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# ISOLATION OF ENDOPHYTIC FUNGI FROM FEW MEDICINAL PLANTS OF MUTHATHI WILD LIFE SANCTUARY AND THEIR ANTIBACTERIAL ACTIVITY

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***Abstract:*** **Endophytic fungi from medicinal plants provide a reservoir of a bioactive metabolites. The present study is an attempt to investigate the ability of endophytic fungi isolated from few medicinal plants procured from Muthathi Wild Life Sanctuary in Mandya District, Karnataka, India to produce secondary metabolites, which acts as antimicrobial agents and to check their potential antimicrobial activity. The bioactive compounds from the endophytic fungi have tremendous antibacterial activity against eight human bacterial pathogens such as *S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella, K. pneumoneae, P. mirabilis* and *L. monocytogens* by using standard protocol of agar well diffusion method. The present study has proven that some medicinal plants may be a rich source of endophytic fungi with antimicrobial prospective. The isolated endophytic fungi may prime to innovative natural product for practice in pharmaceutical industries. This study has demonstrated that the medicinal plants are very noble source of endophytic fungi with potential to produce bioactive compounds having antibacterial effect.**

***Keywords:*** **Endophytic fungi, Antibacterial activity, Human pathogens, Medicinal plants, Muthathi Wild Life Sanctuary.**

## 1. INTRODUCTION

Endophytes are microorganisms that are present in surviving tissue of different plants establishing mutual relationship without superficially any symptom of diseases (1, 2). These endophytes defend their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites (3, 4).

Endophytic fungi are the microorganisms that are present in living tissues of numerous plants, establishing mutual relationship without causing any symptom of diseases. Endophytes are rich sources of bioactive metabolites, which have important promises in medicine, agriculture and industries (5). The endophytic fungi play important physiological and ecological roles in their host life. They give protection and biotic condition to the host; as they produce additional amount of secondary metabolites. These metabolites, when isolated and characterized, have latent for use in industries, agriculture and in medicines (6). The relation between fungal endophyte and host plant is enormous and different, which is range from symbiotic relationship to antagonistic relationship (opportunistic pathogenic) (7, 8). They develop the resistance of host plants to adverse conditions by discharging bioactive metabolite. These metabolites belong to the category of secondary metabolites of plants which include alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones and xanthones (5).

The innovation of novel anti-microbial, anti-cancerous, anti-oxidant, insecticidal and immune modulatory metabolites from fungal endophytes is a significant alternative to overcome the increasing levels of drug resistance by human pathogens (9, 10). The select of endophytic fungi is considered even beneficial for the protection of floral biodiversity which is being overcome for the purpose of drug manufacturing. In association to the plants, endophytic fungi can be cultured promptly and sufficient biomass can be accumulated by large scale fermentation. Production of bioactive compounds can be increased by genetic engineering of endophytic fungi in order to meet demands while keeping biodiversity and sustainable ecosystem (11).

Medicinal plants play a vital role in providing crucial health care to human populations, since the emergence of civilization. The awareness of medicinal plants has been accrued from different medicinal systems such as Ayurveda, Unani, and Siddha. During the last few decades, there has been an increasing interest in the study of these medicinal plants has been viewed in different parts of the world mainly due to many harms supplementary with synthetic drugs and with the emergence of multi-drug resistant pathogens (12). Medicinal plants are known to harbour endophytic fungi that are assumed to be associated with the production of pharmaceutical products (13). Medicinal plants contain a wide variety of radical scavenging molecules such as phenolic compounds, quinones, coumarins, lignins, tannins, alkaloids, amines, vitamins, terpenoids communities produce related therapeutic products and other endogenous metabolites (14, 15, 16). Endophytes within their host plants have therapeutic values, and practice of ancient medicine must have come into existence according to the availability of medicinal plants within which presence of endophytic fungi as well (17). It was assumed that medicinal plants and their fungal endophytic organisms that occur in the tissues of living plants are potential resources of novel natural products for exploitation in pharmaceutical and agricultural industries (18). The present study was carried out to isolate and test antibacterial activity of endophytic fungi which are isolated from few medicinal plants against human pathogenic bacteria.

## 2. MATERIALS AND METHODOLOGY

**Sample Collection:**

For the isolation of endophytic fungi medicinal plant samples were collected from Muthathi Wild Life Sanctuary, Mandya District, Karnataka, India during in rainy season. Fresh and healthy leaves were collected in separate polythene bags, labelled, transported to the laboratory and stored at 10°C.

