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Antagonistic activity and phytochemical screening of endophytic fungi isolated from medicinal plants

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Abstract
Endophytes represent a promising source of biologically active metabolites for pharmaceutical and agricultural application. They are harmless in most plant species, and secreting growth hormones for the development of host plant. The present study was designed to determine antagonistic activity of endophytic fungi from selected medicinal plants, screening of phytochemical compounds, synthesis of silver nanoparticles and enzymes also extracted. Totally 15 medicinal plants were selected from STET herbal garden, Sundarakkottai, Mannargudi. Leaves of the plants were used for this study. The collected leaves of plants namely *Azadirachta indica* L, *Mentha spicata* L, *Ocimum tenuiflonum* L, *Acalypha indica* L, *Solanum virginianum* L, *Chrysopogon ziznioides* L, *Trachyspermum ammi* L, *Phyllanthus niruri* L, *Centella asiatica* L, *Trigonella fornumgraecum* L, *Andrographis paniculata* L, *Vitex trifolia* L, *Sesbania grandiflora* L, *Justicia adhatada* L, *Clitoria ternatea* L. The endophytic fungi were islated from these plants namely *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Alternaria tenuis*, *Rhizopus*, *Penicillum*, *Helminthosporum*, *Helminthosporum oryzea*, *Fusarium*. Colonization frequency was high nearly 9.23% for *Aspergillus niger*. Relative percentage was high in *Curvularia vermiformi* 23.52%. Due to dominant colonization frequency of *A.niger* was subjected to Antagonistic activity of the bacterial species namely *Staphylococcus aureus* (14±0.3) followed *Bacillus subtilis* (11±0.3) but silver nanoparticles synthesis level was very low in *Aspergillus niger*. Extracellular enzymes were also extracted from *Aspergillus niger* namely phosphatase, cellulase amylase, protease, pectinase, lipase and laccase. (80%.60%.70%.63.33%,30%,23.33% and 10%).

Keywords: medicinal plants, endophytic fungi, colonization frequency, antagonistic activity, *Aspergillus niger*

Introduction

India is commonly called the botanical garden of the world, owing to the wealth of herbal medicines. India with its great topographic and climate diversity has a very and diverse flora and funna. The uses of plants as medicines have been practiced from ancient time. Tamil Nadu is ethanobotanically 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 Thanjavur (1hour) and 40km west of Kumbakonam. The region is covered with mainly alluvial or black soil which is conductive for rice cultivation.

An endophyte is an endosymbiont, often a fungus, which lives within a plant for at least part of its life without causing any apparent disease. They form inconspicuous infections within tissues of healthy plants for all or at least a part of their life cycle (Clay 2002) [4]. 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.

Endophytes were first described by the German botanist Johann Friedrich Link in 1809. They were later termed as "microzymas" by the French scientist Bechamp. There was a belief that plants were under sterile conditions and it was not until 1887 that Victor Galippe discovered bacteria normally occouring inside plant tissue (Hardim *et al*., 2015) [9]. Endophytes are microorganisms that are presents in living tissues of various lands (root, fruits, stem, seed, leaf, etc.,). These endophytes protect their hosts from infection 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 scientist as mutualisms that colonize aerial parts of living plant tissues and do not cause symptoms of disease. All vascular plants harbor endophytic organism. These endophytes protect their hosts from infection agents and adverse condition by secreting bioactive secondary metabolites (Azevedo *et al*., 2003) [3].

Materials and Methods Collection of Medicinal plants

15 Medicinal samples were collected from STET Herbal Garden, Mannargudi, Thiruvarur (DT), Tamil Nadu. Health and mature leaf samples were segregated and brought to the Microbiology Laboratory, PG & Research department of Microbiology with almost care and kept in room temperature for further reference. Plant materials were preserved as Herbarium.

