Research Article

Phytochemical and Antimicrobial evaluation of Endophytic *Alternaria alternata* isolated from *Terminalia arjuna* (Roxb.) Wight & Arn.

Jagadevi Shivaputrappa[1], Vidyasagar G. M.[*]

[1]*Research scholar, Medicinal plants and Microbiology research laboratory, Department of Post Graduate Studies and Research in Botany, Gulbarga University, Gulbarga- 585106, Karnataka, India.*

[*]*Professor, Medicinal plants and Microbiology research laboratory, Department of Post Graduate Studies and Research in Botany, Gulbarga University, Gulbarga- 585106, Karnataka, India.*

Received: 14 May 2018 Revised: 6 June 2018 Accepted: 18 June 2018

Abstract

Objective: The present study was carried out to investigate the chemical constituents by preliminary tests and the antimicrobial activity of various extracts of an endophytic fungi *Alternaria alternata* isolated from the stem bark of *Terminalia arjuna*. **Materials and methods:** The phytochemical analysis of all the extracts of fungi was carried out by referring the standard procedures and antimicrobial activity was carried out by agar well diffusion method. **Results:** The phytochemical investigation of all the extracts of fungi showed the presence of alkaloids, phenols, flavonoids, tannins, saponins, steroids, triterpenes and glycosides. The methanol, ethanol and ethyl acetate extracts of endophytic fungi were tested against four human pathogenic Bacteria like, *Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus* and *Escherichia coli* and four Candida species like, *C. albicans* [MTCC 1637], *C. glabrata* [MTCC 3019], *C. haemulonii* [MTCC 1966] and *C. tropicalis* [MTCC 230] by agar well diffusion method. The methanolic and alcoholic extract of *A. alternata* at 40mg/ml showed maximum activity against *S. aureus* (15.6mm) and *C. albicans* (10.6mm), respectively. **Conclusion:** *Alternaria alternata* possess several bioactive molecules which are responsible for antimicrobial potential, may serve as an alternative source for the treatment and control of several microbial infections.

Keywords: *Terminalia arjuna*, *Alternaria alternate,* Endophytic fungi, antibacterial, anticandida

Introduction

Medicinal plants are the one of the easily available, cost effective, and unique source of drug since ancient times and greatly influencing on the economic value of all over the world. Nature is the boon for all the organisms, fortunately blessed us with a very rich flora and favourable climatic conditions. The plant species growing in various parts of the country or the world, in various adverse climatic conditions are capable of producing several potential bioactive molecules. The literature available gives information about the role of the medicinal plants used in curing several health ailments of human beings. Plants used in ayurvedic medicinal system contain several potential bioactive molecules used in treating life threatening diseases of human beings (Alsheikh et al., 2009). Continuous use of a particular plant species results in reduction in number of plants population, ultimately when a particular species of plant population is reduced then its name is included in the red data book (Ahmedullah et al., 1999). A wide variety of medicinal plants used traditionally to cure several ailments. Pathogenic micro-organisms are responsible for causing many infectious diseases and to cure these, there are several drugs are available but the micro-organisms becoming resistant to presently available drugs (Eloff, 2000). For example Methicillin resistant *Staphylococci*, vancomycin resistant *Enterococci*, penicillin resistant *Pneumococci* are the prominent examples of the drug resistance (Finch et al., 2005). In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for natural, cost effective and least toxic antimicrobial substances. Several plants have been systematically investigated for novel compounds, but very

Address for Corresponding Author:

Dr. Vidyasagar G. M.

Professor, Medicinal plants and Microbiology research laboratory, Dept. of PG Studies and Research in Botany, Gulbarga University, Gulbarga - 585 106, Karnataka, India.

