Endophytes are microbes often bacteria or fungi which lives within a plant at least for part of its life without causing any apparent negative effects. They reside inside the tissues of nearly all healthy plants. A wide diversity of fungi was isolated from healthy tissues of most terrestrial and aquatic plants [6]. The leaves appear to house a greater diversity of fungi than the roots. Plant tissues remain entire and functional even though endophytes are residing inside the plant. Endophytic fungi live in plants internally, either intercellular or intracellular and asymptomatically within tissues and are distinguished from mycorrhiza by lacking external hyphae or mantles [7]. Endophytes as organisms that cause symptomatic infections within plants. This definition excludes pathogenic infections and mycorrhizal fungi [8].

A wide range of plants have now been examined for endophytes, and endophytes have been found in almost all of them. Both plants and the endophytes were mutually benefitted by this endo-symbiosis. Endophytes protect their hosts from disease causing agents and adverse environmental conditions by secreting bioactive secondary metabolites [9-11]. Some specific group of endophytic fungi has a close association with grasses. These fungi are transmitted vertically (through seed) and their impact on the economic value of the host has led to study of their biology [12]. The grass endophytes, and related fungi, are considered separately from the more common general endophytes.

Endophytic filamentous fungi represent an important genetic resource for biotechnology. Now a day's fungal metabolites are the biggest challenge to the human beings. This made the scientists to investigate new sources of drugs. In recent days fungi have been used as a source of secondary metabolites.

The discovery of first antibiotic penicillin by Alexander Fleming in 1928 is the milestone in this field. After this several compounds were isolated. Taxol is another promising anti-cancerous drug isolated from Taxomyces andreanae [13]. These filamentous fungi were frequently used for the isolation of secondary metabolites and increased the list of effective drugs from fungal origin. In the current study, we have used the endophytic fungi HHPCYL03 isolated from Cymbopogon flexuosus grass for the extraction of secondary metabolites.

ABSTRACT

Objective: Cymbopogon grass is one of common aromatic grass species used for extraction of essential oil. The endophytic fungus HHPCYL03 isolated from the Cymbopogon flexuosus, a medicinal grass species collected from Kemmannugundi regions of Karnataka.

Methods: Secondary metabolites were extracted from fungi using organic solvent Ethyl Acetate and screened for anticancer assay against breast cancer cell (MDA-MB-231), lung cancer cell (Calu-6) and colorectal cancer cell (HCT116) lines.

Results: The extract showed the positive result against HCT116 cells.

Conclusion: The fungal endophytes certainly become a repository of good economically, socially benefitted bioactive compounds.

Keywords: Endophytic fungi, Anticancer, Inhibition, Metabolite.
**Extraction of secondary metabolites**

The mycelia of different endo-symbiotic fungi were purified on Potato Dextrose Agar medium. Potato Dextrose Broth was used for production of secondary metabolites by the fermentation method. The pure culture of endo-symbiotic fungi was inoculated to 1000 ml conical flasks containing 500 ml of broth medium on a rotary shaker for 15-22 days at 25 °C. The culture filtrate was filtered through a Watmann filter paper and the filtrate was extracted with the same volume of Ethyl Acetate for twice and the extract was air dried for further analysis.

**MTT assay**

The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is [4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution incubated for 24 hrs at 37ºC in 5 % CO2. Dissolved MTT is converted to an insoluble purple formazan by mitochondrial dehydrogenase enzymes of viable cells. Sensitivity of Breast cancer cells (MDA-MB-231, HCT-116 and Calu-6 cells were seeded in a 96 well plates and the extract was air dried for 24 hr. About 8850 µg/well of MTT was added to all the wells and incubated for 3-4 hours. After incubation with MTT reagent the reagent was then discarded and 100 µl of DMSO was added to each well to dissolve the formazan crystals. The optical density (OD) was recorded at 590 nm in a microplate reader.