**Isolation of Endophytic fungi:**

The endophytic fungi were isolated by the following method observed by Vinu and Jayashankar 2017. The healthy plant leaves were surface sterilized as per the protocol described by (19). Samples were cut into 4×5mm long segments. To remove external micro-organisms and dust, samples were surface sterilized by dipping in ethanol (70%) for 1-2 min, followed by a sodium hypochlorite (NaOCl) solution (4% available chlorine) for 1min and then rinsed in ethanol (70%) for nearly 1-2min. After that, it was finally rinsed in distilled water. Sterilized samples were surface dried under sterile condition on placing over sterilized blotting paper.

The surface sterilized leaves were inoculated on PDA (Potato Dextrose Agar) media supplemented with chloramphenicol as an antibiotic (50µg/ml) and incubated between 28±1ºC temperatures for 5-7days. Plates were observed periodically and when hyphae appeared out from plant segments they were sub cultured and brought to pure culture in PDA slants and stored at 4ºC. All isolated endophytic fungi are maintained in the refrigerator.

**Morphological Identification of Endophytic Fungi:**

Standard taxonomic key included colony, diameter, texture, color and the dimensions and morphology of hyphae and conidia (20, 21).

**Fermentation and Mass Production of Antibacterial Metabolites:**

For the production of secondary metabolite, endophytic fungal cultures were grown in Potato Dextrose Broth (PDB) by placing agar blocks of actively growing pure culture (8mm diameter discs) in 250ml Erlenmeyer flask containing 100ml of PDB and incubated at 28±1ºC.at 30°C for 14days. The culture was centrifuged at 10,000rpm for 10 min to collect the cell free supernatant (CFS) and was filter sterilized which was then used for antimicrobial assay.

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**Selected Organisms to Evaluate The Antibacterial Activity:**

The pathogenic cultures *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Shigella sp.*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Listeria monocytogenes* were grown in Brain Heart Infusion (BHI) media for 24h at 37°C under constant shaking (150rpm).

**Antibacterial Assay:**

The *in vitro* antibacterial assay was performed by Agar Well Diffusion method with some minor modifications (22). The bacterial human pathogens were *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogens*, *Escherichia coli*, *Klebsiella pneumoneae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Shigella.* BHI agar plates were prepared by inoculating 1% of freshly grown pathogenic culture. Wells of 4mm in diameter was made in the plate by using sterile cork borer. Then, 70µl of given CFS was added in each well. The sample was allowed to diffuse for 20min at 4°C. Later, plates were incubated at 37°C for 24-48h. After incubation, the zone of inhibition was measured in mm and recorded. Antibiotic chloramphenicol was used as positive control (23). The antibacterial activity of CFS was evaluated by formation of zone of inhibition, which was measured and expressed in mm.

