INTRODUCTION: Endophytic fungi, or endophytes, are microorganisms that colonize healthy plant tissue. They remain there for at least one cycle of their lives without causing any damage to the host plant through a symbiotic relationship of several fungi (Li et al., 2010; Qui et al., 2010), and even endobacterial endophytes (Andreote et al., 2009; Figueiredo et al., 2009) colonize plants. These endophytes can also protect the plant from many biotic and abiotic threats (Azvedo et al., 2000). They colonize intercellular and intracellular spaces as well as the interior of xylem and phloem cells (Hallmann et al., 1997). The relationship between endophyte and plant may have begun with the growth of higher vegetables hundreds of millions years ago, resulting from coevolutionary processes (Strobel et al., 1996; Strobel and Long, 1998). There is increasing effort to characterize and identify endophytic fungi isolated from medicinal plants. Many studies have shown that some medicinal properties of plants may be related to endophytic fungi hosted by these plants (Azevedo et al., 2002).

Oxalis corniculata Linn. is a member of Oxalidaceae Family and is commonly grown as wild in every where, it is a small annual or perennial procumbent or erect herb. Chemical composition of Oxalis corniculata contain Malic, tartaric and citric acids from leaves and stem. Leaves are a good source of vitamin C. Alcoholic extract of leaves is antibacterial. Medicinal uses of Oxalis corniculata were recorded during monsoon followed by winter and summer. Oxalis corniculata is commonly grow as wild in every where, it is a small annual or perennial procumbent or erect herb. Chemical composition of Oxalis corniculata contain Malic, tartaric and citric acids from leaves and stem. Leaves are a good source of vitamin C. Alcoholic extract of leaves is antibacterial. Medicinal uses of Oxalis corniculata were recorded during monsoon followed by winter and summer.

MATERIALS AND METHODS: Plant materials and fungal isolates: In the present study, endophytic fungal diversity was undertaken by selecting Karnataka University Botanical Garden Dharwad. Geographically, Dharwad district is situated in between 14°15’ to 15°5’ North longitude and 74°49’ and 76° 21’ East latitude. There is a marked diurnal temperature difference. The temperature can be below as 20.2°C in June and high as 34.42°C in March. The annual rain fall is 600-850 mm. The climatic regions are semi - humid or humid. Soil is covered with a hard, compact crust having dark brown colour (Lakshman et al., 2013).

Collection of samples: The plant samples of Oxalis corniculata were collected from medium sized healthy plants. Plant materials were brought in closed sterile polythene bag to the laboratory and processed within 24 hours of collection. The plant parts used for isolation of endophytes in Leaf, Petiole, and Stem. Collected plant materials were washed under running tap water to remove the adhering particles. Leaves were cut into sterile blade small segments measuring 0.5-1.0 cm by sterile blade. Surface sterilization was done by washing segments with 70% ethanol for 2 minutes, 2% sodium hypochlorites for 1-2 minutes, 70% ethanol for 30 seconds followed by 2 to 3 rinses of sterile distilled water. Segments were brought to laminar air flow and blotted in sterilized blotting paper before inoculating into the culture medium (Jayshree et al., 2011).

Inoculation of Explants: Surface sterilized plant segments were aseptically sampled. Leaf segments were randomly chosen separately. In each plant material petriplate 4-6 segments were placed on PDA and MEA media on separately. The inoculated petriplates were then placed for incubation.

Isolation and pure culturing: After 8-10 days inoculated petri plates were examined for fungal endophytes. The petri plates were screened to isolate the fungal endophytes. Fungi growing out from the cut ends of plant segments were transferred to fresh PDA media. After purifying the isolates for several times, final pure cultures were transferred to PDA slants in to the test tubes.

Identification of endophytic fungal isolates: The morphological identification of endophytic fungal strains is based on the morphology of the fungal culture colony or hyphae, the characteristics of the spores, and reproductive structures. If, these features were discernible, measurements of all fungal characters were made in water mounts, and the slides were subsequently mounted in lactophenol and sealed with nail vanish. All experiments and observations were repeated at least twice. (Huang et al., 2008). The endophytic fungal colonization frequency was calculated by using the formula of (Kumarsen and suryanarayan, 1998).

RESULTS: Data on the endophytic studies on Oxalis corniculata has produced 7 genera, belongs to 6 endophytic fungal species. Endophytes were identified based on a microscopic observation of mycelia, conidia, cultural characteristics (surface texture etc) and morphology of fruting bodies. By using standard manuals (following the standard manuals by Burnett 2000; Gilmen 2001; Nagmani et al., 2005; Subramaniam 1983) endophytes are identified. On the examination of some cultures that they failed to sporulate, these were grouped as Mycelia sterilia, and failed to sporulate, these were grouped as Mycelia sterilia, and