Isolation and Identification of Endophytic Fungi (Deepthi *et al* 2018) [6]

The collected plant samples (leaves) were immediately brought to the laboratory and use within 8 hours for

isolation of fungal endophytes. Sterile paper bags and stored at 4°C till further use. The voucher specimens were collected and kept in the herbarium of S.T.E.T Women's College, Mannargudi. These plants 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.

Procedure

Placed a drop of LPCB on a clean glass slide. Removed the small portion of colony with a straight wire and placed it in a drop of LPCB. Placed the cover glass and apply gentle pressure. Allowed the preparation of staining for 15 minutes. The excess strain was removed using tissue paper and the cover glass was sealed suing white nail polish. Examined the preparation of microscopically and observe mycelium and conidia.

The classification of the fungi is adopted from Global Biodiversity Information Facility (GBIF).The colonization frequency (CF) percentage of endophytic fungi and

$$\text{Colonization frequency (CF)} = \frac{\text{No of. Species Isolate}}{\text{No of. Segments Screened}} \times 100$$

Relative Percentage Occurrence (RPO), of different groups of fungi

$$\text{RPO} = \frac{\text{Density of Colonization of one Group}}{\text{Total Density of Colonization}} \times 100$$

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 discribed by (Safowora, 1993). Steroid test, 2ml of acetic anydried 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, Trepenoids test, 5ml of each plant leaves extract was mixed in 2ml 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, Glycosides, 5ml of each plant leaves extract was treated with 2ml of Galacial acetic acid containing one drop of Ferric chloride solution. This was under layed with 2ml of concentrated sulphuric acid. A brown ring of the interface was formed indicating the presence of cardiac glycosides Flavonoids test, 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by the addition of concentrated sulfuric acid a yellow colouration was observed indicating the presence of Flavonoides. Saponins test, About 2g of powdered sample was boiled in 20ml of distilled water bath and filtered. 10 ml of the filtered sample is mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The forthing is then mixed with 3 drops of olive oil formation of emulsion indicating the presence of saponins (Kamarian 2013) [10].

Antagonistic Activity

Different concentrations (50and 100) of both culture filtrate extract (CFE) and culture extract (CME) were assayed against bacteria such as *Bacillus subtils, Escheriachia coli, Pseudomonas aeruginosa, Entercoccus faecalis and Staphylococcus aureus.* The selected pathogens were procured from PG and Research Department of Microbiology STET Women's College, Autonomous, Mannargudi. Based on the frequency isolation of *Aspergillus niger* from all selected medicinal plants, Hence this Endophytic fungi was subjected to antibacterial activity. Assay was carried out using well diffusion method using standard procedure. Muller Hinton, Media was used. Gentamycin were used as positive control. The plates were incubated at 37°C. Zone of inhibition around the well was observed after 24h. Triplicates were maintained for all the samples.

Extractcellular Enzyme Assay

The ability of the endophytic fungi to produce extracellular enzymes: amylase, lipase, pectinase, cellulase, protease, laccase and phosphatase, were screened using different solid media 2250. The production of extracellular enzymes was assessed by growing each of the endophytic fungi on PDA for 1 week and 5 mm of these mycelia plugs were placed on the solid media having different substrates for respective enzymes. After incubation for 5 to 7 days at room temperature, the diameter of hydrolysis and fungal colony were measured; enzyme index was calculated (Florencio *et al.*, 2012) [7].

Chitosan Extraction and Purification

Chitosan is a polysaccharide which is found to be present in the cell walls of most species of fungi. Extraction of chitosan from the endophytes were carried out using standard procedure. The chitosan produced from the isolates were characterized in Potassium Bromide (KBr) pellet by using an infrared spectrophotometer in the range of 400 to 4000 cm^{-1}.