Email: gmvidyasagar@gmail.com

DOI: https://doi.org/10.31024/ajpp.2018.4.4.13

few plants have been systematically investigated for endophytic fungi. The endophytic fungi are those microbes that reside in healthy tissues of plants, at least for a part of their life cycle, without causing disease in their host (Petrini, 1991). Endophytes are the proved rich sources of natural compounds with variety of pharmacological and biological activities. Mutualism interaction between endophytes and host plants may result in fitness benefits for both partners (Kogel et al., 2006). Each and every plant on the earth is the host for one or more endophytes (Storbel et al., 2003). The works carried out so for regarding the role of endophytes in host plant shows that they can stimulate plant growth, increase disease resistance, improve plant's ability to withstand or overcome environmental stresses and recycle nutrients (Sturz et al., 2000). Recent reports had shown that fungal endophytes could also produce metabolites similar to or with more activity than their host plant (Storbel, 2002). Since the microbial sources of bioactive compounds are easier and more economical for large-scale production than plant sources (Storbel et al., 2003). Endophytic fungi are the gold mines of plant Kingdom, in future it is the only such source that can fulfill the needs of human health problems. India is the poor country but it has rich biodiversity that is not there in any other country in the world. So, it is the boon for scientists in their research to isolate several bioactive molecules, which helps in curing many dreadful diseases of human population. *Terminalia arjuna*, the versatile traditional medicinal plant belongs to the Combretaceae family, is the rich source of bioactive compounds with diverse chemical structure. The bark of *T.arjuna* has been recommended and used as cardiac tonic and bark powder/decoction is used to treat heart diseases, bone fractures, skin diseases, polyuria, hypercholesterolemia, white discharge, giddiness, fever and worms (Dwivedi et al., 1989; Maheshwari et al., 2011). Lot of work has been done on the photochemistry and pharmacological activity of this plant and very few reports are available on their fungal endophytes. As of now, little work has been done on the fungal endophytes and its biological activity, hence extensive investigation is needed to exploit the bioactive principles of endophytic fungi isolated from stem bark of *T.arjuna* for therapeutic utility.

Materials and methods

Collection of plant materials

Fresh and symptom less stem barks of the *T. arjuna* was collected from the Malkhed near sedam. The plant was authentically identified with the help of Flora of Gulbarga district. A specimen is deposited in the herbarium, Department of Botany, Gulbarga University, Kalaburagi, Karnataka, India. With voucher specimen number HGUG- 140 (Seetharam et al., 2000). The stem barks were cut with the help of a sterile scalpel and placed in sterile plastic bag, then brought to the laboratory and processed within 24 h of collection.

Isolation and identification of endophytic fungi

Sample was washed thoroughly in running tap water before processing. Stem bark was surface sterilized by sequential washes in 70% (v/v) ethanol (1 min) and 3.5% (v/v) NaOCl (2 min), rinsed with sterile water and allowed to surface dry under sterile conditions. The sterile plant material were cut in to small segments (0.5 X 0.5 cm) and placed on PDA medium which is previously poured in sterile petriplates, supplemented with streptomycin and ketochonazole (100 µg/ml).Three segments were placed on PDA medium in each Petri dish. The Petri dishes were sealed using Para film and incubated in a light chamber for 2 weeks at 12h light and dark cycles at 23^0C (Suryanarayanan, 1992). After incubation for 15 or more days, fungal colonies was observed, then individual fungal colonies were picked from the edge with a sterile fine tipped needle and transferred onto PDA and is maintained as pure culture. The fungi were identified based on fungal culture morphology and conidial characters and by using the available literature (Nagamani et al., 2006; Barnett et al., 1972).

Preparation of crude extracts

The isolate is initially cultured on PDA and incubated as described previously. Cultured fungal fragments were obtained from the actively growing margin of the fungal colony culture and inoculated in to 500 ml conical flasks containing 300 ml PD broth (pH 5.8). The inoculated broth was incubated at 21±2°C for a period of 8-10 days kept on shaker for mass cultivation. The culture broth was filtered through three layered muslin cloth to separate out the mycelial mat from the culture filtrate. The mycelial mat was washed with double distilled water to remove the broth content and ground in a pestle and mortar using ethanol, methanol and ethyl acetate separately. The grounded mycelia was then transferred into three different conical flasks containing ethanol, methanol and ethyl acetate, kept shaking for 3-4 days and filtered with cheese cloth. The filtrate was collected and evaporated to dryness. The extract residue was dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C to be used as stock solution for determining the phytochemical and antimicrobial activity.

Phytochemical screening

The preliminary phytochemical studies were performed for the evaluation of different chemical groups present in methanol, alcohol and ethyl acetate extracts of *A. alternata*.