## 3. RESULTS

**Table 1: List of Antibacterial Activity of Isolated Fungal Cultures**

| Culture code | Zone of inhibition(mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***S.aureus*** | ***E.coli*** | ***B.subtilus*** | ***P.aeruginosa*** | ***Shigella*** | ***K.pneumoniae*** | ***P.mirabilis*** | ***L.monocytogens*** |
| C-1 | - | - | - | - | - | - | - | - |
| C-2 | - | - | - | - | - | - | - | - |
| C-3 | - | - | - | - | - | - | - | - |
| C-4 | - | - | - | - | - | - | - | - |
| C-5 | - | - | - | - | - | - | - | - |
| C-6 | - | - | - | - | - | - | - | - |
| C-7 | - | - | - | - | - | - | - | - |
| C-8 | - | - | - | - | - | - | - | - |
| C-9 | - | - | - | - | - | - | - | - |
| C-10 | - | - | - | - | - | - | - | - |
| C-11 | ++ | ++ | + | - | + | - | ++ | + |
| C-12 | - | - | - | - | - | - | - | - |
| C-13 | - | - | - | - | - | ++ | - | - |
| C-14 | - | - | - | - | - | - | - | - |
| C-15 | - | - | - | - | - | - | - | - |
| C-16 | - | - | - | - | - | - | - | - |
| C-17 | - | - | - | - | - | - | - | - |
| C-18 | ++ | ++ | ++ | ++ | - | + | + | + |
| C-19 | - | - | - | - | - | - | - | - |
| C-20 | - | - | - | - | - | - | - | - |
| C-21 | - | - | - | - | - | - | - | - |
| C-22 | - | - | - | - | - | - | - | - |
| C-23 | - | ++ | - | - | - | - | - | - |
| C-24 | - | - | - | - | + | - | - | - |
| C-25 | - | - | - | - | - | - | - | - |
| C-26 | - | - | - | - | - | - | + | - |
| C-27 | - | - | - | - | - | - | - | - |
| C-28 | - | - | - | - | - | - | - | - |
| C-29 | + | + | - | + | + | ++ | + | + |
| C-30 | - | - | - | - | - | - | - | - |
| C-31 | - | - | - | - | - | - | - | - |
| C-32 | - | - | - | - | - | - | - | - |
| C-33 | - | + | - | - | - | - | - | - |
| C-34 | - | - | - | - | - | +++ | - | - |
| C-35 | - | - | - | - | - | - | - | - |
| C-36 | - | - | - | - | - | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| C-37 | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| C-38 | - | - | - | - | - | - | - | - |
| C-39 | - | - | - | - | - | - | - | - |
| C-40 | - | - | - | - | - | - | - | - |
| C-41 | +++ | ++ | + | + | + | +++ | + | + |
| C-42 | - | - | - | - | - | - | - | - |
| C-43 | - | - | - | - | - | - | - | - |
| C-44 | - | - | - | - | - | - | - | - |
| C-45 | - | - | - | - | - | - | - | - |
| C-46 | - | - | - | - | + | - | - | - |
| C-47 | + | - | - | - | - | - | - | - |
| C-48 | - | - | - | - | - | - | - | - |
| C-49 | + | ++ | + | +++ | + | + | ++ | +++ |
| C-50 | - | - | - | - | - | - | - | - |
| C-51 | - | - | - | - | - | - | - | - |
| C-52 | - | - | - | - | - | - | - | - |
| C-53 | + | + | - | ++ | - | + | - | + |
| C-54 | - | - | - | - | - | - | - | - |
| C-55 | - | - | - | - | - | - | - | - |
| C-56 | - | - | - | - | - | - | - | - |
| C-57 | - | - | - | - | - | - | - | - |
| C-58 | - | - | - | - | - | - | - | - |
| C-59 | - | - | - | - | - | - | - | - |
| C-60 | - | - | - | - | - | - | - | - |
| C-61 | + | + | + | + | ++ | + | + | + |
| C-62 | - | - | - | - | - | - | - | - |
| C-63 | - | - | - | - | - | - | - | - |
| C-64 | - | - | - | - | - | - | - | - |
| C-65 | - | - | - | - | - | - | - | - |
| C-66 | - | - | - | - | - | - | - | - |
| C-67 | - | - | - | - | - | - | - | - |
| C-68 | - | - | - | - | - | - | - | - |
| C-69 | + | ++ | + | + | - | + | + | + |
| C-70 | - | - | - | - | - | - | - | - |
| C-71 | - | - | - | - | - | - | - | - |
| C-72 | - | - | - | - | - | - | - | - |
| C-73 | - | - | - | - | - | - | - | - |
| C-74 | - | - | - | - | - | - | - | - |
| C-75 | - | - | - | - | - | - | - | - |
| C-76 | - | - | - | - | - | - | - | - |
| C-77 | - | + | ++ | + | - | + | + | - |
| C-78 | - | - | - | - | - | - | - | - |
| C-79 | - | - | - | - | - | - | - | - |
| C-80 | - | - | - | - | - | - | - | - |
| C-81 | - | - | - | - | - | - | - | - |
| C-82 | - | - | - | - | - | - | - | - |
| C-83 | + | - | + | - | + | + | - | + |
| C-84 | - | - | - | - | - | - | - | - |
| C-85 | - | - | - | - | - | - | - | - |
| C-86 | - | - | - | - | - | - | - | - |
| C-87 | - | - | - | - | - | - | - | - |
| C-88 | ++ | ++ | ++ | ++ | - | + | + | - |
| C-89 | - | - | - | - | - | - | - | - |
| C-90 | - | - | - | - | - | - | - | - |
| Chloramphenicol | 20 | 26 | 22 | 20 | 24 | 20 | 23 | 20 |

