International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 07-05-2021, Accepted: 19-05-2021, Published: 01-06-2021
Volume 6, Issue 3, 2021, Page No. 521-526

Isolation and characterization of endophytic microbes from selected medicinal plants and its antimicrobial activity

N Uma Maheswari, P Kokila
PG and Research Department of Microbiology, Sengamala Thayaar Educational Trust Women's College, (Autonomous) Sundarakkottai, Mannargudi, Thiruvarur, Tamil Nadu, India

Abstract
Endophytes are ubiquitous and have been found in all the species of plants and their tissues such as stem, leaves, roots and petioles *etc*. Endophytes may benefit host plants by preventing pathogenic organisms from colonizing them. Now a day, studies on endophytes of medicinal plants had been given due to attention as they tend to produce natural products beneficial for a human being. Hence the present study was planned to isolate endophytic bacteria and fungi from fresh leaves of medicinal plants, collected from Herbal Garden, STET Women's college, Mannargudi, Thiruvarur (Dt) namely *Ocimum tewurflorum L, Cardiospermum halicababum L, Acalypha indica L, Mukia maderaspatana L, Eclipta prostrate L, Phyla noditlora L, Solanum nigrum L, Andrographis paniculata L, Boerhavia diffusa L, Centella Asiatica L, Lawsonia inernis L, Solanum Trilobatum L, Clitoria ternatea L, Phyllanthus nuiri L, Mentha spiata L, Justicia adhatoda L, Vitex negunda L, Murraya koenigi L, Coriandum Sativum L, Catharanthus roseus L.* ,screening of phytochemical consitutuents ,and antimicrobial activity was also done. Although Totally 20 medicinal plants were selected ,bacterial species isolated from medicinal plants and it was identified as *Bacillus subtilis, Streptococcus pyogenes, E. coli, Staphylococcus aureus, Staphylococcus saprophiticus, Micrococcus* by Gram staining and biochemical characteristics. Phytochemical constituents such as steroids, terponoids, glycosides, flavonoids and saponins were present in some of these medicinal plants. The isolates were also screened for extracellular biosynthesis of Silver nanoparticles for which *UV-VIS* absorption was recorded. Among the isolates *A. Niger and E. coli* was present in all medicinal plants.

Keywords: endophytes, medicinal plants, antimicrobial activity, silver nano particles

Introduction
Medicinal plants are gaining worldwide attention owing to the fact that the herbal drugs are cost effective, easily available and with negligible side effects. Medicinal plants harbour endophytic microflora and they are valuable source of Bioprospecting endophytes. Endophytes are the microorganisms that inhabit interior of plant tissues that shows no apparent harm to host. Endophytic fungi from medicinal plants are known as Promising Source of bioactive novel metabolites which has significant role in pharmacology, agriculture and in industries. (Chathudevi 2016) [1] India is commonly called the Botanical garden of the world, owing to the wealth of herbal medicines. India with its great topographic and climatic diversity has a very rich and diverse flora and fauna. The uses of plants as medicines have been practiced from ancient time. Tamil Nadu is ethano boanically very rich, having a wide variety of medicinal plants. With its (Cauvery) diverse topographical condition, the region is well situate for arrange of medicinal plant species. Mannargudi is located at 380km North of Chennai, 80km east of Tiruchirappalli, 35km east of Thanjavur (1hour) and 40km west of Kumbakonam. The region is covered with mainly alluvial or black soil which is conducive for rice cultivation. Endophytes are microorganisms that are presents in living tissues of various lands (root, fruits, stem, seed, leaf etc.,) Establishing mutual relationship without apparently any symptom of disease. These endophytes protect their hosts from infectious agents and adverse condition by secreting bioactive secondary metabolites.