1) Test for Alkaloids

Dragendorff's test: To 2 mg of the extract 5 ml of distilled water was added, 2ml Hydrochloric acid was added until an

acid reaction occurs. To this 1 ml of Dragendorff's reagent extract was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

Mayer's test: To 2 mg of the extract taken in a test tube, a few drops of Mayer's reagent was added. Formation of a yellow / white precipitate confirmed the presence of alkaloids.

Wagner's test: 2 mg of the extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids (Saldanha, 1984).

2) Test for Phenols

Ellagic Acid test: The test solution was treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) NaNO2 solution. The solution turned muddy or Niger brown precipitate occurred in the extract. It indicates the presence of phenol solution.

Ferric chloride test: 0.5 ml of FeCl3 (w/v) solution was added in 2 ml of test solution, formation of an intense color indicates the presence of phenols (Memelink et al., 2001).

3) Test for Flavonoids

Shinoda's test: In a test tube containing 0.5 ml of the extract 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

Ferric chloride test: Test solution with a few drops of ferric chloride solution shows intense green colour.

Zinc-Hydrochloric acid reduction test: Test solution with zinc dust and a few drops of hydrochloric acid shows magenta red colour.

Alkaline reagent test: Test solution when treated with sodium hydroxide solution, shows an increase in the intensity of yellow colour which becomes colourless on addition of a few drops of dilute acid.

Lead acetate solution test: Test solution with a few drops of lead acetate (10%) solution gives a yellow precipitate.

4) Test for Triterpenoids

Liebermann - Burchard's test (LB test): 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.

Salkowaski test: When a few drops of concentrated sulphuric acid were added to the test solution, shaken and allowed to stand, lower layer turns yellow indicating the presence of triterpenoids.

5) Test for Saponins

Foam test: In a test tube containing about 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

6) Test for Steroids

Liebermann-Burchard's test: 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.

Salkowaski reaction: 2 mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red colour indicated the presence of steroids.

7) Test for Tannins

Ferric chloride test: To 1-2 ml of the extract, few drops of 5% w/v FeCl3 solution were added. A green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudotannins.

Gelatin test: Test solution when treated with a gelatin solution gives white precipitate. colour This confirmed the presence of a naphthoquinon (Gibbs, 1974).

8) Test for glycosides

Baljet test: The test solution was treated with sodium picrate gives orange color.

Keller-Killiani test: The test solution was treated with a few drops of ferric chloride solution and mixed. When concentrated sulphuric acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green.

Raymond's test: Test solution when treated with dinitro-benzene in hot methanolic alkali, gives violet color.

Bromine water test: Test solution when treated with bromine water gives yellow precipitate.

Legal's test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red color.

Source of test microorganisms

Antimicrobial activity was carried out using the ethanol, methanol and ethyl acetate crude extracts of endophytic fungi against four bacteria and Candida strains using agar well diffusion method. Bacteria strains like *S. aureus, E. coli, S. typhi* and *K. pneumoniae* and four candida species like *Candida albicans, Candida glabrata, Candida haemulonii* and *Candida tropicalis* were used for antimicrobial activity. The above mentioned bacteria strains were obtained from the Medicinal plants and Microbiology laboratory, Department of Botany, Gulbarga University,

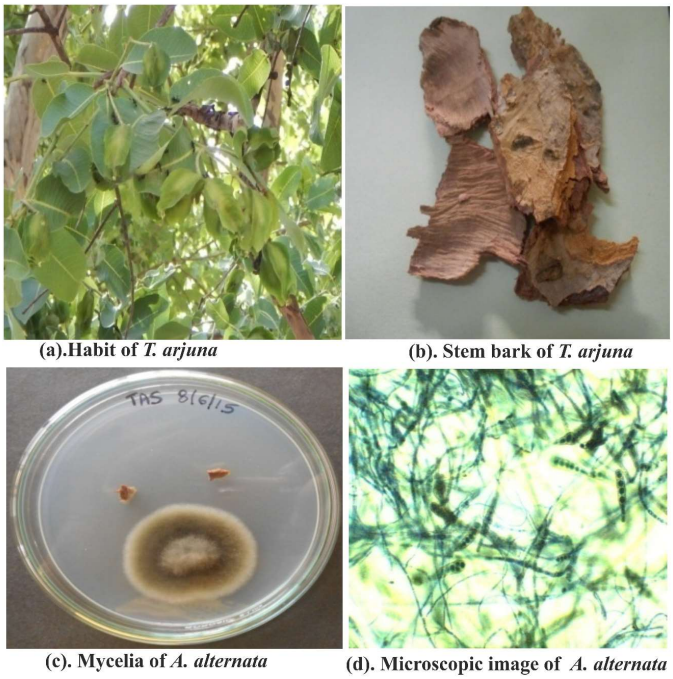
Kalaburagi, Karnataka, India. And candida species were taken from microbial type culture collection (MTCC) Chandigarh, India.