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**Table 2: List of Medicinal plants isolated the Endophytic fungi shows the Zone of inhibition against Bacterial Pathogens**

| Plants Name/ Family | Local name | Habitat | Part used | Endophytic fungi |
|---|---|---|---|---|
| Withania Somnifera (L) Dunal<br><br>Solanaceae | Ashwagandha | Shrub | Leaves | C-11 |
| Centella asiatica (L) Urban<br><br>Apiaceae | Ondelaga | Herb | Leaves | C-18 |
| Cleome gynandra L.<br><br>Cleomaceae | Naribele | Herb | Leaves | C-29 |
| Plectranthus amboinicus Spreng<br><br>Lamiaceae | Doddapatre | Herb | Leaves | C-37 |
| Madhuca longifolia J.F.Macbr<br><br>Sapotaceae | Hippe | Tree | Leaves | C-41 |
| Coccinia grandis (L.) VOIGT<br><br>Cucurbitaceae | Thonde | Climber | Leaves | C-49 |
| Solanum nigrum L<br><br>Solanaceae | Ganike | Herb | Leaves | C-53 |
| Vitex Negundo L<br><br>Verbenaceae | Lakki | Shrub | Leaves | C-61 |
| Manihot esculenta Crantz<br><br>Euphorbiaceae | Maragenasu | Shrub | Leaves | C-69 |
| Tridax procumbens L<br><br>Asteraceae | Addike soppu | Herb | Leaves | C-77 |
| Ocimum sanctum L<br><br>Lamiaceae | Srirama tulsi | Shrub | Leaves | C-83 |
| Cissus quadrangularis L<br><br>Vitaceae | Narale kudi | Shrub | Leaves | C-88 |

**Table 3: Broad Spectrum Activity of some Isolated Endophytic fungi**

| Culture code | Zone of inhibition(mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *S.aureus* | *E.coli* | *B.subtilus* | *P.aeruginosa* | *Shigella* | *K.pneumoniae* | *P.mirabilis* | *L.monocytogens* |
| C-11 | ++ | ++ | + | - | + | - | ++ | + |
| C-18 | ++ | ++ | ++ | ++ | - | + | + | + |
| C-29 | + | + | - | + | + | ++ | + | + |
| C-37 | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| C-41 | +++ | ++ | + | + | + | +++ | + | + |
| C-49 | + | ++ | + | +++ | + | + | ++ | +++ |
| C-53 | + | + | - | ++ | - | + | - | + |
| C-61 | + | + | + | + | ++ | + | + | + |
| C-69 | + | ++ | ++ | + | - | + | + | + |
| C-77 | - | + | ++ | + | - | + | + | - |
| C-83 | + | - | + | - | + | + | - | + |
| C-88 | ++ | ++ | ++ | ++ | - | + | + | - |

**Inhibition Zone:** - **:** No activity, + **:** Weak activity indicates the clear zone 5~9mm, ++ **:** Moderate activity indicates the clear zone 10~ 12mm, +++ **:** High activity indicates the clear zone 13~16mm and indicates the clear zone >16mm.

**Note:** C11 to C88 are the fungal cultures isolated from selecetd medicinal plants.

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**Antibacterial Activity of Endophytic fungi showing Zone of inhibition against Bacterial Human pathogens.**