Silver Nanoparticles from Endophytes (Ahmad and, Mukherjee, 2003) [1] For the synthesis of silver nanoparticles, the fungi isolates were grown in 250 ml flask containing 100ml potato dextrose broth (PDB) at room temperature for 72 hour and then the biomass was harvested and filtered through Whattman filter paper. The fungal mat was washed with distilled water for 48h. After 48hr of incubation, the cell filtrate was separated by filtration. The fungal cell filtrate was then collected, and it was challenged with the $AgNo_3$ salt (final conc. 1mM). After 24 hrs of incubation, the formation of silver nanoparticles were screened by visual observation of colour that changes from pale white to brown. Then it was further confirmed by subjecting the reaction mixture to UV-Visible spectrophotometer analysis. The spectrum was scanned at the resolution of 1nm, between 200-800 nm for each sample.

Statistical Analysis (Gupta, 2004)

The infection index was calculated from the relation between the number of fragments from which the endophytic fungi emerged and the total number of fragments used in the experiment. All the experiments were repeated as triplicates. The results obtained in the present study was subjected to statistical analysis such as Mean (X). Using the following formula,

Mean (X) = Σx/N

Where,
Mean (X) - Sum of all Values of the variable
N - Number of observation

Results and Discussion

The present study was planned to isolate the endophytic flora of the medicinal plants in around STET herbal Garden, Mannargudi, Thiruvarur (Dt). A total of 15 medicinal plants were screened for the presence of endophytic fungi.

The fresh leaves of *Azadirachta indica* L, *Mentha spicata* L, *Ocimum tenuiflonum* L, *Acalypha indica* L, *Solanum virginianum* L, *Chrysopogon ziznioides* L, *Trachyspermum ammi* L, *Phyllanthus niruri* L, *Centella asiatica* L, *Trigonella fornumgraecum* L, *Andrographis paniculata* L, *Vitex trifolia* L, *Sesbania grandiflora* L, *Justicia adhatada* L, *Clitoria ternatea* L. Medicinal plants were collected from STET Herbal Garden, STET Womens college, Mannargudi, Thiruvarur (Dt). Endphytic fungi were isolated from these plants. The medicinal plants were preserved as herbarium in terms for future reference.

Isolation and Identification of Endophytic Fungi

Totally 15 medicinal plants were selected isolation, endophytic fungi, collected from STET Herbal Garden, STET Women's college, Mannargudi, Thiruvarur (Dt). The isolated and identified endophytic fungi such as *Rhizopus*, *Aspergillus niger*, *Aspergillus oryzea*, *Aspergillus flavus*, *Aspergillus terrues*, *Apergillus fumigatus*, *Alternaria tenuis*, *Helminthosporum*, *Helminthosporium oryzae*, *Fusarium*, *Penicillum citrinum*

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 *Aspergillus fumigatus* cause disease and smooth conidial surface, *Aspergilus flavus* size range from 400-800 pale brown surface and smooth conidial surface, *Aspergillus niger* range from 400-3000 slightly brown and irregular conical surface, *Aspergillus fumicatus* 200-400 size grayish smooth walled surface smooth and slightly rough conidial surface, *Aspergillus fumicatus* mycelium produce pigment are called fumigatin, *Aspergillus terreus* 100-250 size and uncoloured smooth walled conidial surface.

Penicillum is commonly known as green or blue mould. Usually grow as a saprophytes on decaying fruits and vegetables. *Penicillum citrinum* rapid growth dark green and pale yellow sepatate hyphae.

Helminthosporum size range 430-580 brown coloured straights conidia and *H.oryzae*, size range from 430-580, brown coloured straight conidia are straight flexuousconidia. *Helminthosporum mycelium* and spores of some mould possess he 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. Alternaria occurs universally and grow mostly as saprophytes on plant debris and drying parts of plants.The mycelium in 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 phytomycetes. 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 namels *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 13 types of medicinal plants.

The *Aspergillus flavus* are present 8 medicinal plants namely, *Cardiospermum halicababum*, *Acalypha indica*, *Phyllanthus nuiri*, *Vitex negunda*, *Murraya koenigiri*, *Coriandum sativum*, *Ctharanthus roseus*, *Boerhavia diffusa*.