Preparation of sample

For the preparation of test sample, the crude extract of endophytic fungi was dissolved in Dimethyl sulphoxide (DMSO). The corresponding concentration was expressed in terms of mg of extract per ml of solvent (mg/ml).

Antimicrobial activity

The crude extract from the endophyte *A. alternata* were tested against four human pathogenic bacteria like *S. typhi, S. aureus, K. pneumoniae* and *E. coli* and four candida species like *C. albicans, C. glabrata, C. haemulonii* and *C. tropicalis* using a concentration of 100µl as inoculum for antimicrobial activity. The antimicrobial activity was carried out by agar well diffusion method. Nutrient agar and YPD plates were inoculated with an overnight culture of each bacteria and candida suspension**.** The inoculated organisms were evenly spread out using sterile cotton swabs. The wells were bored with 6mm cork borer and wells were poured with 40, 30, 20, and 10 mg/ml concentration of the sample. In other wells, supplements of DMSO (-ve) and reference antimicrobial drug Streptomycin (+ve) for bacteria and Ketoconazole (+ve) (100µg/ml) for fungi were used as negative and positive controls, respectively. The experiment was carried out in triplicate. The plates were incubated at 32ºC for 24h and results were recorded as zone of inhibition in mm.



(a).Habit of *T. arjuna* (b). Stem bark of *T. arjuna*

(c). Mycelia of *A. alternata* (d). Microscopic image of *A. alternata*

Figure 1. (a). Habit of *T. arjuna* (b). Stem bark of *T. arjuna* (c). Mycelia of *A. alternata* (d). Microscopic image of *A. alternata*

Results

The figure a & b represents habit and stem bark of *T. arjuna*. The figure c & d, showed mycelial morphology of endophytic fungi arising from plant segment and microscopic image of fungal conidia. Phytochemical screening of crude extracts of the endophyte *A. alternata* isolated from the stem bark of *T. arjuna* was done for the presence of various bioactive molecules. Alcohol extract contains alkaloids, flavonoids, steroids, phenol, saponins, terpinoids, tannins and glycosides and methanol extract contains all the biomolecules except tannins, whereas in ethyl acetate extract except saponins, steroids and tannins, all the components are present which is shown in table 1. The antibacterial and anticandida activity of various extracts of *A. alternata* and its comparison with reference drugs is showed in table 2. Among four bacteria and four candida strains tested, *S. aureus* and *C. albicans* showed maximum zone of inhibition to methanol and alcoholic extract of *A. alternata* respectively. The inhibition zones were nearly as comparable to reference drug (Streptomycin and Ketoconazole). The results shows that bioactive principles having antibacterial and anticandida activity are present in the culture filtrate of the endophytic fungi.

Table 1. Phytochemical Screening of different extracts of endophytic *A. alternata* isolated from stem bark of *T. arjuna*

Phytochemical Tests	Methanolic extract	Ethanolic extract	Ethyl acetate extract
Alkaloids			
Dragendorff's test	-	-	+
Wagner's test	-	-	+
Mayer's test	+	+	-
Flavonoids			
Shinoda test	-	-	+
Ferric chloride test	+	+	+
Zinc Hydrochloric acid	-	-	-
reduction test	+	+	-
Alkaline reagent test	+	+	+
Lead acetate test			
Phenols			
Feric chloride test	+	+	+
Ellagic acid test	-	-	+
Terpinoids			
Liebermann Burchard's test	+	+	+
Salkowaski test	+	+	+
Saponins			
Foam test	+	+	-
Steroids			
Liebermann - Burchard's test	+	+	-
Salkowaski test	-	-	-
Tannins			
Ferric chloride test	-	+	-
Gelatin test	-	-	-
Glycosides			
Baljet test	-	-	-
Keller-Killiani test	+	+	+
Raymond's test	-	-	-
Bromine water test	+	+	-
Legal's test	-	-	+

