"ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC FUNGI ISOLATED FROM THE LEAVES OF *Cassia alata*"

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Abstract: Endophytic fungi are the coloniser of healthy plant tissues that get nutrition and shelter from host and in response produce many functional metabolites. These metabolites have anti-feedant activity and provide resistance against various biotic and a biotic stresses, and which may enhance the host fitness. Many reports are there that endophytes in medicinal plants have antimicrobial, antioxidant as well as anti-cancerous property. In the present study two major endophytic fungal organisms were isolated from the leaves of *Cassia alata*, based on the morphological characters they were identified as *Aspergillus* species and *Penicillium* species. The significance of the study is exploiting the endophytic fungi in the area of microbial diversity realising the capability of microorganism to produce diverse bioactive molecules and the existence of unexplored microbial diversity, research is under way to isolate and screen microbes of diverse habitat and unique environment for discovery of novel metabolites. The isolates were then screened for their antibacterial activities. On the basis of antimicrobial activities of mycelia mass, free extract broth solution of fungal endophytes, it was evident that they shows significant antibacterial activity against almost all bacterial pathogens, *Penicillium* species, is more effective than *Aspergillus* species in terms of zone of inhibition produced against pathogens.

Index Terms- Endophytic fungi, secondary metabolites, FTIR, antimicrobial activity.

I. INTRODUCTION

Endophytes are microorganisms that live within plants for atleast a part of their life cycle without causing any visible manifestation of disease (Bacon and white, 2000). The term endophyte (Gr. Endon – within; phyton - plant) was first coined by De Bary in 1866. Common endophytes include a variety of bacteria, fungi and actinomycetes and they can be isolated from wild or cultivated crops of either the monocots or dicots. Among the microbial group the most frequently isolated endophytes are fungi (Maroof Ahmed *et al.*, 2012). The drug resistance, toxicity is problems caused by synthetic drugs can overcome by the secondary metabolite by using plant containing endophytic fungi (Kajal *et al*., 2016). Endophytes may affect plant litter quality organisms that control litter decomposition and the availability of nutrients in plant communities (Saikkonen *et al*., 2015)

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 (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 (Guo *et al.*, 2008).

Endophytic fungi spend the whole or parts of its life cycle colonizing inter or intracellularly inside the healthy tissues of the host plant. Some species of endophytic fungi have been identified as sources of anti-cancer, anti-diabetic, insecticidal, immunosuppressive and biocontrol compounds. A large number of secondary metabolites have been isolated and characterized from endophytic fungi (Dadgale, 2012). Endophytes have been shown to prevent disease development through endophyte-mediated *de novo* synthesis of novel compounds and antifungal metabolites. Investigation of the biodiversity of endophytic strains for novel metabolites may identify new drugs for effective treatment of diseases in humans, plants and animals (Strobel *et al.*, 2004).

Bioactive metabolites find wide-ranging application as agrochemicals, antibiotics, immune suppressants, antiparasitics, antioxidants and anticancer agents (Gunatilaka, 2006). (Jalgaonwala *et al.*, 2010) assayed 142 endophytic fungal isolates from various parts of medicinal plants belonging to Jalgoan, Maharashtra for evaluation of antimicrobial activity against various pathogenic and opportunistic microbes, 78 fungal isolates exhibited antimicrobial activity. Reis (2006) and Alves *et al.*, (2008) assayed technique for determination of minimum inhibitory concentration (MIC) and it is often considered as the best methodology for assessing antibiotics susceptibility or resistance of bacteria to antibiotics.

Generally, endophytic bacteria and fungi can promote plant growth and yield and can act as biocontrol agents. Endophytes can also be beneficial to their host by producing a range of natural products that could be harnessed for potential use in medicine, agriculture or industry (Schulz *et al.,* 2002; Schulz *et al.,* 1999; Schulz and Boyle, 2005; Ryan *et al.,* 2008). In addition, it has shown that they have the potential to remove soil contaminates by enhancing phytoremediation and may play a role in soil fertility through phosphate solubilisation and nitrogen fixation (Ryan *et al.,* 2008). Biocontrol refer to the eco-friendly way of reducing plant pathogens that may cause damage to agriculture crops through natural antagonists (Rybakova, 2015). The researchers are currently paying more attention to the drug development from the endophytic fungi isolated from medicinal plants (Tan and Zou, 2001)

II. MATERIALS AND METHODS

2.1 Collection of Plant sample

Healthy leaves are collected from different branches of *Cassia alata* from Ottapalam, palakkad district, Kerala. Under sterile condition leaves are collected and selected for the isolation of endophytic fungi to reduce the chance of contamination. Concentration of the surface sterilization agent and time period required for surface sterilization was optimized by trial and error method. Leaves wear surface sterilized by distilled water followed by that leaves were cut into small pieces using sterile scissors and forceps then wash with 10% NaOCl for 1 minute.