The term "endophyte'' originally introduced by De Bary 1886 rears to the any organisms occurring thin plant tissues, deistic from the epiphytes that live on plant surfaces. Endophytes have been defined by various scientists as mutualisms that colonize aerial parts of living plant tissues and do not cause symptoms of disease. All vascular plants harbor endophtic organisms (Zhang *et al.,* 2006) [2]. These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites (Azevedo *et al.,* 2000) [3] Endophytes existing in plants have a wide range of antimicrobial strains, which are the important potential sources of antimicrobial substances (Strobel 2003) [4]

Materials and methods
Samplecollection
Medicinal plants were collected from STET Herbal Garden, STET Women's college, Mannargudi, Thiruvarur (Dt), Tamil Nadu, Healthy and mature leaf samples were segregated and brought to the Microbiology laboratory, PG & Research department of Microbiology with utmost care and kept in room temperature for further experiments. The healthy leaves of the medicinal plants were used for preliminary phytochemical analysis and isolation of endophytic fungi. Plants with no visible symptoms of disease were carefully selected after physical examination. The plant materials was brought to the laboratory in sterile bags and processed within hours after sampling. The plants are; *Ocimum tewurflorum L, Cardiospermum halicababum L, Acalypha indica L, Mukia maderaspatana L, Eclipta*

prostrate L, Phyla noditlora L, Solanum nigrum L, Andrographis paniculata L, Boerhavia diffusa L, Centella Asiatica L, Lawsonia inernis L, Solanum Trilobatum L, Clitoria ternatea L, Phyllanthus nuiri L, Justicia adhatoda L, Vitex negunda L, Murraya koenigi L, Coriandum Sativum L, Catharanthus roseus L, Mentha spicata L.

Surface sterilization of leaves (Ellis, 1976) [5]

In order to isolate the endophytic fungi and bacteria, the collected healthy leaves were thoroughly washed in tap water. Then the leaves were cut into small segments (about 1cm) including midrib portion. The leaf samples were surface sterilized 0.1% mercury chloride for 60 seconds and then rinsed in sterile distilled water for 10 seconds (three times).

Isolation of endopnytic fungi

The collected plant sample (leaves) were immediately brought to the laboratory and used within 8 hours for isolation of fungal endophytes. Sterile paper bags and stored at 4ºC till further use. The isolation of endophytic fungi was done according to the standard method. (Petrini 1986)[6].These plant samples were thoroughly washed in running tap water to remove soil particles and adhered debris, and finally with distilled water. From each samples sub samples were prepared for further isolation of endophytes.

Identification of endophytic fungi

The isolated fungi were identified based on the morphology of surface texture, pigmentation and spores at the hyphal tips which were used to identify the endophytic fungi at species level using standard manual (Joseph Gilman 2001) [7]. The microscopic examination was also done to study their reproductive spores (Anitha *et al*., 2013) [8]. The identified fungal isolates from the respective plant material were isolated and then subculture in a petridish which slant and incubated at 4˚ C.

Isolation of endophytic bacteria

The leaves were cut in to small pieces of 5 × 5 mm with sterile blades in the laminar air flow. The pieces were placed on Nutrient agar plates supplemented with antifungal agent (bavistin, 30µg/ml) and incubated for 2-4 days at 37°C. Surface sterilized samples were also crushed in sterile distilled water using a sterile mortar and pestle and then streaked on nutrient agar plates supplemented with antifungal agent (bavistin, 30µg/ml) and incubated for 2-4 days at 37°C. After incubation, morphologically different bacterial colonies were selected and subcultured in order to get isolated colonies. The bacterial isolates were studied for their Gram nature and colony characters.