Table 2. Antimicrobial efficacy of different extracts of endophytic *A. alternata* isolated from stem bark of *T. arjuna*

Test Organisms	Zone of Inhibition (mm) in different solvent system												Standards (100µg/ml) Streptomycin/ Ketochonazole
	Ethyl acetate extract				Methanolic extract				Alcoholic extract				
	40	30	20	10	40	30	20	10	40	30	20	10	
S. aureus	4.3	3.5	2.5	-	15.6	14.2	13.3	12.3	6.5	4.6	3.3	2.3	18.3
K. pneumoniae	5.6	4.2	3.6	2.5	-	-	-	-	8.3	7.5	6.3	5.5	11.5
S. typhi	4.3	3.5	2.6	-	4.2	3.6	3.3	2.5	4.6	3.6	2.6	2.3	12.3
E. coli	-	-	-	-	5.3	4.6	3.6	2.6	-	-	-	-	11.5
C. glabrata	3.6	2.6	2.3	-	6.6	5.3	4.6	4.3	5.6	4.3	2.6	-	11.5
C. tropicalis	5.2	3.6	2.6	2.3	6.3	5.6	4.3	3.6	-	-	-	-	13.3
C. haemulonii	-	-	-	-	5.6	5.3	2.6	-	6.6	6.3	3.6	3.3	11.6
C. albicans	7.6	6.3	6.5	5.6	-	-	-	-	10.6	8.6	7.6	6.5	16.3

The methanolic and alcoholic extracts of *A. alternata* at 40 µg/ml showed maximum activity against *S. aureus* (15.6 mm) and *C. albicans* (1637) (10.6 mm) respectively

Discussion

Medicinal plants are the warehouse of several potential bioactive molecules and are well known for curing several human ailments. The available literature describes about the efficacy of *T. arjuna* leaf, fruit and stem bark extracts on many human pathogenic microorganisms. The n-butanolic extract of leaf shows maxium inhibition against *B. subtillis* (Riazunnisa et al., 2013) and the alcoholic extract of leaf-fruit showed maximum activity against *coliform spp* (Abdullah-Al-Emran et al., 2011). Chloroform, Methanolic and water extract of stem bark were used for screening antimicrobial assay, here chloroform extract is ineffective against all tested organisms and methanolic extract shows greater potency towards gram-negative bacteria, as compared to water extract (Sukalyani et al., 2003). Keeping in view of the medicinal properties of *T. arjuna,* the endophytic fungi was isolated and identified by morphological and microscopic study. The Phytochemical screening of different extracts of endophytic *A. alternata* isolated from stem bark of *T. arjuna* consists several bioactive molecules which are also present in plant. This proves that the endophytic fungi have the capacity to produce same compounds as that of its host plant. Many previous reports give information about endophytic fungi producing novel bioactive natural products from medicinal plants (Huang et al., 2010; Song et al., 2005). The endophytic fungi have been isolated from all the parts like leaf, fruit, root and stem or inner bark tissues (Kusari et al., 2009). The present study proves that the methanol and alcoholic extracts of this fungus could be a possible source of new therapeutic agent against various infectious diseases. Hence the endophytic fungi *A. alternata* isolated from stem bark of *T. arjuna* has ability to produce several bioactive molecules which will be useful in curing several human health problems.

Conclusion

It is interesting to note that crude extracts of *A. alternata* possess several bioactive molecules which are responsible for antimicrobial potential, which might be further explored to be used as an alternative source for the treatment and control of some microbial infections. Further investigations are needed to develop a standard anti-microbial drug.

References

Abdullah-Al-Emran, Farzana Ahmed, Md. Sanuwarul Kabir, Md. Mahabubur Rahaman, Shahed SM. 2011. Investigation of antimicrobial activity of ethanolic leaf fruit extract of *T. Arjuna* against multi-drug resistance bacteria in Bangladesh. Journal of Applied Environmental and Biological Sciences, 1(5):90-95.

Ahmedullah M, Nayar MP. 1999. Red data book of Indian plants (Peninsular India), Vol. 4. Botanical Survey of India, Calcutta.