%CF = Number of tissue segments colonized by a fungus X 100
Total number of tissues segments placed
Aspergillus niger Tiegh., Aureobasidium pullulans (de Bary) Arnaud.Les., Cladosporium epiphyllum Person., Cunninghamamella blacksleeana Lender., Hymenula affinis Fautrey and Lambotte., Papulaspora Preuss., Rhizopus nigricans Var. verticillatum. All the isolated and identified fungus was stored in Botany Department Microbiology Laboratory Karnatak University Dharwad (BDMLKUD). The important cultured endophytes were photographed shown in (plate I and Plate II). In the present investigation, among the endophytes; Cunninghamamella blacksleeana was dominant which is followed by Aspergillus niger Tiegh. The colonization frequency significantly higher Cunninghamamella blacksleeana (73.26%) and low from Aspergillus niger Tiegh. (53.2%), Hymenula affinis Fautrey and Lambotte. (33.3%) which was followed by Aureobasidium pullulans (de Bary) Arnaud.Les. (30.18%). In addition to three more endophytes are recorded viz; Rhizopus nigricans Var. verticillatum., Papulaspora Preuss., Cladosporium epiphyllum Person., However, highest colonization frequency were observed during the monsoon, this is mainly due to the leaves are mature and there was very little precipitation, endophytic species can be affected by season. Then it is followed by winter and summer seasons. Significant variation in the colonization frequency of endophytic species at different seasons observed, indicating the environmental factors such as rainfall and atmospheric humidity and their effect on host plant similar results were obtained by (Selvanathan et al., 2011). Therefore, surveys of endophytic fungal communities at different seasons have different specific colonization frequency. In many instances leaves sampled during wet season harbored more endophytes than those screened during dry season shown in (Table 2). In the present study, all the seven endophytic fungi were isolated from the leaves, Petiole and Stem of O. corniculata. Highest numbers of endophytes were recorded in monsoon season followed by winter and summer seasons respectively. There results are consistent with earlier contribution of Selvanathan et al., 2011.

Description of Endophytic Fungi:
Species 1: Aspergillus niger Tiegh.
Colonies growing moderately on PDA or MEA, 3.5-4.5cm in 10 days, with abundant submerged mycelium, conidial heads carbon black, exudates lacks, conidial heads large and black, at first globose and then radiate or splitting in well defined columns in age, up to 700-800µm in diam; conidiophores arising directly from the substratum, smooth, non septet, thick walled, 1-2mm ×15-20µm; vesicles globose, walls thick, commonly 45-75µm in diam; occasionally longer bearing two series of fully packed phialides, brownish; metulae mostly 20-30×5-6µm, often reaching 60-80×8-10µm, rarely septate; phialides 7-10×3.5µm; conidia globose, spinulose with colouring substance, black 4-5µm; dehisce to subglobose (Plate II-1).

Species 2: Aureobasidium pullulans (de Bary) Arnaud. Les.
Colonies growing moderately on PDA or MEA, 3.5-4.5cm in 10 days, with abundant submerged mycelium, conidial heads carbon black, exudates lacks, conidial heads large and black, at first globose and then radiate or splitting in well defined columns in age, up to 700-800µm in diam; conidiophores arising directly from the substratum, smooth, non septet, thick walled, 1-2mm ×15-20µm; vesicles globose, walls thick, commonly 45-75µm in diam; occasionally longer bearing two series of fully packed phialides, brownish; metulae mostly 20-30×5-6µm, often reaching 60-80×8-10µm, rarely septate; phialides 7-10×3.5µm; conidia globose, spinulose with colouring substance, black 4-5µm; dehisce to subglobose (Plate II-1) sporangiophores, each terminating in a sporangium. Sporangiophores 1-2mm, high, the sporangia are globose 100-200µ in diameter (Plate I).

Table 1: Colonization frequency (%) of endophytes in Oxalis corniculata on different culture media

<table>
<thead>
<tr>
<th>S.N</th>
<th>Endophytic fungi</th>
<th>Colonization frequency (%)</th>
<th>Total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MEA</td>
<td>PDA</td>
</tr>
<tr>
<td>1</td>
<td>Aspergillus niger Tiegh.</td>
<td>133</td>
<td>6.66</td>
</tr>
<tr>
<td>2</td>
<td>Aureobasidium pullulans (de Bary) Arnaud. Les.</td>
<td>6.66</td>
<td>133</td>
</tr>
<tr>
<td>3</td>
<td>Cladosporium epiphyllum Person.</td>
<td>6.66</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Cunninghamamella blacksleeana</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Hymenula affinis (Fautrey and Lambotte)</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Papulaspora Preuss.</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Rhizopus nigricans Ehrenberg.</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| TOTAL | | 256.5 |

* L-Leaf, P-petiole, S-stem
Species 2: Aureobasidium pullulans (de Bary.) Arnaud, Les.
Colonies growing fast, reaching 4cm in 7 days at 24°C on MEA, mid to dark brown; Vegetative hyphae hyaline, up to 12µm wide; pigmented hyphae distinctly constricted at the septa; Conidiophores mostly 6-8µm wide, dark brown, with small lateral protuberances which become short, open ended necks of phialides; Conidia hyaline ellipsoidal, often with distinct basal apiculation, variable in size and shape, straight, mostly (7.5-)9-11X(3.5-)4-5X(7-)µm, but may be bigger in old colonies; secondary conidia and endoconidia similar, but smaller (Plate II).