### Table 1: Percentage inhibition of HHPCYL03 extract on different cancer cell lines

<table>
<thead>
<tr>
<th>Conc. µg/ml</th>
<th>MDA-MB-231</th>
<th>HCT-116</th>
<th>Calu-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.1±1.00</td>
<td>3.0±2.12</td>
<td>5.76±0.73</td>
</tr>
<tr>
<td>10</td>
<td>16.7±0.57</td>
<td>16.6±0.75</td>
<td>7.3±0.24</td>
</tr>
<tr>
<td>20</td>
<td>23.4±1.09</td>
<td>28.3±0.91</td>
<td>8.9±0.81</td>
</tr>
<tr>
<td>40</td>
<td>31.6±0.99</td>
<td>48.4±0.79</td>
<td>27.5±1.41</td>
</tr>
<tr>
<td>80</td>
<td>44.2±0.48</td>
<td>49.4±0.16</td>
<td>47.8±1.09</td>
</tr>
<tr>
<td>160</td>
<td>54.3±0.63</td>
<td>50.1±0.51</td>
<td>67.3±0.93</td>
</tr>
<tr>
<td>320</td>
<td>65.6±0.12</td>
<td>52.1±1.01</td>
<td>76.4±3.36</td>
</tr>
</tbody>
</table>

Table 01 shows variation in the cell inhibition rate by fungal extracts at various concentrations. In case of all the three cell lines inhibition percentage was increased with increase in concentration of the test extract. This indicates the dependence on the concentration gradient.

### Table 2: MTT assay values

<table>
<thead>
<tr>
<th>Log (inhibitor) vs. response</th>
<th>HHPCYL03</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA-MB-231</td>
</tr>
<tr>
<td>Bottom</td>
<td>77.0±0.54</td>
</tr>
<tr>
<td>Top</td>
<td>3.4±0.63</td>
</tr>
<tr>
<td>LOGIC50</td>
<td>1.78±0.146</td>
</tr>
<tr>
<td>IC50</td>
<td>6.9±0.95</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The per cent of inhibition in our study is lower compared to an earlier study, where the extracts inhibited the proliferation of HeLa cells in a dose-dependent manner [17]. The cytotoxicity of HeLa cell lines using MTT assay showed an IC50 of 92.2±0.23 and 88.5±1.23 µg/ml for the extracts of Fusarium sp. and A. fumigatus. The extract showed significant result at 320 µg/ml against Calu-6 cells. The cytotoxic effect of fungal endophyte isolated from Barringtonia acutangula was tested by the MTT assay, which showed the effect of its secondary metabolites on the cell viability in HT29, human colon cancer cell line [18]. Fungi-derived natural products have been an excellent source of pharmaceuticals as well. Antibacterial penicillins, cholesterol-lowering lovastatin, antifungal echinocandin B, and immunosuppressive cyclosporin A, serve to illustrate the importance of investigating fungal sources for new medicines [19].

Extract of HHPCYL03 treatment showed the minimum level of growth inhibition on MDA-MB-231, HCT-116 and Calu-6 cell lines.
the IC50 values are shown in the table 1. The extract has shown significant dose dependant growth inhibition of HCT-116 cells compared to MDA-MB-231 and Calu-6 cell lines. Most of the bioactive metabolites isolated from endophytic fungi were the potential source of anticancer activity [20-22]. This was evident that bioactive metabolites isolated from endophytic fungi were the IC50 values are shown in the table 1. The extract has shown bioactive compound produced by endophytes could be substitute approaches for finding of novel anticancer drugs [23, 24].

These results suggest that crude extract of HHPCYL03 has more cytotoxic effect on HCT-116 cells with the IC50 value ~ 7.0 as shown in the table 2 and could be a potential candidate against colorectal cancer.

CONCLUSION

In our study, the extract from fungi HHPCYL03 showed the positive result against colorectal cancer cells. Nowadays cancer is the major problem to mankind more and more novel anticancer drug discovery is the only way to combat the deadly cancer. In this regard more and more bioactive anticancer compounds have to be isolated from fungal sources. The fungal endophytes certainly become a repository of good economically, socially benefitted bioactive compounds.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Biotechnology, Govt. of India, New Delhi for financial assistance through its grant F. No: BT/PR/14396/NDB/52/179/2010 and Kuvempa University for administrative and laboratory support. We were also thankful to Mr. Shravana Kumar S, Ashwini HS, Srinivas SG for their help in the collection of environmental samples from the field.

CONFLICT OF INTERESTS

Declared None

REFERENCES