Sample Pre-Treatment

For the pre-treatment of leaf samples and isolation of endophytic bacteria all the leaf samples were excised and subjected to a surface sterilization procedure described by (Arunachalam and Gayathri 2010) [9] The procedure for sample pre-treatment is shown below;

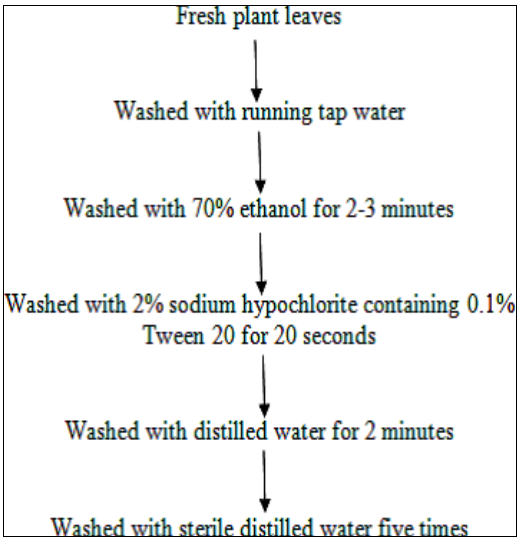

Fig 1

The efficiency of surface sterilization was check by imprint method (Schulz *et al.*, 1999) [10]. The samples were also washed with distilled water and inoculated into nutrient broth containing bavistin (30µg/ml) media as a control to check growth in liquid medium.

Phytochemical analysis

Processing of plant samples

The leaves of the plants are properly washed in tap water and then rinsed in distilled water. The rinsed leaves are dried in an oven at temperatures of 35-40^{0}C for three days. The rinsed leaves of each plant are pulverized; using a sterile electrical blender to obtain a powdered form of these plants is stored in grass containers, protected from sunlight until required for analysis.

Preliminary phytochemical screening of medicinal plants

Phytochemical tests were carried out on the aqueous and ethanol extract and on the powdered specimens using standard procedures to identify the constituents as described by (Safoware, 1993) [11], (Harborne, 1857) [12]

Test for steroids

2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml sulfuric acid. The colour change from violet to blue or green indicating the presence of steroids.

Test for terpenoids

5ml of each plant leaves extract was mixed in 2 ml of chloroform, and concentrated sulfuric acid (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed indicating the presence of terpenoids.

Test for cardiac glycosides

5ml of each plant leaves extract was treated with 2 ml of Glacial acetic acid contain one drop of ferric chloride solution. This was under layered with 2 ml of concentrated with sulfuric acid. A brown ring of the interface was formed indicating the presence of cardiac glycosides.

Test for flavonoids
5 ml of dilution ammonia solution were added to a portion of the aqueous filtrate of each plants extract followed by the addition of concentrated sulfuric acid. A yellow colouration was observed indicating the presence of flavonoids.

Test for saponins
About 2g powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtered sample is mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The forthing is then mixed with 3 drop of olive oil formation of emulsion indicating the presence of saponins.

Extracellular synthesis and characterization of silver nanoparticles from endophytes (Chathurdevi. *et al*2016)
For the synthesis of silver nanoparticles, the fungal isolates were grow in 250 ml flask contains 100 ml potato dextrose broth (PDA) at room temperature for 72 hrs. and then the biomass was harvested and filtered through Whattman filter paper No.1. The fungal mat was washed with distilled water to remove media component and suspended in 100 ml distilled water for 48 h. After 48 hrs of incubation, the cell filtrate was separated by filtration. The fungal cell filtrate was the collected, and it was challenged with the $AgNO_3$ salt (final conc. 1mM). After 24 hrs of incubation, the formation of silver nanoparticles was screened by visual observation of colour that changes from pure white to brown. Then it was further confirmed by subjecting the reaction mixture to UV- Visible spectrophotometer analysis.

Antimicrobial activity
Different concentrations (50 µl and 100 µl) of both culture filtrates extract (CFE) were Assayed against bacteria such as *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa. Staphylococcus aureus, Salmonella typhi.* and same concentrated extract was assayed against fungi such as *Aspergillus rayzae, A. flavus, Pencillium citrinum, Rhizopus.* Assay was carried out using well diffusion method, Muller Hinton media was used. Gentamycin were used as positive control. The plates were incubated after 24 hrs.

