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Antibacterial Activity of Fungal Endophytes Isolated from *Wattakaka volubilis* (Linn.f.), A medicinal plant from Telangana, India.

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***Abstract*—** Endophytic fungi are known to produce a wide range of metabolites of pharmacological importance. Endophytes associated with medicinal plants are targets for intensive research. This research paper deals with the endophytic fungi isolated from *Wattakaka volubili* also known as Dudipala, a high valued medicinal plant in traditional medicine. A total of 39 fungal species representing mostly imperfect fungi were isolated from different plant parts. The fungal species varied with geographic and environmental conditions of the same host species. Among different genera, *Aspergillus, Penicillium, Fusarium, Phoma* and mycelia sterilia were dominant. Solvent extracts of five endophytic fungi viz *Aspergillus oryzae, Diplodia andamanensis, Fusarium graminearum, Penicillium rubrum and Rhizoctonia bataticola* were tested against four human pathogenic bacteria, Antibacterial activity varied with the fungus as well as solvent. The inhibitory activity is very prominent on *E.coli*. Hexanol and chloroform extracts activity is very close to streptomycin. In light of these observations it is recommended that the pure extracts need to further test for antibacterial activity.

Keywords*—Wattakaka volubilis, endophytic fungi; antibacterial activity.*

I. INTRODUCTION

Endophytic fungi have been found in all woody plants that have been examined for endophytes [1, 2, and 3]. Studies of fungal endophytes in trees, shrubs and ferns reveal that individual species and even individual plants typically harbor scores of fungal species [4, 5]. Despite their great diversity and abundance, their interactions with their host plants have received less attention. In addition, there has been little attempt to integrate ecological and evolutionary aspects of the endophytic fungi [6]. Endophytic fungi are known to produce a wide range of metabolites that find applications in human health [7]. A recent comprehensive study has indicated that 51% of biologically active substances isolated from endophytic fungi were previously unknown. The novel bioactive compounds include anticancer, anti microbial, antioxidants, immunomodulators, anti-inflammatory, etc. As a result, a number of investigators devoted their attention to investigate the endophytes from different plant species.

Wattakaka volubilis (= *Dregea volubilis; Marsdenia volubilis*) (family Asclepiadaceae) commonly Known as Jutiki' is a tall, woody climber occurring throughout the hotter parts of India. Only one species is reported from India. It is a well recognized medicinal plant in folklore and traditional medicines like Ayurveda, Siddha and Unani [8, 9]. Traditionally, leaves are used as an application to boils and abscesses. Different plant parts are used in cold, eye diseases and snake bites [10]. The roots and tender stalks are considered emetic and expectorant [11]. Many phyto constituents like steroids, steroidal glycosides, sugars, triterpenoids, flavonoids, phenolic compounds and some alkaloids are found in the plant [12] .Medicinal plants are also reported to host endophyte fungi that are known to be involved in the co-production of active metabolites [13].

These fungi may also contribute to the biological activities exhibited by the plants.

Though there are reports on endophytic fungi associated with ethnomedicinal plants [14, 15] antibacterial activity has been a major challenge to health care system globally. Studies have shown that microbes have developed resistance to antibiotics through various molecular mechanisms such as prevention of access to drug targets and modification of the drug [16, 17]. Thus this global problem has led to the increase in researches featuring endophytic fungi, particularly those isolated from medicinal plants for their potential as source of new antibiotics. Reports on the bioactivity of endophytic fungi isolated from *Wattakaka volubilis* are scarce. The present study is aimed to assess the efficiency of endophytic fungi associated with this plant for antimicrobial activity.

II. MATERIAL AND METHODS

Collection of plant samples:

Tissue samples of root (40), stems (80), leaves (70) and bark (50) were collected from healthy *Wattakaka volubilis* plant from different geographic locations of Telangana state, India (Warangal 17°58N, 79°35E 263m; Karimnagar18°26N 79°07E 277m and Khammam 17°14N, 80°08E 139m elevation) in different seasons (summer, winter and rainy) during 2017. Healthy and old mature plant parts were carefully chosen for sampling. Samples were placed in Ziploc bags, maintained at 4º C in on thermocol ice box during transport and processed within 24 hours.