The *Acalypha indica*, *Solanum nigrum*, *Boerhavia diffusa*, *Centella asiatica*, *Clitoria ternatea* are having in *Aspergillus fumigates.*

Ocimum teiurflorum, *Cardiosperum halicababum*, *Eclipta prostrate*, *Centella Asiatica*, *Solanum trilobatum*, *Justicia adhatoda* are having *A. rugulosus Alternaria tenuis* are present in *Eclipta prostrate*, *Andrographis paniculata*, *Centella asiatica*, *Solanum trilobatum*, *Mentha spicata*, *Vitex negunda*.

Rhizopus are present in only *Boerhavia diffusa*, *Vitex negunda*, *Cantharanthus roseus.*

Penicillum citrinum are present in *ocimum tewurflorum*, *Cardiospermum halicababum*, *Eclipta prostrate*, *Solnanum nigrum*, *Centella asiatica*, *Lawsonia inernis*, *Phyllanthus nuiri*, *Justicia adhatoda*, *Murraya koenigii* and *Coriandum sativa.*

The *Helminthosporum* are present in only 2 plants namely, *Boerhavia diffusa* and *Coriandum sativum.*

Finally the *Fusarium* was present in *phyllanthus nuiri*, *Mentha asiatica*, *Justicia adhatoda*, *Vitex negunda* and *Coriandum sativum* (Table-1).

Table 1: Details of Isolated Endophytic Fungi from Selected Medicinal Plants

S.No	Host Plants	Tamil Name	Collection of plants Materials	Fungi
1.	*Azadirachta indica*	Veppilai	Leaves	*Aspergillus oryzea, Aspergillus niger, Penicillum citrinnum*
2.	*Mentha spicata*	Pudhina	Leaves	*Aspergillus niger, Aspergillus flavus, Penicillum citrinum.*
3.	*Ocimum tenuiflonum*	Tulsi	Leaves	*Aspergillu oryzae, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus.*
4.	*Acalypha indica*	Kuppaimeni	Leaves	*Aspergillus oryzae, Aspergillus niger*
5.	*Solanum virginianum*	Kantakari	Leaves	*Aspergillus oryzae, Aspergillus niger, Aspergillusfumigatus, Penicillum citrinum*
6.	*Chrysopogon zizaniodes*	Vetiver	Leaves	*Aspergillus oryzae, Aspergillus niger, Aspergillusfumigatus, Penicillum citrinum.*
7.	*Trachyspermum*	*Omavali*	Leaves	*Aspergillus oryzae, Alternaria tenuis, Helminthosporum oryzae*

	ammi			
8.	*Phyllanthus niruri*	*Keelanelli*	Leaves	*Aspergillus oryzae, Aspergillus niger, Aspergillus flavus, Penicillum Fusarium. citrinum,*
9.	*Centella asiatica*	*Vallarai*	Leaves	*Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus, Alternaria tenuis, Penicillum citrinum.*
10.	*Trigonella graecum foenum-*	*VendhaiyaKee Rai*	Leaves	*Aspergillus Aspergillus flavus niger,*
11.	*Andrographis paniculata*	*Siriyanangi*	Leaves	*Aspergillus niger, Alternaria tenuis, Helminthosporum oryzae.*
12.	*Vitex negunda*	*Nochi*	Leaves	*Aspergillus Aspergillus flavus. niger,*
13.	*Sesbania grandiflora*	*Agathi*	Leaves	*Alternaria tenuis, Helminthosporum oryzae.*
14.	*Justicia adhatoda*	*Adathoda*	Leaves	*Aspergillus Aspergillus Penicillum Fusarium. oryzae, niger, citrinum,*
15.	*Clitoria ternate*	*Sangupoo*	Leaves	*Aspergillus fumigatus.*

Phytochemical Constitution in Medicinal Plants

In phytochemical screening steroid are commonly present in the plants in the plants namely *Mentha spicata, Ocimum tenuiflonum, Solanum virginianum, Andrographis paniculata, Justicia adhatada, Clitoria ternatea.*