Calculation of colonization frequency
Colonizing frequency was calculated as per standard procedure (Suryanarayanan. *et al.,* 2003) [13].

$$\text{Colonization frequency} = \frac{\text{No of species isolated}}{\text{No. of segment screened}}$$

Results and Discussion
Collection of medicinal plants
Endophytic bacteria are ubiquitous in most plant species, residing latently or actively colonizing plant tissues locally as well as systemically. The present study was planned to isolate the endophytic microbes from the medicinal plants in around STET herbal Garden, Mannargudi, Thiruvarur (Dt). Totally 20 medicinal plants were screened for the presence of endophytic fungi. The fresh leaves of *Ocimum tewurflorum L, Cardiospermum halicababum L, Acalypha indica L, Mukia maderaspatana L, Eclipta prostrate L, Phyla noditlora L, Solanum nigrum L, Andrographis paniculata L, Boerhavia diffusa L, Centella Asiatica L, Lawsonia inernis L, Solanum Trilobatum L,Clitoria ternatea L, Phyllanthus nuiri L, Mentha spiata L, Justicia adhatoda L, Vitex negunda L, Murraya koenigi L, Coriandum Sativum L, Catharanthus roseus L.* Medicinal plants were collected from STET Herbal Garden, STET Women's college, Mannargudi, Thiruvarur(Dt). Endophytic fungi were isolated from these plants.

Isolation and identification of endophytic fungi
Totally 20 medicinal plants were selected isolation, endophytic fungi,were *Aspergillus oryzae, Aspergillus niger, Aspergillus flavus, Aspergillus focus, Aspergillus fumigates, Aspergillus nidulans, Aspergillus. rugulosus, Aspergillus terreus, Alternaria tenuis, Rhizopus sps, Penicillum citrinum, Pencillium notatum, Helminthosporum, H. oryzae, Fusarium, Bipolaris*.

Morphology of the isolated organisms
Aspergillus fungus is also called Eurotinum. It is chiefly a saprophytic fungus which is widely distributed. *Aspergillus* is commonly found in rotting orange and phyllanthus fruits. *Aspergillus flavus* and *Asperillus fumigatus* cause disease in animal and plants. *Aspergillus flavus* size range from 400-800 pale brown surface and smooth conidial surface, *Aspergillus niger* size range from 400-3000 slightly brown and irregular conical surface, *Aspergillus fumicates* 200-400 size grayish smooth walled surface smooth and slightly rough conidial surface, *Aspergillus fumigatus* mycelium produce pigment are called fumigatin. *A. nidulans* 70-150 size brown and smooth walled surface and smooth conida, *A. terreus* 100-250 size and unclored smooth walled and smooth walled conidial surface.
Penicillum is commonly known as green or blue mould. Usually grows as a saprophytes on decaying fruits and vegetables. *Penicillum citrinum* rapid growth dark green and pale yellow sepatate hyphae, *P. notatum* rapid growth olive green and off white smooth hyphae. They produce blue colour spores of mycelium. They are produce pigment is known as Spirulosin. *Helminthosporum* size range 430-580 brown coloured straight conidia and *H. oryzae,* size range from 430-580, brown coloured straight conidia are straights to flexuousconidia. *Helminthosporum* mycelium and spores of some mould Posses the pigments they are called Catenarin. Genus of *Fusarium* are saprobic or saprophytic. Some are only mild facultative parasites. *Fusarium* white cream gray surface with micro and macro conidia. The hyphae are septate or branched. *Bipolaris* size range from 2-14 and fast growing grey to blackish brown and it have large conidia. *Alternaria* occus universally and grow mostly as saprophytes on plant debris and drying parts of plants. The mycelium is shot, septate, light brown but becoming darker with age. *Alternaria tenuis* size ranging from 100-250 uncoloured and having large conidia. *Rhizopus* is a saprophytic phymycete. The hyphae are loosely entangling and white fluffy mycelium. *Rhizopus sps* size ranging from growing dark green colour and septate hyphae.