Isolation of fungal endophytes:

The plant parts were washed thoroughly in tap water followed by sterilized water for few minutes to remove dirt and debris [18]. A total 1,920 number of segments were selected for different parts of plant sampling. In root maturation zone of elongation were cut in to vertically in 4 to 8 cm segments, The mature internodal parts of stem were collected and cut in to 0.4 -0.6 cm. Healthy and mature leaves lateral parts and midrib were cut into approximately 1cm segments.[19]. The mature and healthy bark samples were cut in to the 1.5-2.0 above ground level [20].

The samples from leaves were washed previously with neutral detergent and rinsed with sterile water .They were then immersed in 70% (v/v) alcohol for 1 min, 2% sodium hypochlorite for 3 min and again in sterile water for 2 min. Surface sterilization of stem, root, and bark parts were carried out by Fisher [21,22] method. The segments were immersed first in 75 % ethanol for 60 seconds, followed by 4% sodium hypochlorite for 180 seconds, again in 75 % ethanol for 30 seconds and finally rinsed in sterile distilled water for 10 seconds. The samples were kept on sterile filter paper to remove excess moisture. The externally sterilized segments were placed on agar plates (potato dextrose agar medium) supplemented with 120 mg of streptomycin per liter, and then Petri dishes were sealed using parafilm.

Identification:

Each Petri dish containing 4 segments was incubated at 27± 2 ºC at 12-h light/dark cycle [23]. After 7-12days, the petri-dishes were monitored every day to check the growth of endophytic fungal colonies from the plant sample. The sample fragments were isolated and transferred to fresh tubes of PDA slants for preservation of fungal isolates and were stored at 4℃ for further studies. Colony morphology of each fungus was recorded. Slides of fungal colonies were made with lactophenol cotton blue and observed under microscope. Mycelia, spore characteristics were recorded. Photomicrographs were taken under fluorescent microscope and fungi were identified with the help of standard manuals [24, 25].

Fungal culture and extraction:

Fungal crude extract was prepared using the protocol of Radji *et al*., 2011 [26]. Prospective endophytic fungi isolates were inoculated into 1000ml Erlenmeyer flasks containing 450 ml of potato dextrose broth. (450 g of potato extract, 20 g of dextrose, and 20 g of sucrose in 1000 ml of distilled water) and incubated at 27±℃ for 21 days under stationary conditions with intermittent shaking. The fungal broth culture was filtered through whatman no.1 filter paper to give clear filtrate and mycelia and exposed to the extraction process. Liquid supernatant was extracted with an equal volume of organic solvents (Chloroform, Hexane, and Methanol) and the extract was then evaporated under reduced pressure using rotary evaporator. The crude extracts were used for preliminary evaluation of antibacterial activity using disc diffusion method.

Antimicrobial activity:

The antimicrobial activity of Crude extract of fungal endophytes was carried out by disc diffusion method. The

phyto pathogenic bacteria were selected, two gram positive bacteria *Staphylococcus aureus* (ATCC- 6538) and *Micrococcus luteus* ATCC-4698) and two gram negative bacteria *Escherichia coli* (ATCC-8739) and *Klebsiella pneumonia* (MTCC- 3384) were obtained from the Department of Microbiology (KU) Warangal, maintained on nutrient agar medium at 37℃ and stored at 4℃ .

All bacterial suspension was standardized to contain approximately 1.5×10^8 CFU /ML. The bacteria were inoculated on dried surface nutrient agar medium.

The dried crude extract from different solvents (Hexane, Methanol and Chloroform) of 5 different fungal species was dissolved in 0.1% acetone to a concentration of 100mg/ml. Then 30 ml of the dissolved extract was pipeted to 5mm diameter (Sterile whatman no 1 filter paper) circular discs and allowed to dry in incubator at 35℃. In each plate three paper disks with the extract were placed and negative (sterilized water) and positive (Streptomycin) control was separately maintained. All plates were incubated at 37℃ in incubator for 24 hours. After incubation the diameter of the zone of inhibition (ZOI) was measured using a measuring scale.