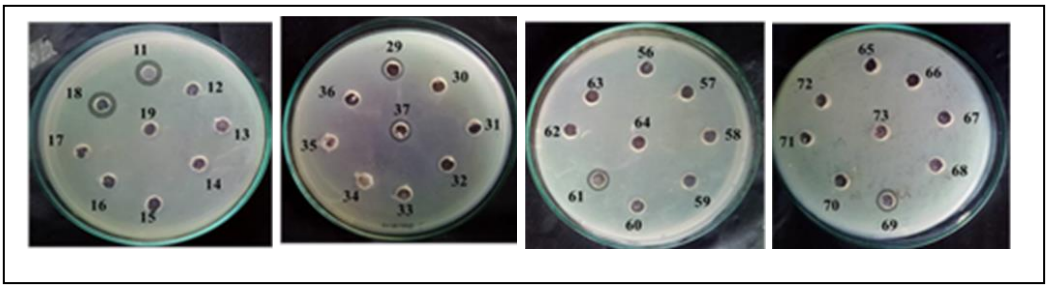

**Fig (a). Zone of inhibition against *S.aureus* strain**

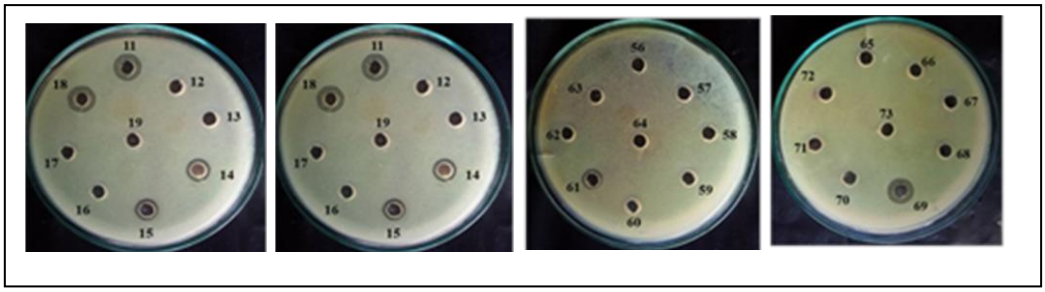

**Fig (b). Zone of inhibition against *E.coli* strain**

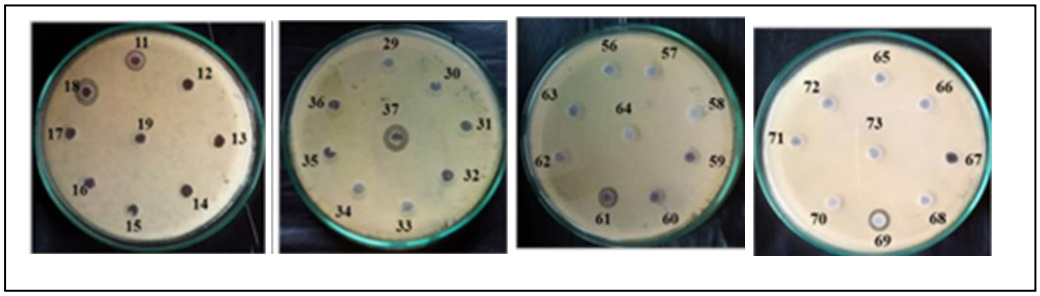

**Fig (c). Zone of inhibition against *B.subtilis* strain**

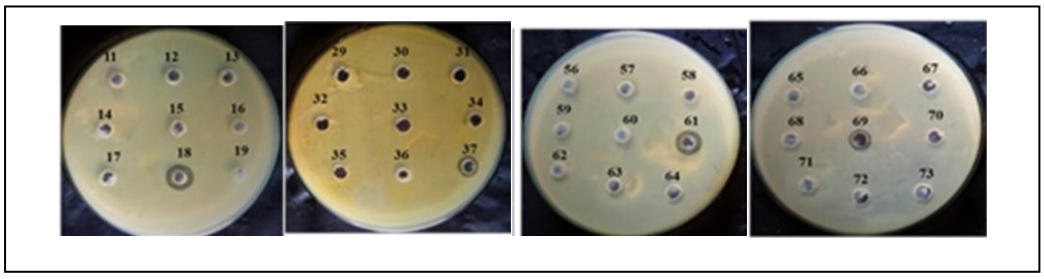

**Fig (d). Zone of inhibition against *P.aeruginosa* strain**

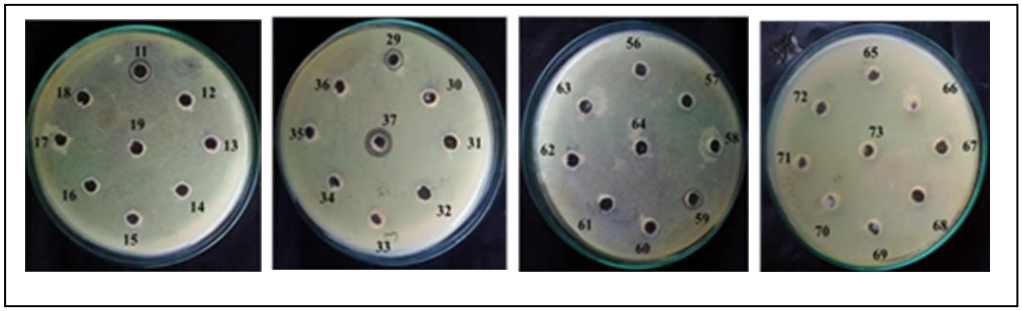

**Fig (e). Zone of inhibition against *Shigella* strain**

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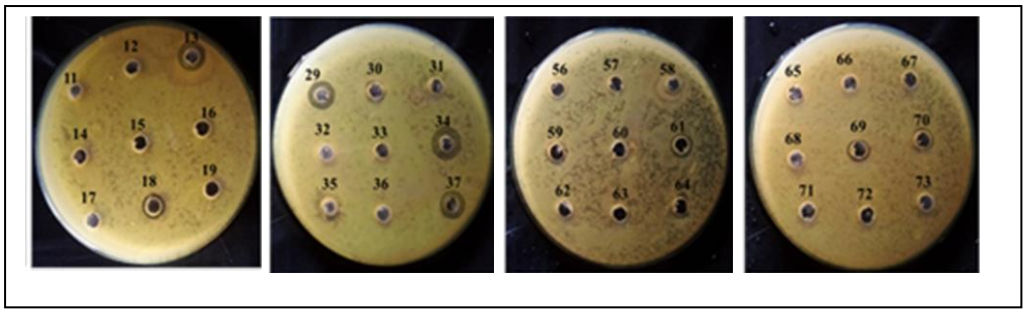