2.2 Isolation of pure culture

The surface sterilized leaves are placed in PDA (Potato dextrose agar) plates supplemented with chloramphenicol (100 g mL^{-1}). Plates were incubated at 28˚C until growth initiation and pure culture were maintained on PDA for further studies. The endophytic fungi were morphologically characterized according to their microscopic structure (using lacto phenol cotton blue staining) and identified by their colony morphology.

2.3 Antimicrobial activity

The fungal endophytes isolated from the *Cassia alata* plant were cultured on Potato dextrose broth for liquid fermentation. The mycelia agar pluge were inoculated into a 250 ml Erylen Meyer flask containing 100 ml of potato dextrose broth at 28˚C for 14 days. After incubation, the fermentation broth was filtered to remove the mycelia mass using Whatmann No.1 filter paper which was then screened for antimicrobial activity against selected pathogens such as *Staphylococcus* sp, *Pseudomonas* sp, *Klebsiella* sp, *Escherichia coli, and Salmonella* sp. All the bacterial samples were obtained from Lakshmi Hospital Palakkad. The bacterial cultures were spread on MHA (Mueller-Hinton agar) plates then wells were created and filtered broth containing fungal metabolites were inoculated on the wells and incubate at 37˚C for 24 hours for zone production.

2.4 FTIR analysis

The Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out in the mycelia free filtrate of fungal cultures to know the different functional groups present in the fungal extracts. The absorbance was measured at 400-600nm for the identification and quantification of organic species were identified according to standard infrared chart. The experiment data were processed statistically by analysis of variance in Randomized Block Design.

III. RESULT

3.1 Pure culture isolation

The surface sterilization protocol combined with the imprint technique was effective in removing epiphytic organisms and that the bacterial and fungal isolated strains can be considered to be true endophytic organisms. A total of two endophytic fungal organisms were isolated from healthy plant leaves of *Cassia alata* (Jalgonwala *et al.,* 2010) and the fungal isolates were identified as *Aspergillus* sp and *Penicillium* sp based on the morphological characters under the light microscope and colony morphology on the growth medium employed are shown in plate 1.

Aspergillus sp easily grow on potato dextrose agar at 25˚C. Colonies starts white to pale yellow but quickly form jet-black conidia; and characteristically has profuse conidiation so that circumferential conidia obscure vesicle. The conidia are spherical and roughen with maturity. Microscopically, it is identified by its hyaline, septate hyphae. *Penicillium* sp produce greenish colonies and its branching or simple conidiophores supporting phialides in rush- like clusters known as penicillin. It is differentiated from paecilomyces by its phialides lacking long, pointed apical extensions. On microscopic observation septate hyaline hyphae were observed.

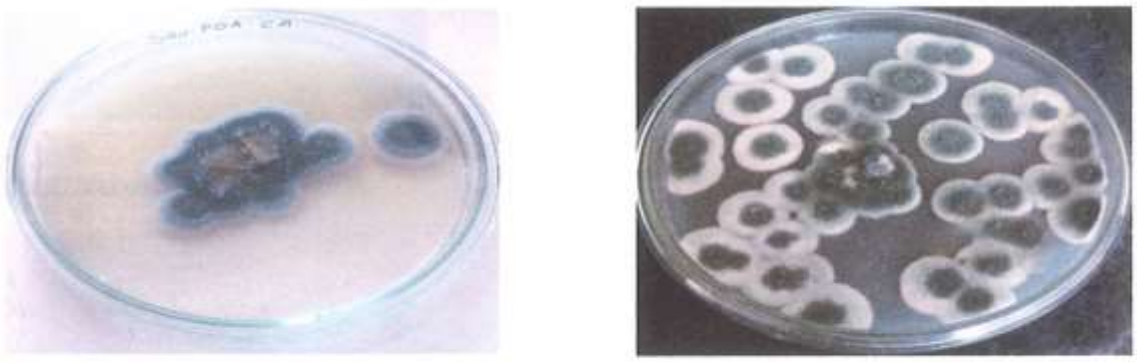
3.2 Antimicrobial activity

On the basis of antimicrobial activities of mycelia mass free extracts of fungal endophytes, by using well diffusion method on MHA, (Reis (2006) and Alves *et al.,* (2008)) it was evident that they shows significant antibacterial activity against almost all bacterial pathogens such as *Staphylococcus* sp, *Klebsiella* sp., *Pseudomonas* sp., *Escherichia coli, Salmonella* sp. High