List of endophytic fungi from medicinal plants
Aspergillus oryzae was present in the endophytic plants namely *Ocimum tewurflorum, Acalypha indica, Mukai maderaspatana, Solanum nigrum, Centella Asiatia, Phyllanthus nuiri, Mentha spicat, Justicia adhatoda,* and *catharanthus* respectively. *Aspergillus Niger* were present in all 20 types of medicinal plants. The *Aspergillus flavus* are

present8 medicinal plants namely *Cardiospermum halicababum, Acalypha indica, Phyllanthus nuiri, Vitex negunda, Murraya koenigii, Coriandum sativum, Ctharanthus roseus, Boerhavia diffusa.*

Aspergillus fumigatus are present in *Ocimum tewurflorum, Acalypha indica, Eclipta prostrate, Lawsonia inernis, Solanum trilobatum, Clitoria ternatea, Phyllanthus nuiri, Vitex negunda,* and *Corandum sativum.* The *Acalypha indica, Solanum nigrum, Boerhavia diffusa, Centella asiatica, Clitoria ternatea* are having in *Aspergillus fumigates. Aspergillus nidulans* are present in *Phyla noditlora, Solanum nigrum, Centella Asiatica, Mentha spicata, Justicia adhatoda,* and *Murraya koenigii.*

Next, *A. rugulosus was present inOcimum tewurflorum, Cardiospermum halicababum, Eclipta prostrate, Centella Asiatica, Solanum trilobatum, Justicia ahhatoda Aspergillus terreus* are present in only six medicinal plants namely *Acalypha indica, Phyla noditlora, Centella asiatica, Lawsonia inernis, Coriandum sativum, Catharanthus roseus. Alternaria tenuis* are present in *Eclipta prostrate, Andrographis paniculata, Centella asiatica, Solanum trilobatum, Mentha spicata, Vitex negunda, Murraya koenigii. Rhizopus* are present in only three medicinal plants *viz Boerhavia diffusa, Vitex negunda, Catharanthus roseus.*

Pencillium citrinum are present in *Ocimum tewurflorum, Cardiospermum halicababum, Eclipta prostrate, Solnanum nigrum, Centella Asiatica, Lawsonia inernis, Phyllanthus nuiri, Justicia adhatoda, Murraya koenigii* and *Coriandum Sativm. Pencillium notatum* are present in *Cardiospermum halicababum, Solanum nigrum, Bhringraj Eclipta Atba, Vitex negunda, Murraya koenigii. Helminthosporum* are present in only 2 plants they are; *Boerhavia diffusa* and *Coriandum sativum. Eclipta prostrate, Andrographis paniculata, Centella asiatica, Phyllanthus nuiri* and *Catharanthus* are having *H. oryzae* respectively. *Fusarium* was present in *Phyllanthus nuiri, Mentha spicata, Justicia adhatoda, Vitex negunda,* and *Coriandum sativum.*

Finally the *Bipolaris* are only present in *Ocimum tewurflorum.*

Isolation and identification of endophytic bacteria

The bacterial isolates were identified by using Bergey's manual of Determination bacteriology. Distinct developing on the culture plates were observed for their pigmentation, margin, elevation and opacity. Gram's staining and motility was done using 24 hours pure culture. The Gram's reaction, shape, arrangement and size of the cells were examined. Biochemical characteristics of the isolates were determination by employing the following tests such as Catalase, Coagulase, Citrate utilization, Urease, Indole and Triple sugar ion. The bacterial colonies were isolated from medicinal plants and it was identified as *Bacillus subtilis, Streptococcus pyogens, E. coli, Staphylococcus aureus, Staphylococcus saprophiticus, Micrococcus*. Predominantly *E.coli* was isolated in all the plants.

Morphological characteristics of the isolated bacteria from medicinal plants

Colony morphology of *Bacillus subtilis*- Creamy white raised with rough edges, Gram positive bacilli.