III. RESULTS

Geographic distribution and variation in climatic conditions affect both host and the endophytes. Environmentally, the endophytes may be metabolically aggressive by affecting host defense chemicals. Synthetic ability of endophytes may account for hostile environment faced by the host. Thos perhaps explains the apparent anamoly observed when a species of endophyte isolated from a host plant produces a bioactive compound but fails to do so when isolated from another plant species [27]. It may also be true for the endophytes of species growing in different geographical locations and experiencing different climatic conditions [28]. Hence it is not only host plant as well as endophytes important but also ecosystem in which they are growing since in different ecosystems. The mode of interaction between the host and endophyte may shift drastically; keeping these aspects in mind three different geographic locations were selected. Similarly samples were collected in different seasons (Table – I). A critical observation of the table reveals that there is a variation in the geographic co-ordinates. The difference between the altitudes of sampling locations varied between 98 and 441, that implies great variation. Average annual and seasonal rainfall also varied significantly. The rainfall shows profound effect on the survival, growth and reproduction of plant species indirectly effecting the endophytic diversity and distribution. The temperatures of three selected zones varied seasonally and also according to location. The difference in different seasons was ± 3 ℃. The variation of seasonal temperature is about 12 ℃.

In the present investigations, an attempt has been made to isolate the endophytic fungi from different organs of the test plant i.e. *Wattakaka volubilis.* For this purpose random samples of plant parts were selected and the isolations of endophytic fungi were made. Colonization frequency, dominant frequency and endophytic infection rates (Table-2) were estimated from the number of fungi isolated from four plant parts; root, stem, leaf and bark. A total of 320 root segments were examined for endophytic fungi, out of which 107 segments were found to be positive for one or other funge. Colonization frequency varied with the fungus. The highest colonization frequency was observed with *Aspergillus* species. Similarly, *Alternaria, Aspergillus* and *Penicillium* species were dominant out of many fungi isolated. Endophytic infection rates were noticed for only few fungi. In case of the stem out of 640 segments isolated 143 were found to be positive for endophytic fungi. The endophytic fungi isolated from stem were not exactly similar to root. Same in the case with colonization frequency, dominant frequency and endophytic infections rates. The percentage of positive segments in leaf was almost equal to stem. Colonization frequency, dominant frequency and endophytic infection rates of leaf varied with the fungi and they were comparatively less than the corresponding parameter values of stem. In bark, the positive segment for endophytic colonization was 22.5. Bark appears to be colonized by fewer fungi. Likewise, CF, DF and EIR values were also found to be less than root, stem and leaf. Colonization by fewer fungi and less frequencies can be attributed to dry conditions of the bark than with other parts of the plant.

Qualitative analysis of the endophytic fungi associated with different parts of the plant reveals that roots are colonized by maximum number of fungal species followed by stem, bark and leaf. However the fungal species varied with the plant parts. For example *Alternaria* species were recovered from the root and stem but not from leaf and bark. Among different genera, *Aspergillus, Penicillium, Fusarium, Phoma* and Mycelia sterelia were dominant. Interestingly mycelia sterelia could not be isolated from the leaf segments.

Antibacterial Assay:

Many endophytic fungi have been reported to produce novel antibacterial, antifungal, antiviral, anti - inflammatory, antitumor and other compounds. The production specificity of these compounds can partly be attributed to host from an ecological perspective. In the present investigation five endophytic fungi viz. *Aspergillus oryzae*, *Diplodia andamanensis*, *Fusarium graminarium*, *Penicillium rubrum* and *Rhizoctonia bataticola*, the most frequently occurring fungi from different plant parts were selected and extractions made in three different solvent, i. e hexane, chloroform and methanol were tested for antibacterial activity against two Gram +ve (*M.luteus* and *S.aureus*) and two Gram -ve (*E.coli* and *K.pneumoniae*) bacteria. The results are presented in (Table - 3). A critical perusal of the table reveals that all the extracts have shown some anti bacterial activity against all the four test bacteria. However, the antibacterial activities varied with endophtye, the solvent used for extraction and also test bacterium. Chloroform extracts of *A. oryzae* have shown activity on *M.luteus* but not on *S.aureus*. All the extracts from three solvents inhibited the growth of *E.coli* and interestingly but not on *K.pneumoniae*. All three extracts of *Diplodia andamanensis* have inhibited the growth of three test bacteria *M.luteus*, *S. aureus* and *E. coli* but not on *K.pneumoniae*. In contrary, hexane and methanol extract of *F. graminarium* have shown inhibitory activity against *S. aureus* and *E. coli* respectively. All three types of extracts of *P. rubrum* have shown antibacterial activity on three bacteria but not on *S.aureus*. Further, the inhibitory activity is very prominent on *E. coli* and hexane and chloroform activity is very close to Streptomycin. Solvents extracts of *R.bataticola* have shown inhibitory activity against *S. aureus* but not against all the three remaining bacteria. Even that activity was also very poor. In all the cases wherever inhibitory activity was observed it was far less than the control streptomycin (10ug/ml).