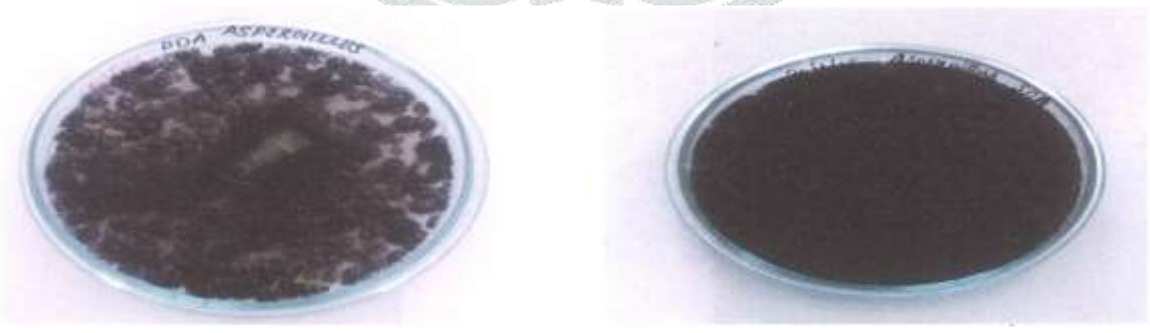
concentration of endophytes shows better activity than the reference antibiotic disc (Ciprofloxacin) used and given in table 1, figure 1 and 2.

PLATE 1

Cassia alata plant



Growth of *Penicillium* sp. on Potato dextrose agar



Growth of *Aspergillus* sp. on Potato dextrose agar

TABLE 1. ZONE OF INHIBITION PRODUCED BY ENDOPHYTIC FUNGAL STRAINS AGAINST PATHOGENIC ORGANISMS.

Bacterial pathogen	Zone of inhibition (in mm)							
	Aspergillus sp.				*Penicillium* sp.			
	Dilution			Ciprofloxacin	Dilution			Ciprofloxacin
	50µl	100 µl	150 µl		50µl	100 µl	150 µl	
Staphylococcus sp.	15	18	20	15	14	18	21	15
Salmonella sp.	14	16	17	16	15	19	23	16
Klebsiella sp.	17	18	20	10	15	23	24	10
Escherichia coli	13	15	16	8	16	17	18	8
Pseudomonas sp.	9	11	12	22	9	12	13	23

FIGURE 1.ZONE OF INHIBITION PRODUCED BY *Aspergillus* sp.