Trepenoids was present in the *Azadirachta indica, Mentha spicata, Ocimum tenuiflonum, Solanum virginianum, phyllanthus niruri, Centella asiatica, Trigonella fornumgraecum, Andrographis paniculata, Clitoria ternatea.*

Glycosides present in *the Chrysopogon zizaniodes, Trigonella foenum-graecum Acalypha indica, Andrographis paniculata, Vitex trifolia, Clitoria ternatea, Phyllanthus niruri, Justicia adhatoda.*

Flavonoides are also present in *Chrysopogon zizaniodes, Acalypha indica, Andrographis paniculata, Clitoria ternatea, Phyllanthus niruri, Justicia adhatoda.*

Sponins present in the *Chrysopogon zizaniodes, Acalypha indica, Andrographis paniculata, Clitoria ternatea, Phyllanthus niruri, Justicia adhatoda,* (Table-2).

Table 2: Details of Phytochemical Screening of medicinal plants

Plants	Steriods	Terponoids	Glycosides	Flavonoids	Saponins
Azadirachta indica L	-	-	-	-	-
Mentha spicata L	+	+	-	-	-
Ocimum tenuiflonum L	+	+	-	-	-
Acalypha indica L	-	-	+	+	+
Solanum virginianum L	+	+	-	-	-
Chrysopogon zizniodes L	-	-	+	+	+
Trachyspermum ammi L	-	-	-	-	-
Phyllanthus niruri L	-	+	+	+	+
Centella asiaticia L	-	+	-	-	-
Trigonella fornumgraecum L	-	-	+	-	-
Andrographis paniculata L	+	+	+	+	+
Vitex trifolia L	-	-	+	-	-
Sesbania grandiflora L	-	-	-	-	-
Justicia adhatada L	+	+	+	+	+
Clitoria ternatea L	+	+	+	+	+

Colonization Frequency- Moreover it was observed that the colonization frequency (CF) of endophytic fungi was notice *Aspergillus flavus* (12.04%), *Aspergillus fumigatus* (9.23%), *Aspergillus niger* (12.08%), *Aspergillus terreus* (4.47%), *Alternaria tenuis* (7.04%), *Rhizopus* (14.00%), *Penicillum* (19.04%), *Helminthosporum* (34.07%), *Helminthosporum oryzea* (23.09%), Fusarium (21.00%),(Figure-1)

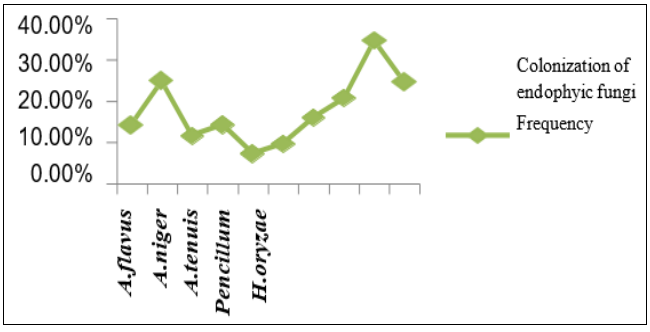

Fig 1: Colonization Frequency of Endophytic fungi

Endophytic fungi

RPO- Moreover it was observed that the RPO of endophytic fungi was noticed *Aspergillus niger* (17.64 %), *Alternaria alternate* (11.76%), *Curvularia vermiformis* (23.52%), *Cladosporium* (05.88%), *Penicillum citrinum* (11.76%), *Rhizopus*(17.64%), (Figure-2)