Staphylococcus pyogenes- Creamy, slightly raised with smooth edges. *E.coli*- Whitish raised with rough edges, Gram negative bacilli. *Staphylococcus aureus*- Golden yellow, slightly raised with smooth edges. *Staphylococcus saprophiticus*- Creamy white, raised with rough edges, Gram positive cocci in cluster. *Micrococcus*- Creamy deep yellow, slightly raised with smooth edges, Gram positive cocci.

Phytochemical screening of medicinal plants

In phytochemical screening steroids are commonly present in the plants namely *Ocimum teuflorum, Mukia maderapata, Eclipta prostrate, Solanum nigrum, Andrographis panicutata, Lawsonia inernis, Mentha spicata, Justicia adhatoda* and *Catharanthus roseus*.

Trepenoids was present in the *Ocimum teuflorum, Cardiosperum halicababum, Mukia maderapata, Eclipta prostrate, Solanum nigrum, Anderographis panicutata, Lawsonia inernis, Phyla noditlora, Boerhavia deffusa, Centella Asiatica, Solanum tribatum, Clitoria ternatea, Mentha spicata, Negunds* and *Catharanthus roseus.* Glycosides present in the *Cardiosporum halicababum, Acalypa indica, Mukia mederaptata, Andrographis paniactata, Boerhavia diffusa, Lawsonia inanis, Clitoria ternatea, Phyllanthus nuri, Justicia badhatoda, Negunds,* and *Cantharanthus roseus.* Flavonoides are also present in the *Acalypa indica, Mukia mederaptata, Andrographis paniactata, Boerhavia deffusa, Phyllanthus nuri,* and *Coryandam sataivam.* Next saponins present in the *Cardiosporum halicababum, Acalypa indica, Mukia mederaptata, Andrographis paniactata, Boerhavia diffusa, Lawsonia inanis, Clitoria ternatea, Phyllanthus nuri, Justicia badhatoda,*. (Table -3)

Colonization frequency for endophytic fungi

Moreover it was observed that the colonization frequency (CF) of endophytic fungi was notice *Aspergillus oryzae* (12.05%), *A. niger* (77.02 %), *A. flavus* (9.33%), *A. fucus* (19.66%), *A. fumicates* (12.09%), *A. nidulans* (4.45%), *A. terreus* (48.09 %), *Alternaria tenuis* (14.00%), *Rhizopus* (19.04%), *Pencillium citrinum* (34.07%), *P. notatum* (23.08%), *Helminthosporium* (21.00%), *H. oryzae* (17.13%), *Fusarium* (12.63%), *Bipolaris* (23.54 %). (Fig-1).

Colonization frequency for endophytic bacteria

Similarly colonization frequency (CF) of endophytic bacteria is *Bacillus subtilis* (20.00%), *E.coli* (29.66 %), *Staphylococcus pyogenes* (12.05 %), *S. aureus* (14.09 %), *S. saprophiticus* (14.45 %), *Micrococcus* (7.08 %) respectively. (Fig- 2).