**Fig (f). Zone of inhibition against *K.pneumonia* strain**

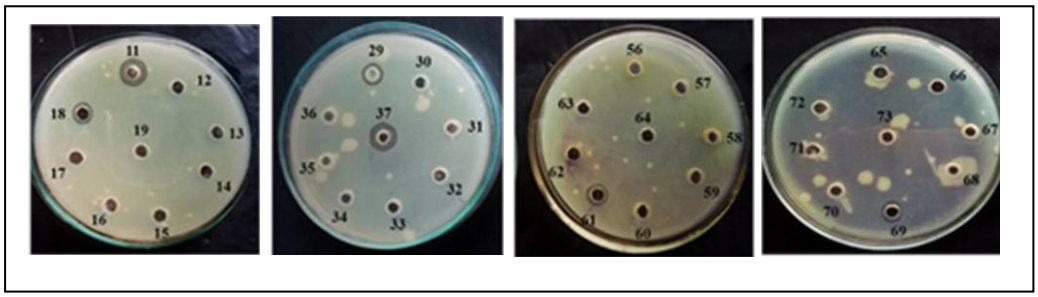

**Fig (g). Zone of inhibition against *P.mirabilis* strain**

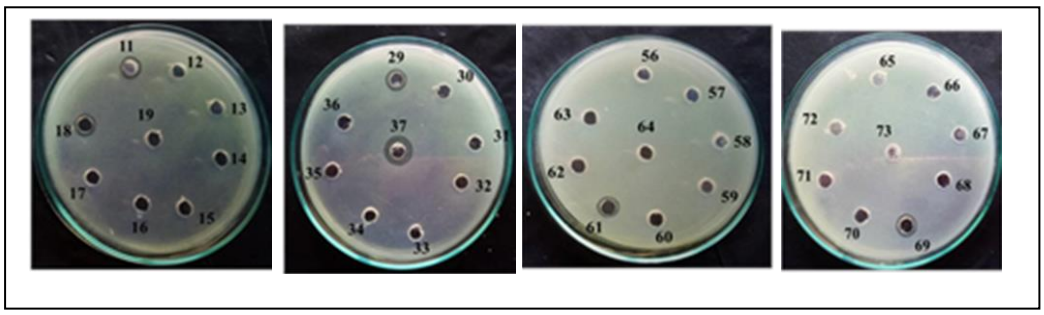

**Fig (h). Zone of inhibition against *L.monocytogen* strain**

A total of 90 endophytic fungi were isolated from the 30 different medicinal plants of Muthathi Wild Life Sanctuary (MWLS), Mandya (Table 1). In the present research, results were based on the evaluation of secondary metabolite produced in stationary condition as well as directly diffused through agar wells. Screening of endophytic fungi was done on the basis of their antibacterial activity against clinically significant eight human bacterial pathogens such as *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, *Shigella*, *K. pneumoneae*, *P. mirabilis* and *L. monocytogens* by using standard protocol of agar well diffusion method. The range of antibacterial activity was expressed in diameter of inhibition zones (mm), shown in (Table 3). The endophytic fungi were followed by cultures like C11, C18, C29, C37, C41, C49, C53, C61, C69, C77, C83 and C88 showed good zone of inhibition against all eight bacterial strain but finest results were seen by culture C37, C41, C49 and C61 against all the clinical pathogens. Rest of culture C18, C29, C69 and C88 showed strong potential activity results against eight of seven or six pathogens shown in (Table 3). On the other hand C11, C53, C77 and C83 exhibited slightly moderate activities. Rest of the other fungal strains showed very less or negligible activity against all eight pathogenic bacteria.

## 4. DISCUSSIONS

Endophytes are microorganisms that are present in surviving tissue of different plants establishing mutual relationship without rapidly any symptom of diseases. Endophytic fungi are the microorganisms that are present in living tissues of numerous plants, establishing mutual relationship without causing any symptom of diseases. These ubiquitous fungi interact absolutely with their environment. In addition, they are the group of organism with very good potential for application in plant enhancement and disease control. Isolation of endophytic fungi from medicinal and other plant results

to produce bioactive compound which has greater activity against various pathogenic microbes. Hence, large scale production of these bioactive compounds must be crucial to accomplish the needs of agriculture and pharmaceutical industries. The colonization of fungi inside the living floral tissues without any important damage is endophytic fungi.