A comparison of efficacies of three solvents reveals that there is not much difference as far as the antibacterial properties are concerned. However few variations could be observed with methanol extracts of four endophytic fungi have shown inhibitory activity. In contrary the same solvent extract of *P. rubrum* has shown antibacterial activity against *K.pneumoniae.*

IV. DISCUSSION

Plants serve as a reservoir of large numbers of microorganisms (fungi and bacteria) known as endophytes [29]. Endophytic fungi are an ecological, polyphyletic group of highly diverse fungi, mostly belonging to ascomycetes and anamorphic fungi [30, 31]. It has been estimated that there may be as many as one million different endophytic fungal taxa, thus endophytes may be hyperdiverse [32]. The host endophyte relationship cab be described in terms of host specificity or host preference [33, 34]. Host specificity implies that complex biochemical interactions occur between host and its associated hosts [35, 36]. Endophytes are able to colonize multiple host species of the same plant family within the same habitat. Similarly, the endophytes of the same species growing in different habitat and environmental conditions may vary. In this context Tan and Zou and Schulz [37, 38] felt that more attention should be given to studying endophytic biodiversity, the chemistry and bio activity of endophytic metabolites, and the relation between endophytes and their host plants, against this information. In the present investigations endophytic fungi of selected plants growing in different geographical locations and also different seasons were studied. Similarly, the endophytic fungi from different plant parts were investigated.

Antimicrobial metabolites (Antibiotics) are low molecular weight organic compounds produced by microorganisms. Many endophytic fungi were demonstrated to produce many known and novel antibiotics [39]. They are produced by the endophytes as on adaptation for specific functions in their ecological niche [40]. It is believed that screening for antimicrobial compounds from endophytes is a promising way to overcome the increasing threat of drug resistant microbes of human and plant pathogens [41].In this context endophytic fungi associated with proved medicinal plants are of great attraction [42] reported 30% of tested isolates exhibited and antifungal activity. Several studies have confirmed that crude extracts of endophytic fungi exhibited anti bacterial activity [43] published a remarkable review of anti microbial metabolites isolated from endophytes. They further concluded that there is a great opportunity to utilize endophytes as a new source of production of reliable and novel antimicrobial agents. In the present investigations, therefore, an opportunity was taken to screen some selected endophytic fungi against two Gram +ve and two Gram –ve bacteria.

V. CONCLUSION

The present investigations have demonstrated that the diverse endophytic fungi of *W. volubilis* varied with the host species, as well as geographic and environmental conditions

of the same host species. It conveys the message that to get the full spectrum of endophytic fungi. It is essential to select the different host species as well as the same species growing in different locations. The present medicinal plant harboured a rich endophytic mycoflora in its different organs. Antimicrobial activity of endophytic fungi revealed that the extracts are capable of inhibiting bacteria, however with different capacities. The crude extracts were showing antibacterial activity, if the crude extracts purified they may show a high antibacterial activity. The work in this direction is in progress.