Antimicrobial activity of plant extract

The inhibitory effect of extracts from 20 medicinal plants was noticed and their antibacterial activity tested against diverse entric pathogens (*Bacillus subtilis*, *Staphylococcus pyogens*, *E.coli*, *Staphylococcus aureus*, *Staphylococcus saprophiticus*, *Micrococcus*, *Salmonella*) at a concentration of 10 ml, resulted in different extract of inhibition the strains *Bacillus subtilis* (11±0.3 mm), *Staphylococcus pyogenes* (8±0.2mm), *E.coli* (16±0.4), *Staphylococcus aureus* (7±0.2), *Staphylococcus saprophiticus* (14±0.3), *Micrococcus* (6±0.3), *Salmonella* (11± 0.4) were inhibited by the extract of medicinal plants. The antibacterial activity against the 7 tested pathogens. Extracts of exhibited potential antifungal activity against *Aspergillus oryzae* (14± 0.3), *A. niger* (11±0.4), *A. flavus* (9±0.2), *A. focus* (7.0.6), *A. fumigatus* (4± 0.5), *A. nadulans* (11± 08), *A. rugulosus* (7±0.1), *A. terreus* (12±0.9), *Alternaria tenuis* (9±0.6), *Rhizopus* (13±0.5), *Pencillium citrinum* (12±01), *P. notatum* (13±0.1), *Helmenthosporum* (9± 0.5), *H. oryzae* (14±0.1), *Fusarium* (5± 00), *Bipolaris* (7± 0.3)did not exhibit and inhibitory activity against the tested fungal strains (Table- 1). Our study reports was agreed to Mrunali Bind 2019 [14] isolate and identify endophytic bacteria from Pigeon pea and evaluating their antimicrobial potential against *Fusarium udum*. In the related to the findings of Anushi Jain 2017 [15] endophytic bacteria were isolated from leaves of *Codiaeum variegatum var pictum (Croton)*, *AdhathodavasicaNees (Adulsa)*, *Neolamarckiacadamba (Kadamba)*, *Azadirachtaindica (Neem)*, *Curcuma longa (Turmeric)*, *Hibiscus rosa-sinensis (Hibiscus)*, *and Saracaasoca (Ashoka)*.

Sixteenbacterial strains were isolated and were studied for their Gram nature and colony characters. Hence our study concluded that 20 Species of fungi, 6 bacterial species were isolated from the medicinal plants. They were also tested for the production of extracellular enzymes and increase the fertility of the soil was also checked.

Synthesis of silver nanoparticles by fungal endophytes

The rapid colour change of dark brown in both fungal cell filtrates (after 24 h) with the addition of silver nitrate solution was observed from our results. The appearance of a colour change indicates the synthesis of silver nanoparticles. The formation of silver nanoparticles in fungal cell filtrate was further characterized by using UV-VIS Spectrophotometer. The reaction mixture of *Pencillium citrinum* showed at 552 nm and *Aspergillus oryzae* showed at 439 nm. The *A. flavus* and *A. focus* at 345 nm and *A. fumigatus* at 458 nm and *A. rugulosus* at 234 nm. The *Helminthosporum* at 452 nm. Traditional medicinal plants namely *Acalypha indica*, *Eclipta prostrate*, *Mentha spicata*, *Justicia adhatoda*, and *Solanum nigrum* showed maximum endophytic flora. Antimicrobial activity was effectively performed especially *Aspergillus Niger* and *E.coli* are controlled by (14± 0.2) concentration. Silver nanoparticles synthesis 552 nm in *Pencillium citrinum* (Table- 2).Our study similar to Jia-li-Duan2013 [16], Six endophytic bacterial strains, which belong to genera of *Pseudomonas*, *Rhizobium*, *Bacillus* and *Novosphingobium*, were isolated from the root of healthy *S. miltiorrhiza.*