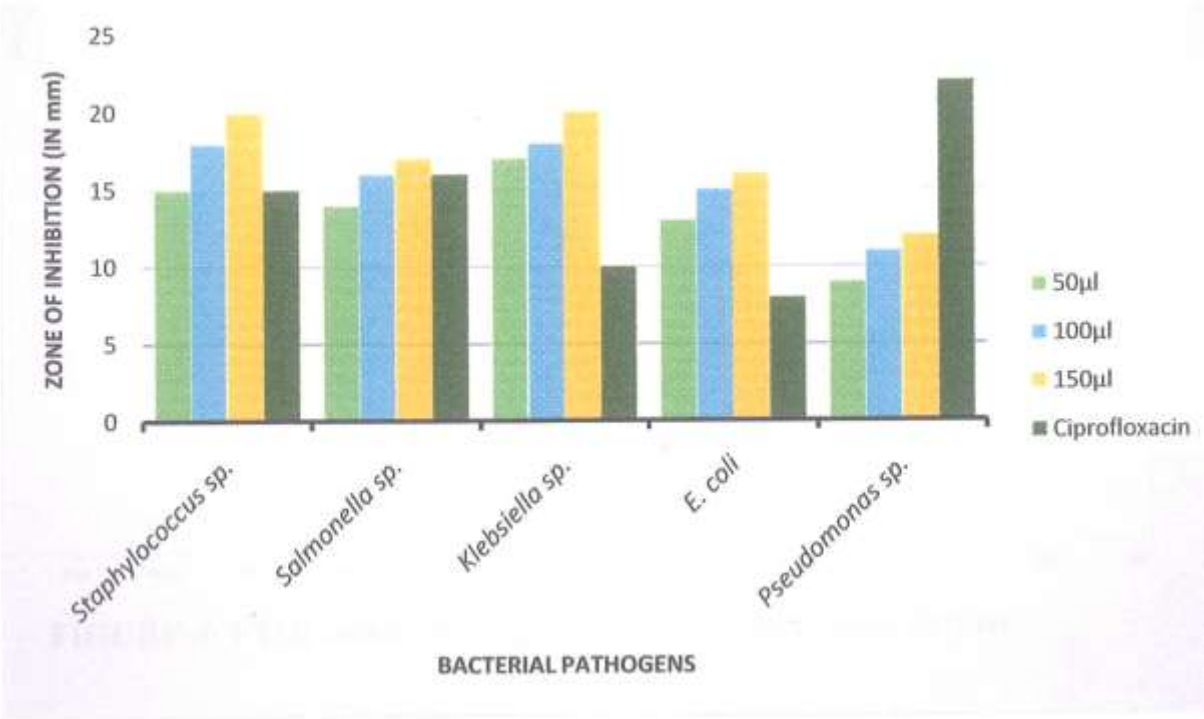
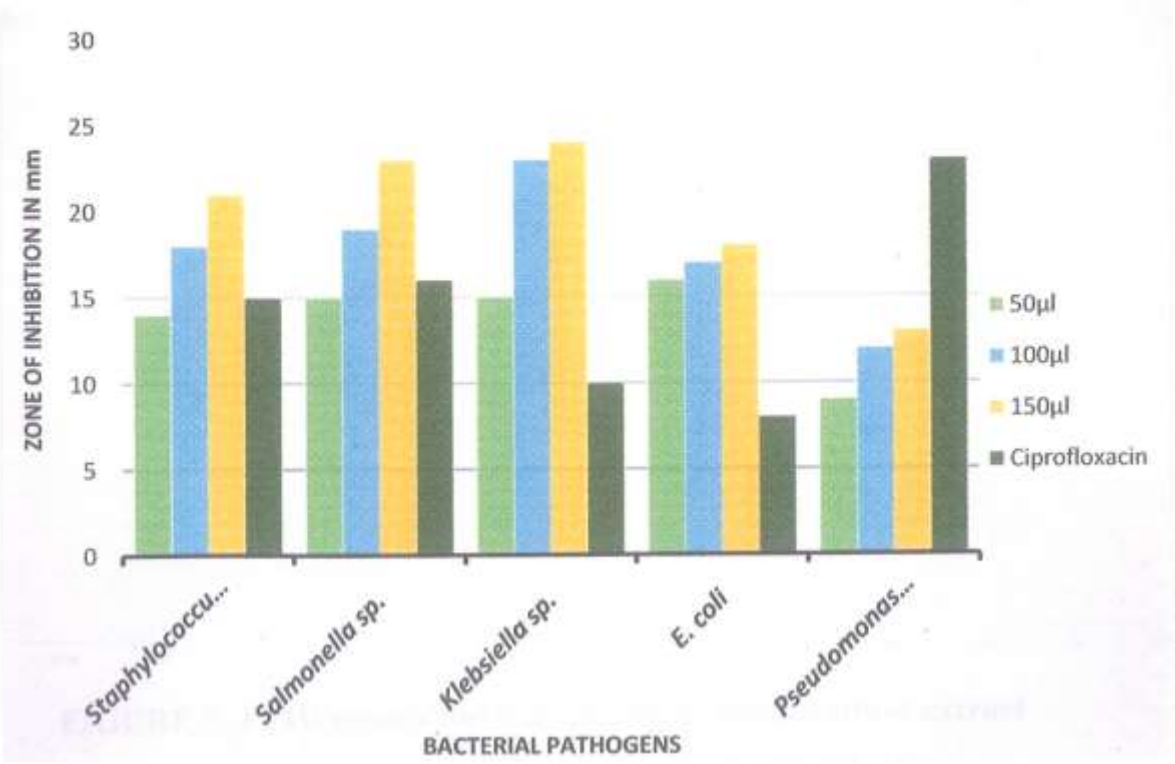


FIGURE 2. ZONE OF INHIBITION PRODUCED BY *Penicillium* sp.



3.3 FTIR analysis

The FTIR analysis of the broth culture for *Aspergillus* sp. is given in Figure 3and the result for *Penicillium* sp. Are given in Figure 4.