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Table.1 Sampling locations and their geographic and climatic features of *Wattakaka volubilis* (L.f.) Stapf

	Sampling locations	Sampling Season	Geographic coordinates		Altitude (m)	Annual rainfall (mm) (2017)	Mean temperature (℃)
			North	*East*			
Zone-I	Warangal	Summer may-June Rainy July- October Winter November February	17°5824	79°3538	263	83.0mm	34.5℃
	Geesugonda		17°5758	79°4147	264	244.8mm	25.3℃
	Hasanparthi		18°0403	79°3132	262	Nil	24.4℃
	Narsampet		17°5544	79°5345	247		
Zone-II	Karimnagar	Summer may-June Rainy July- October Winter November February	18°26'34"	79°07'42"	277	115.8mm	33.3℃
	Husnabad		18°07'50"	79°12'30"	365	63.5mm	27.5℃
	Mulkanoor		18°05'14"	79°22'11"	313	Nil	27.3℃
	Siddipet		18°09'08"	78°56'36"	441		
Zone-III	Khammam	Summer may-June Rainy July- October Winter November February	17°14'51"	80°08'58"	139	36.2	34.0℃
	Kothagudum		17°32'30"	80°37'27"	115	28.6	24.0℃
	Palvancha		17°35'54"	80°41'50"	99	Nil	37.7℃
	Tallada		17°12'55"	80°25'17"	98		

Table. 2. Frequency of endophytic fungi isolated from Different parts of *Wattakaka volubilis* (L. f.) Stapf

Sl. No	Fungal endophytes	Number of isolates	Root			Number of isolates	Stem			Number of isolates	Leaf			Number of isolates	Bark		
			CF %	*DF %*	*EIR %*		*CF %*	*DF %*	*EIR %*		*CF %*	*DF %*	*EIR %*		*CF %*	*DF %*	*EIR %*
1.	*Alternaria alternate*	5	1.5	4.6	-	5	0.7	3.4	-	-	-	-	-	8	2	8.8	0.7
2.	*A. fasciculate*	9	2.8	8.4	0.9	-	-	-	-	-	-	-	-	-	-	-	-
3.	*A. solani*	3	0.9	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	*Aspergillus candidus*	7	2.1	6.5	-	-	-	-	-	15	2.6	0.1	0.8	4	1.2	5.5	-
5.	*A.flavus*	5	1.5	4.6	3.1	10	1.5	6.9	0.3	-	-	-	-	10	2.5	11.1	0.5
6.	*A.nidulans*	-	-	-	-	5	0.7	3.4	-	7	1.2	0.5	0.3	-	-	-	-
7.	*A.niger*	-	-	-	1.5	3	0.4	2.0	0.1	-	-	-	-	5	1.2	5.5	-
8.	*A.ochraceus*	-	-	-	-	2	0.3	1.3	-	-	-	-	-	-	-	-	-
9.	*A.oryzae*	12	3.7	11.2	-	3	2.0	9.0	0.7	2	0.3	1.6	-	2	0.5	2.2	-
10.	*A.versicolor*	1	0.3	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-
11.	*Chaetomium gracile*	4	1.2	3.7	2.5	-	-	-	-	3	0.5	2.4	0.3	-			
12.	*Colletotrichum dematium*	3	0.9	2.8	-	-	-	-	-	-	-	-	-	-	-	-	-
13.	*C. falcatum*	-	-	-	-	4	0.6	2.7	-	8	1.4	6.4	0.7	-	-	-	-
14.	*Cylindrocladium scoparium*	-	-	-	-	9	1.4	6.2	0.4	-	-	-	-	-	-	-	-
15.	*Drechslera spicifera*	6	1.8	5.6	0.9	14	2.1	9.7	0.6	5	0.8	0.4	-	3	0.7	3.3	-
16.	*Diplodia andamanensis*	-	-	-	-	-	-	-	-	-	-	-	-	11	2.7	12.2	0.5
17.	*Epicoccum nigrum*	-	-	-	-	5	0.7	3.4	-	11	1.9	8.8	1.4	-			
18.	*Emericella nidulans*	-	-	-	-	3	0.4	2.0	-	9	1.6	7.2	0.8	-			
19.	*Fusarium graminearum*	2	0.6	1.8	-	-	-	-	-	1	0.1	0.8	-	6	1.5	6.6	-