In the current work 90 endophytic fungi were isolated from different plants found in Muthathi Wild Life Sanctuary, Mandya. 12 endophytic fungi from the plants *Centella asiatica* (L) Urban, *Withania Somnifera* (L) Dunal, *Plectranthus amboinicus* Spreng, *Madhuca longifolia* J.F.Macbr, *Coccinia grandis* (L.) VOIGT, *Solanum nigrum* L, *Vitex Negundo* L, *Manihot esculenta* Crantz, and *Ocimum sanctum* L were the main isolates (24). Similarly, isolation of endophytic fungi was done by (19).

The antibacterial activity of endophytic fungi isolated from plants of Muthathi Wild Life Sanctuary, Mandya was done by Agar well diffusion assay, against eight clinically significant pathogenic bacteria *S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella, K. pneumoneae, P. mirabilis* and *L. monocytogens*. The metabolites show considerable zone of inhibitions (mm). The antimicrobial activity of *A. niger* and *A. alternata* showed significant effect on different gram positive and gram negative bacteria and also on different fungi was reported (25). Similarly, these endophytes reduce the growth of pathogenic bacteria by different mode of action in antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant *Trichilia elegans* (26). The antimicrobial activity of fungal secondary metabolites produced by endophytes from *Luehea divaricata* against the human pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. None extracts showed antagonistic activity against *S. aureus*, while some extracts inhibited the *E. coli* growth (27). The antimicrobial potential of endophytic fungi *Phomopsis Alternaria*, *Colletotrichum*, *Nigrospora* and sterile mycelia isolated from the leaf tissues of *Tectona grandis* and *Samanea saman* (28). Antimicrobial activity in cultures of isolated endophytic fungi from five medicinal *Garcinia* plants and verified that the metabolites produced by 70 fungal isolates and extracted with ethyl acetate showed antimicrobial activity by agar well diffusion method against: *Staphylococcus aureus, Candida albicans, Cryptococcus neoformans* and *Microsporum gypseu*. (29), in other research work studied the antibacterial activity of endophytic fungi isolated from plant *Calotropis procera* Linn against six human pathogenic bacteria (1).

## 5. CONCLUSION

The secondary metabolites present in endophytic fungi from different medicinal plants of Muthathi Wild Life Sanctuary may act as potential antimicrobial agents. These medicinal plants were harbours several endophytic fungi which produce biologically active antimicrobial substances with selective antimicrobial properties. A total of 90 endophytic fungi were isolated from 30 different medicinal plants (30). Therefore, there is a need of advance in depth studies of these isolated fungal endophytes. The natural bioactive compounds obtained exclusively from the endophytic fungi have been largely unexplored. Efforts must be made to confirm safe, effective and inexpensive treatments for wide range of diseases by traditional methods which use locally available medicinal plants. The systematic and trustworthy researches on these characteristics are to be done in order to achievement traditional knowledge of ethno medicinal plants.

**Conflict of Interest:**

No conflict of interest declared.

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## REFERENCES

[1] Sandhu SS, Aharwal RP, Kumar S. Isolation and antibacterial property of endophytic fungi isolated from Indian medicinal plant *Calotropis procera* Linn. World J Phar Pharmacy Science (2014); 3(5):678-691.

[2] Strobel G and Daisy B. Bio prospecting for microbial endophytes and their natural products. Microbiol Molecular Biology Rev (2003); 67: 491-502.

[3] Carroll GC and Carroll FE. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can J Bot. (1978); 56: 3032-3043.

[4] Strobel GA. Microbial gifts from rain forests. Can J Plant Pathology (2002); 24: 14- 20.

[5] Tan RX, Zou WX. Endophytes: a rich source of functional metabolites Nat. Prod. Rep (2001); 8: 448-459.

[6] Kumar S, Aharwal RP, Shukla H, Rajak RC, Sandhu SS. Endophytic fungi: as a source of antimicrobials Bioactive compounds. World Journal of Pharmacy and Pharmaceutical Sciences (2014); 3(2): 1179-1197.

[7] Schulz B, Boyle C. The endophytic continuum. Mycology Res, (2008); 109: 661-686.

