, pp197-204.
4. Redman, R.S., Sheehan, K.B., Stout, R. G., Rodriguez, R.J. and Henson, J. M., 2002, *Science*, Vol 298, pp 1581-1582.
5. Jensen, G, Wandall, D.A. and Gaarsler, K., 1996, *Eur. J. Clin. Microbiol. Infect. Dis.* Vol 15, pp872-886.
6. Ong, H.C. and Noralina, J., 1999, *Fitoterapia* Vol 70 ,502-513.
7. Dobranic J.K., Johnson J.A. and Alikhan Q.R., 1995, *Can. J. Microbiol*.,Vol 41, 194-198.
8. Bills,G.F., Polishook, J.D.. 1992, Sydowia, 44: pp.1- 12.
9. Ellis, M.B., 1971, *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England.
10. Sutton, B. C., 1973, Vol4A, pp: 513-582.
11. Hata, K. and Futai, K., 1995, *Can.J.Bot*.,Vol 73, pp384-390.

12. Suthep, W,. Nongluksna, A., Nuntawan, T.,Kannawat, D., Nijsiri, R., Rithaya, M., 2004, *W.J.Microbiol and Biotechol*., Vol 20, pp 265-272.
13. Becerro M.A., N.I. Lopez, X. Turon and M.J. Uniz., 1994, *J. Exp. Mar. Biol. Ecol.*, Vol. 179, pp195-205.
14. Bauer, A.W., Kirby, W.M. and Sherris, J.C., 1999, *Am. J. Clin. Pathol*., Vol 45, pp 493-496.
15. Balandrin, M.F., Kinghorn, A.D. and Farnsworth, N.R., 1993, Plant-derived natural products in drug discovery and development. In: Kinghorn, A.D. and Balandrin, M.F., eds. Human Medicinal Agents from Plants. American Chemical Society, Washington, D.C.
16. Cabral,D., Stone, J.K., and Caroll, G.C. 1993, *Mycol.Res*. Vol 97, pp367-376.
17. Donnell. O, J. and Dickinson, C.H., 1980, *Trans.J.Mycol*., Vol 74, pp 335-342.
18. Suryanarayanan, T. S., and Kumaresan, V., 2000, *Mycol Res,* Vol 104, pp 1465-1467.
19. Sakamura, S. ITO, J. And Sakai, R., 1971, *Agr.Biol. Chem.*, Vol35, pp 105-110.
20. Radu, Son and Cheah, Yoke Kqueen, 2002, *Malaysian J.Med.Sci*. Vol 9, pp23-33.

Corresponding Author:

K. Sowparthani*,

Email: *sowpavenkat@gmail.com*