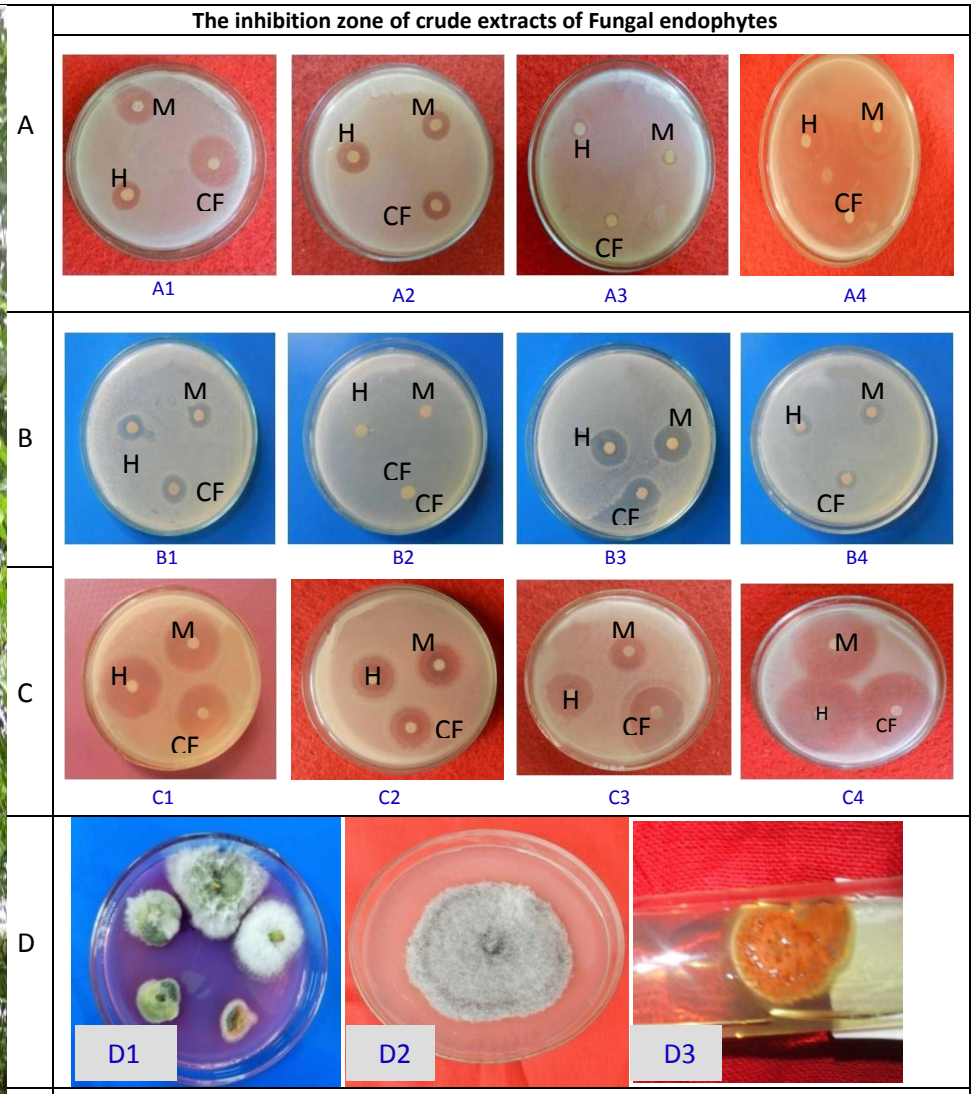
20.	*F.oxysporum*	1	0.3	0.9	-	-	-	-	-	-				-	-	-	-
21.	*F. merismoides*	-	-	-	-	5	0.7	3.4	0.4	5	0.8	0.4	-	2	0.5	2.2	-
22.	*F. solani*	-	-	-	-	6	0.9	4.1	0.9	-				-			
23.	*Geotrichum albidum*	5	1.5	4.6	-	-	-	-	-	10	1.7	8	0.8	-			
24.	*Gliocladiopsis sagariensis*	6	1.8	5.6	0.6	-	-	-	-	3	0.5	2.4	-	-			
25.	*Helminthosporium sativum*	3	0.9	2.8	-	6	0.9	4.1	-	-	-	-	-	-			
26.	*Neurospora crassa.*	2	0.6	1.8	-	-	-	-	-	-				4	1	4.4	0.2
27.	*Nigrospora sphaerica*	-	-	-	-	5	0.7	3.4	0.3	-				-	-	-	-
28.	*Penicillium chrysogenum*	2	0.6	1.8	-	11	1.7	7.6	0.9	3	0.5	2.4	-	2	0.5	2.2	-
29.	*P. citrinum*	7	2.1	6.5	1.2	9	1.4	6.2	-	-	-	-	-				
30.	*P. glabrum*	4	1.2	3.7	-	-	-	-	-	-	-	-	-	9	2.2	10	1.5
31.	*P. rubrum*	-	-	-	--	1	0.1	0.6	-	18	3.2	14.4	1.6	1	0.2	1.1	-
32.	*Phoma crysanthemicol*	1	0.3	0.9	-	-	-	-	-	-	-	-	-	6	1.5	6.6	1.2
33.	*Phoma.destructiva*	4	1.2	3.7	-	-	-	-	-	15	2.6	12	2.5	-	-	-	-
34.	*Phoma glomerata*	-	-	-	-					6	1.0	4.8	-	8	2	8.8	0.7
35.	*Rhizoctonia bataticola*	2	0.6	1.8	-	20	3.1	13.9	0.7	4	0.7	3.2	-	2	0.5	2.2	-
36.	*R.soloni*	8	2.5	7.4	1.8	5	0.7	3.4	-	-	-	-	-	-			
37.	*Sterile mycelium 1*	-	-	-	-	8	1.2	5.5	1.2	-	-	-	-	5	1.2	5.5	0.5
38.	*Sterile mycelium 2*	2	0.6	1.8	-	-	-	-	-	-	-	-	-	2	0.5	2.2	-
39.	*Sterile mycelium 3*	3	0.9	2.8	0.3	4	0.6	2.7	-	-	-	-	-	-	-	-	--
40.	Total %	33.4 %				22.3 %				22.3 %				22.5%			