Alsheikh M, Saleh AI, Mohammad Al-Dosari S, Abdul M, Maged Abdel-Kader S. 2009. Evaluation of the Hepatoprotective Effect of *Fumaria parviflora* and *Momordica balsamina* from Saudi Folk Medicine against Experimentally Induced Liver Injury in Rats. Research Journal of Medicinal Plants, 3(1):9-15.

Barnett HL, Hunter BB. 1972. Illustrated genera of imperfect fungi. 3rd Edition., Burgess Publishin. Co. p. 241.

Dwivedi S, Udupa N. 1989. *Terminalia arjuna*: Pharmacognosy, phytochemistry. pharmacology and clinical use: A review. Fitoterapia, 60:413-20.

Eloff JN. 2000. On expressing the antibacterial activity of plant extracts-a small first step in applying scientific

knowledge to rural primary health care. South African Journal of Science, 96:16-118.

Gibbs RD. 1974. Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen's University Press, Montreal and London.

Huang WY, Cai YZ, Hyde KD, Corke H, Sun M. 2010. Endophyticfungi from Nerium oleander L (Apocynaceae): main constituents and antioxidant. World Journal Of Microbiology And Biotechnology, 23(9):1253-1263.

Kogel KH, Franken P, H¨uckelhoven R. 2006. Endophyte or parasite-what decides? Current Opinion in Plant Biology, 9(4):358–363.

Kusari S, Zuhlke S, Spiteller M. 2009. Endophytic Fungus from *Camptotheca acuminata* that produces camptothecin and analogues. Journal of Natural Products, 72:2–7.

Lai HY, Yau YY, Kim KH. 2010. Blechnumorientale Linn-a fern with potential as antioxidant, anticancer and antibacterial agent BMC Complement. BMC Complementary and Alternative Medicine, 10:15.

Maheshwari VL, Patil RH, Prakash K. 2011. Hypolipidemic effect of *Terminalia arjuna* (L.) in experimentally induced hypercholesteremic rats. Acta Biologica Szegediensis, 55(2):289-293.

Memelink J, Verpoort R, Kigine JW. 2001. Organisation of jasmonate responsive gene expression in alkaloid metabolism. Trends in Plant Science, 6(5):212-9.

Nagamani A, Kunwar IK, Manoharachary C. 2006. Hand book of Soil fungi. IK International Pvt. Ltd.

Norrby RS, Nord CE, Finch R. 2005. Lack of development of new antimicrobial drugs: a potential serious threat to public health. Lancet Infectious Diseases, 5:115-119.

Petrini O. 1991. Fungal endophytes of tree leaves, in Microbial Ecology of Leaves. In Andrews JA, Hirano SS (Eds), Springer –Verlag, New York, p 179.

Riazunnisa K, Chandraobulu Y, SaiSudha G, Habeebkhadrian C. 2013. In vitro antibacterial activity and phytochemical studies of some medicinal plants. International Journal of Pharmaceutical Sciences Review and Research, 23(2):77-80.

Saldanha CJ. 1984. Flora of Karnataka, Oxford and IBH Publishing Co, Vol 1, New Delhi.

Seetharam YN, Kotresha K, Uplaonkar SB. 2000. Flora of Gulbarga District, First edition.

Song YC, Huang WY, Sun C, Wang FW, Tan RX. 2005. Characterization of graphislactone a as the antioxidant and free radical scavenging substance from the culture of CephalosporiumspIFB-E001, an endophytic fungus in *Trachelospermum jasminoides*. Biological and Pharmaceutical Bulletin, 28:506-509.

Storbel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews, 67:491-502.

Storbel GA. 2002. Rainforest Endophytes and Bioactive Products. Critical Reviews in Biotechnology, 22:315.

Sturz AV, Nowak J. 2000. Endophytic communities of Rhizobacteria and the strategies required creating yield-enhancing associations with crops. Applied Soil Ecology, 15:183.

Sukalyani D, Diganta D, Sudipta H, Subhalakshmi G, Ratnamala R, Banasri H. 2003. Antibacterial and antifungal activity of *Terminalia arjuna* Wight & Arn. bark against multi-drug resistant clinical isolates. Journal of Coastal Life Medicine, 1(4):315-321.

Suryanarayanan TS. 1992. Light-incubation: a neglected procedure in mycology. The Mycologist, 6:144.