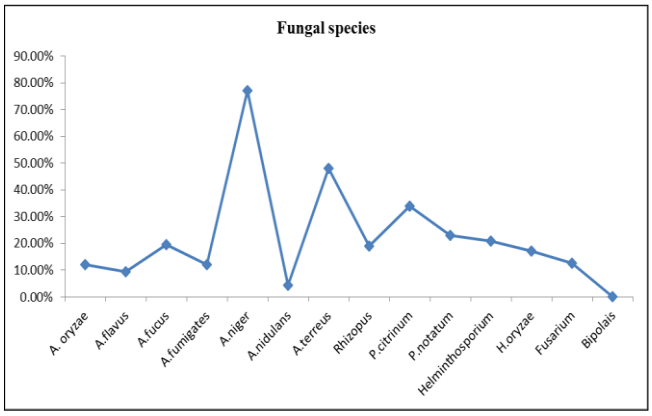

Fig 2: Colonization frequency for endophytic fungi

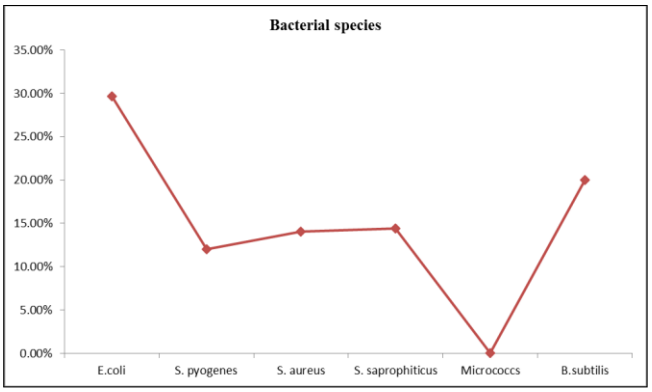

Fig 3: Colonization frequency for endophytic bacteria

Table 1: Antimicrobial activity of the medicinal plants against pathogens

Name of the Organisms		Zone of inhibition(mm)	
Fungi	**Bacteria**	**Fungi**	**Bacteria**
Aspergillus oryzae	*Bacillus subtilis*	14± 0.3	11±0.3
Aspergillus niger	*Staphylococcus pyogenes*	11±0.4	8±0.2
Aspergillus flavus	*E.coli*	9±0.2	16±0.4
Aspergillus focus	*Staphylococcus aureus*	7±0.6	7±0.2
Aspergillus fumigates	*S. saprophiticus*	4±0.5	14±0.3
Aspergillus terreus	*Micrococcus*	10±00	6±0.3
Alteraria tenuis	-	11±0.8	-
Rhizopus sps	-	13±0.5	-
Pencillium citrinum	-	12±0.1	-
Pencillium notatum	-	13±0.1	-
Helminthosporum	-	9±0.5	-
H. oryzae	-	14±0.1	-
Fusarium	-	5±00	-
Bipolaris	-	7±0.3	-

Table 2: Details of Synthesis of silver nano particles by fungal endophytes

Name of the fungal isolates	Values of silver nano particles(nm)
Pencillium citrinum	552
Aspergillus oryzae	439
Aspergillus flavus	345
Aspergillus focus	345
Aspergillus fumigates	458
Aspergillus niger	439
Helminthosporum	452

Table 3: Details of Phytochemical screening of medicinal plants

Plants	Steriods	Treponoids	Glycosides	Flavonoids	Saponins
Ocimum tenuiflorum	+	+	-	-	+
Cardiospermum halicacabum	-	+	+	-	+
Acalypha indica	-	-	+	+	+
Mukia maderaspatana	+	-	+	+	-
Eclipta prostrate	+	+	-	+	+
Phyla nodiflora	-	+	-	-	+
Solanum nigrum	+	-	-	-	+
Andrographis paniculata	+	+	+	+	+
Boerhavia diffusa	-	+	+	-	+
Centella asiatica	-	+	-	+	-
Lawsonia inermis	+	+	+	-	-
Solanum procumbens	-	+	-	-	-
Clitoria ternatea	-	+	+	+	+
Phyllanthus niruri	-	+	+	+	-
Justicia adhatoda	+	+	-	-	+
Vitex negundo	-	+	+	+	+
Murraya koenigii	-	-	+	-	+
Coriandum sativum	-	-	-	-	+
Catharanthus roseus	+	-	-	+	-
Mentha spicata	+	+	-	+	-

Conclusion

The endophytes can be a potent natural source of bioactive compounds which can be considered to be prolific resource for drugs and extraction of bioactive compounds. This study highlights the occurrence and diversity of cultural endophytes in a large number of plant species. The successful colonization of several crops with such microbes suggests that they can be utilized in future applications, such as delivery of derivative enzymes for controlling certain plant diseases or other useful products. Bacterial and fungal endophytes can be useful in agriculture under integrated crop production technology and can be used as biocontrol agents. Further research will be focus on the strain improvement and genomic variation among these isolates.

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