The FTIR analysis of *Aspergillus* sp. Broth shows peaks at 3680.18, 3649.32, 3622.32, 3564.45, 3502.73, 3448.72, 3390.86, 2981.95 and 2947.23 indicates the presence of OH group (indicating the presence of alcohol). The peak at 2364.73 and 2322.29 shows the presence of NH group. The peak corresponding to 1913.39 and 1739.79 indicates C=O stretch. The peak at 1647.2 shows NH stretch, while the peak at 1516.05 shows of C=C group (preferably belonging to a benzene ring).

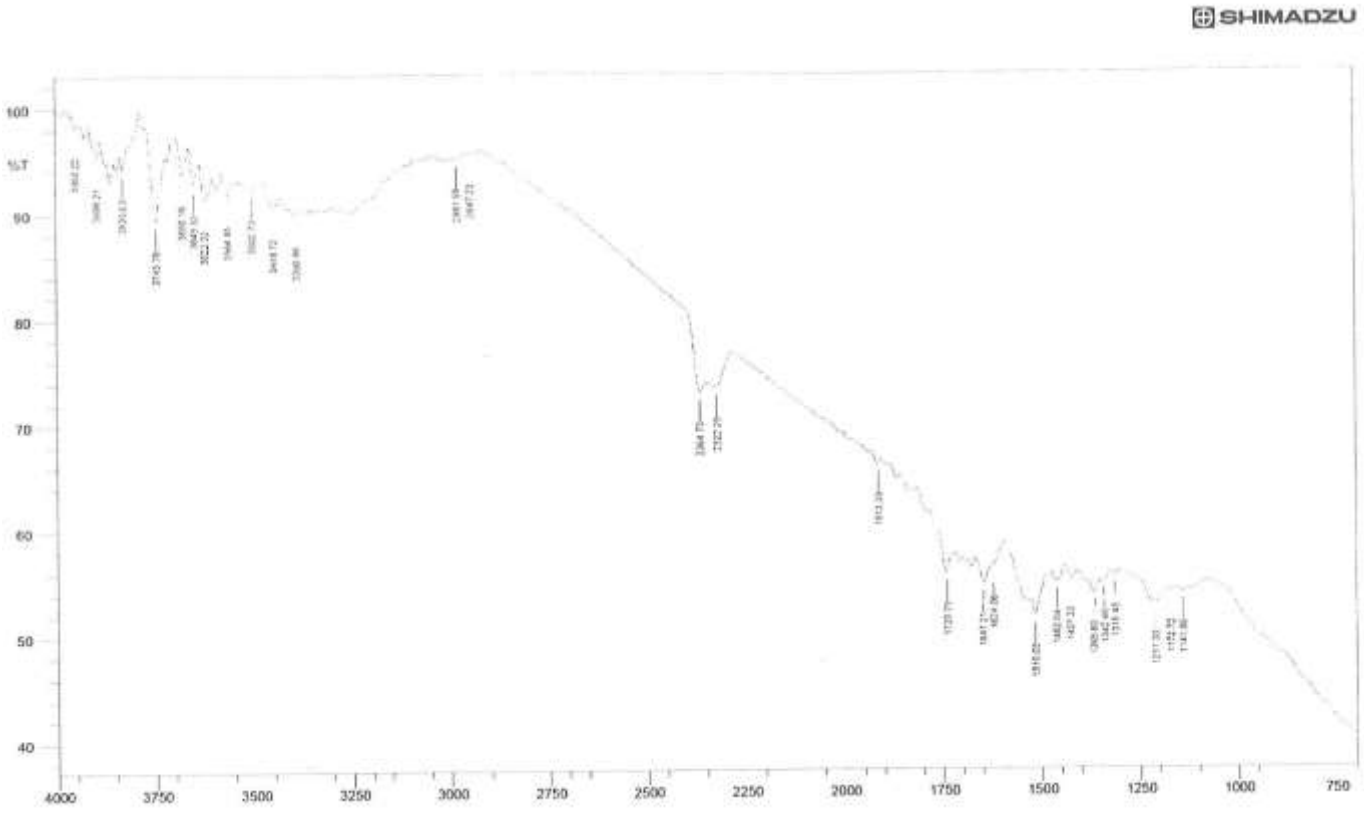


FIGURE 3: FTIR results for *Aspergillus* sp. Broth culture extract

The FTIR analysis of *Penicillium* sp. Broth shows peaks at 3680.18, 3649.32, 3622.32, 3564.45, 3525.88, and 2978.09 indicates the presence of OH group. The peaks at 2927.94 indicate the presence of OH stretch. The peaks at 2364.73 and 2322.29 indicate NH stretch. The peak at 1739.79 shows the presence of C=O stretch. The peak value of 1516.05 indicates the presence of C=C group (preferably belonging to benzene ring).