Species 3: Cladosporium epiphyllum Person.
Colonies greyish-black, large, thick; conidiophores at first erect, then falling, pale green; conidia very numerous, soon falling from the chain, at first one-celled, then two-to more-celled, olive-green, 10-22X4-6µ thick (Plate II).

Species 4: Cunninghamamella blakesleeana Lender.
Colonies growing rapidly on PDA and MEA, white, later becoming yellow, loose, erect, 2-4cm in height; sporangiolophores long, simple or regularly verticillately branched, lateral branches of the sporangiolophores variable in length and number, usually less than 50µm long; terminal vesicles, globose to subglobose, 40-60µm; lateral vesicles usually smaller than terminal vesicles, 19-28µm; sporangiolophores hyaline, echinulate or smooth, ovoid ones 7.5-10µ; in diam (excluding spines), ellipsoidal ones 10-12.5X7-8µm(excluding spines) (Plate II).

Species 5: Hymenula affinis Fautrey and Lambotte.
Conidia straight, somewhat dorsiventral near apex, apiculated, typically one-septate, 10.2±2.8µ(9.1-11.1X4.6-3.9)µ usually in a continuous smooth or slightly roughened, slimy-layer, from hyaline to pale salmon-colored on a globose agar. Conidiophores from simple to sparingly branched, septate. Mycelium hyaline. No chlamydospores (Plate II).

Genus 6: Papulaspora Preuss.
Conidiophores and conidia lacking, reproductive units consisting of irregular clusters of cells(bulbils); bulbils without organization frequently pigmented; pale brown or orange, often appearing like microclerotia; vegetative hyphae hyaline. In this genus no true spores are formed. The hyaline vegetative hyphae bearing "bulbils" are characteristic of the genus (Plate II).

Species 7: Rhizopus nigricans Var. verticillatum.
Sclerotia creeping, recurring to the substrate in the form of arachnoid hyphae, which are strongly raised and distant from the substrate and implanted at each node by means of rhizoids (Plate II).

DISCUSSION:
Medicinal plants are one of the oldest forms of health care known, every plant on earth is known to harbor at least one endophytic microbe. These are one of the most unexplored and diverse group of organisms having symbiotic association with higher life forms and may produce beneficial substances for host (Weber,1981).

The leaves of the host plant exhibited highest endophytic association than the petiole and stem samples. Endophytes isolated from leaf samples exhibited greater diversity and high colonization frequency compared to the endophytes of the other plant parts examined. Oxalis corniculata being a tropical plant and no report on biodiversity. A recent meta-analysis found that leaf endophytes are indeed more species-rich in the tropics than in temperate regions (Arnold et al., 2005). Most of the leaf samples finding more number of endophytic diversity in the examined plants. One of the possible reasons for the differences in the colonization rates between plants is the structure and substrate which influence the colonization and distribution of endophytic fungi (Okane et al., 1997). Similarly, Kumar and Hyde (2004) have reported that the colonization rate in the leaves was found to be significantly higher than those in other parts studied of the host. Present studies clearly exhibited that the number of endophytic fungi was higher in leaves followed by petiole and stem. Diversity of endophytes on a single plant often differs greatly in the dominant members of their endophytic communities (Chaverri et al., 2010, Gazis et al., 2010, Hofman et al., 2008, Pocasangre, 2000), and may even show functional differences. As in case of Alfalfa plants of leaves, stems and roots are colonized by distinct fungi that produce different ranges of secondary metabolites (Weber et al., 2006). Similar observation was noted in the present study even with a single plant different leaves may differ significantly in community composition (Gamboa et al., 2001, Fisher et al., 1996). Single leaves of a tropical forest tree, manilkara bidentata, showed fine scale variation of endophyte isolation rates and identity. In this respect, plants are genetic mosaics because each organ may have a unique combination of genes in its micro biome (Herre et al., 2007.). However, some endophytes are restricted to single cell and tissues in the leaf endophytes in different tissues may not interact (Stone JK. 1987). The overall colonization frequencies differed with different organs. Similar results were obtained in the endophytic diversity of Thalvaipandian et al., 2011.

In conclusion; the understanding of the number of endophytic diversity associated with an Oxalis corniculata plant in leaves, petiole and stem exhibited a significant variation in colonization frequency of endophytic species. Oxalis corniculata significantly greater number of colonization frequency was documented on leaves. Our culture results showed that the culture media results shows as endophytes grows more in PDA compare to MEA media. Roots do not have endophytes association. In the contest of seasonal distribution the highest number of endophytes colonization during monsoon and it followed by winter and summer seasons was determined on the Oxalis corniculata.

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