Table. 3. Antibacterial activity of the solvent extracts of five endophytic fungi isolated from *Wattakaka volubilis* (L. f.) Stapf

Sl.No.	Fungal endophytes	*Micrococcus luteus*			*Staphylococcus aureus*			*Escherichia coli*			*Klebsiella pneumonia*		
		H	**Cf**	**M**	**H**	**Cf**	**M**	**H**	**Cf**	**M**	**H**	**Cf**	**M**
1	*Aspergillus oryzae*	-	3.8 ± 0.05	-	-	-	-	3.6±0.10	5.8±0.07	3.6±0.07	-	-	-
2	*Diplodia andamanensis*	5.7 ± 0.12	13.8±0.47	11.8 ± 0.06	10.9±0.31	9.4±0.29	9.4±0.26	3.8±0.08	2.8±0.05	3.7±0.18	-	-	
3	*Fusarium graminearum*	-	-	-	4.7±0.17	-	-	-	-	2.9±0.03	-	-	-
4	*Penicillium rubrum*	5.5±0.11	4.7±0.13	5.8±0.13	-	-	-	11.9±0.03	9.9±0.04	11.8±0.13	4.9±0.07	3.7±0.17	3.7±0.6
5	*Rhizoctonia bataticola*	-	-	-	3.2±13.0	3.7±0.06	3.8±0.03	-	-	-	-	-	-
6	Streptomycin 10ug /ml	18.5±0.02	19.8±0.03	19.7±0.13	13.7±0.15	13.8±0.01	13.6±0.15	13.6±0.20	11.7±0.26	21.8±0.06	24.9±0.07	23.7±0.12	24.6±0.76
7	Water10ug /ml	-	-	-	-	-	-	-	-	-	-	-	-

Activity was expressed as inhibitory zones (mm).The values are average of triplicates with standard errors.
*H-Hexane; CF-Chloroform; M-Methanol

A. *Wattakaka volubilis*



1 = Crude extract of **A**. *Diplodia andamanensis*, **B**. *Penicillium rubrum* **C.** Streptomycin (Control) and D. (D1) : Colony morphology of Endophytic fungi (D2) : *Diplodia andamonsis* (D3): *Penicillium rubrum*

2. Inhibition zone found in tested bacterial pathogens *Bacillus subtilis (A1,B1,C1), Staphylococcus aureus* (A2,B2,C2), *Escherichia coli (* A3,B3,C3) and *Klebsiella pneumonia* (A4,B4,C4)

Figure 1. Antibacterial activity of Endophytic fungi from ***Wattakaka volubilis* (L.f.) Stapf**