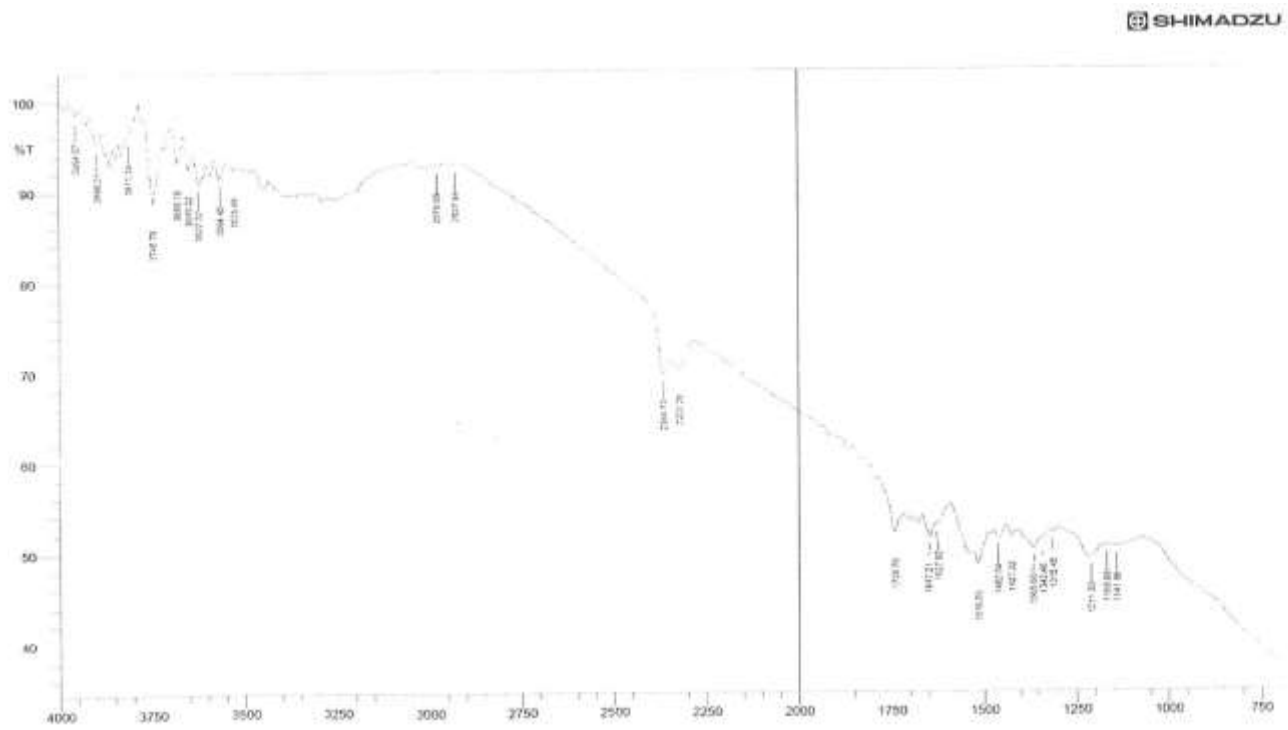


FIGURE 4: FTIR result for *Penicillium* sp. Broth culture extract

IV. CONCLUSION

The production of bioactive compounds by endophytes, especially those exclusive to their host plant it is not important for an ecological perspectives but also from a bio-chemical stand point. Plants have long provided mankind with a source of medicinal agent with natural product once serving as source of all drugs. The significance of the study is exploiting the endophytic microorganism in the area of microbial diversity. Realizing the capability of microorganism to produce diverse bioactive molecules and the existence of unexplored microbial diversity, research is under way to isolate and screen microbes of diverse habitat and unique environment for discovery of novel metabolites. The isolates were then screened for their antibacterial activities. On the basis of antimicrobial activities of mycelia mass, free extracts broth solution of fungal endophytes, was evident that they shows significant antibacterial activity against almost all bacterial pathogens. *Penicillium* sp. is more effective than *Aspergillus* sp. In terms of zone of inhibition produced against pathogens.

V. ACKNOWLEDGEMENT

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