[8] Arnold AE. Understanding the diversity of foliar endophytic fungi: Progress, Challenges and Practice. Fungal biology Reviews (2007); 21: 51-56.

[9] Song JH. What's new on the antimicrobial horizon? International Journal of Antimicrobial Agents (2008); 32(4): 207-213.

[10] Demain AL, Sanchez S. Microbial drug discovery: 80 Years of progress. Journal of Antibiotics (2009); 62(1): 5-16.

[11] Onifade AK. Research trends: Bioactive metabolites of fungal origin. Res. J. Biol. Science (2007); 2: 81-84.

[12] Gritto MJ, Nandagopalan V, Doss A. Ethno-botanical study on the traditional healers in Pachamalai hill of Eastern Ghats, Tamilnadu, South India. *J Med Plants Study* (2015); 3 (2): 80-85.

[13] Zhang Yi , Mu J, Feng Y, Kang Y, Zhang J, Gu PJ, Wang Y, Ma LF and Zhu YH. Broad-Spectrum Antimicrobial Epiphytic and Endophytic fungi from Marine Organisms: Isolation, Bioassay and Taxonomy. Mar. Drug (2009); 7: 97- 12.

[14] Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. *J Agriculture Food Chemistry* (1999); 47 (10):3954-3962.

[15] Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* (2001); 49 (11):5165-5670.

[16] Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Science* (2004); 74 (17): 2157-2184.

[17] Vinu AK and M Jayashankar. Seasoning of Endophytic fungi: Reasoning of medicinal use. IJCMS, Vol 3, (2017): pp 794-797.

[18] Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK. Prospecting endophytic fungal assemblage of digitalis lanata Ehrh. (Foxglove) as a novel source of digoxin: a cardiac glycoside. *Biotechnology* (2013); 3: 335-340.

[19] Gond S, Verma V, Kumar A, Kumar V, Kharwar R. Study of endophytic fungal community from different parts of *Aegle marmelos* Correae (Rutaceae) from Varanasi (India). World Journal of Microbiology and Biotechnology (2007); 23: 1371-1375.

[20] Joseph C, Gilman. A manual of soil fungi. 2nd edition. Biotech Books, Delhi (2001).

[21] Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. APS press. St. Paul Minneesota, USA (1998).

[22] Lorian V. Antibiotics in laboratory medicine. Williams and Wilkins, Baltimore, (1996).

[23] Xie J, Zhang R, Shang C, GuoY. Isolation and Characterization of a Bacteriocin produced by an isolated *Bacillus subtilis* LFB112 that exhibits Antimicrobial activity against domestic Animal pathogens. Afr J Biotechnology (2009); 8 (20)5611e9.

[24] Aharwal RP, Kumar S, Sandhu SS; Isolation and antibacterial property of endophytic fungi isolated from Indian medicinal plant *Calotropis Procera* (Linn.) R.Br. World journal of pharmacy and pharmaceutical sciences (2014); 3(5): 678-691.

[25] Verza M, Arakawa NS, Lopes NP, Kato MJ, Pupo MT, Said S, Carvalho I. Bio-transformation of a tetrahydrofuranlignan by the endophytic fungus *Phomopsis* sp. Journal of the Brazilian Chemical Society (2009); 20:195–200.

[26] Rhoden SA, Garcia A, Bongiorno VA, Azevedo JL, Pamphile, JA. Antimicrobial Activity of Crude Extracts of Endophytic Fungi Isolated from Medicinal Plant *Trichilia elegans*. Juss. J App Pharmacy Science (2012); 02(8): 57-59.

[27] Bernardi-Wenzel J, Garcia A, Rubin-Filho CJ, Prioli AJ, Pamphile JA. Evaluation of foliar fungal endophytes diversity and colonization of medicinal plant *Luehea divaricate*. Biology Res (2010); 43: 375-384.

[28] Sukanyanee C, Piapukiew J, Thienhirun S, Sihanonth P and Whalley AIS. Endophytic Fungi of Teak leaves *Tectona grandis* L. and rain tree leaves *Samanea saman* Merr. World J Microbiol Biotechnology (2006); 22:481-486.

[29] Phongpaichit S, Rungjindamai N, Rukachaisirikul V and Sakayaroj J. Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. FEMS Immunology Med Microbiology (2006); 48:367- 372.

[30] Gayathri Pai, Chandra M. Screening of Phytochemicals and Isolation of Endophytic Fungi from Medicinal plant *Helicteres isora* L. *J Pharm (IOSRPHR)* (2017); 7 (12): 01-05.