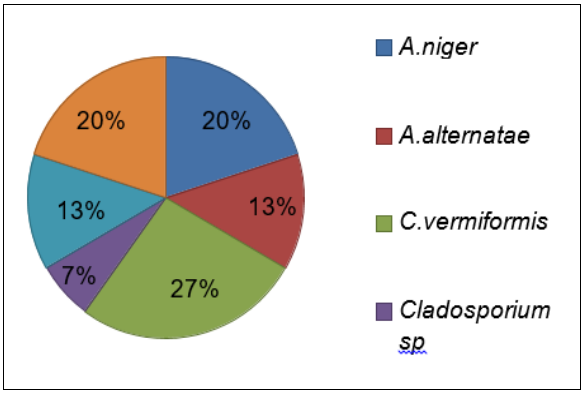

Fig 2: RPO of Endophytic Fungi Isolated from *Azardirachta indica*

Antagonistic Activity

The results of the inhibitory action of various concentrations (50 µl, 100µl) of both Culture Filtrate Extract (CFE) and Culture Mycelial Extract (CME) of both endophytes were tested against bacteria (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus*) at *Bacillus subtilis* (11±0.3), *Escherichia coli* (8±0.2), *Pseudomonas aeruginosa* (16±0.4), *Enterococcus faecalis*(7±0.2) and *Staphylococcus aureus* (14±0.3) were inhibited by the extract of medicinal plants, (Figure-3).

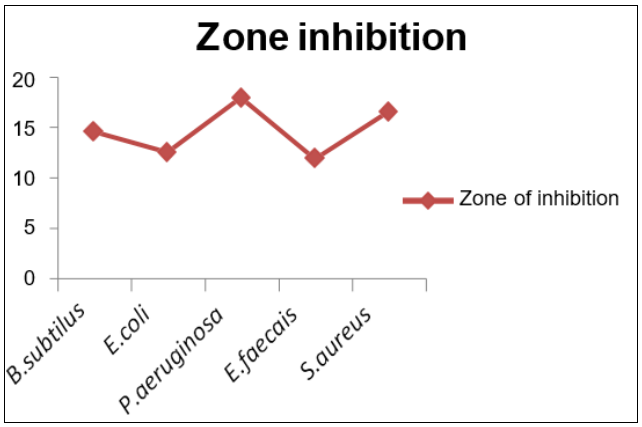

Fig 3: Antagonistic activity of fungi

Synthesis of Silver Nanoparticles by Fungal Endophytes

The rapid colour change of dark brown in both fungal cell filtrates (after 24 hrs) with the addition of silver nitrate solution was observed from our results. The appearance of the 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 *Penicillum citrinum* showed at 552nm and *Aspergillus oryzae* showed at 439nm. The *A.flavus* at 345nm and *A.fumigatus* at 458nm and *Helminthosporum* at 452nm,(Table-3).

Table 3: Details of Synthesis of Silver Nanoparticles by Fungal Endophytes

Name of the fungal isolates	Values of Silver Nanoparticles (nm)
Penicillum citrinum	552
Aspergillus oryzae	439
Aspergillus flavus	345
Aspergillus fumigates	458
Helminthosporum	452

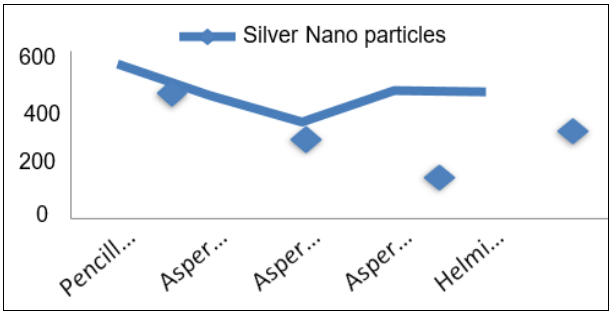

Fig 4: Synthesis of silver Nano Particles

Endophytic fungi

Medicinal plants namely *Acalypha indica*, *Mentha spicata*, *Justicia adhatoda*, *and Solanum virginianum*, *Clitoria ternatea* showed frequency colonization of *Aspergilus niger*. Colonization frequency was high in *Helminthosporum oryzae*. RPO % was high in *Curvularia vermiformis*. Altogether 11 species of endophytic fungi were isolated from the selected medicinal plants from the Herbal Garden STET Women's College, Autonomous, Mannargudi. *Aspergillus niger* was effectively controlled the pathogen. Amylase, Pectinase, cellulase was extracted from the dominant colonization of *A.niger* Our study revealed that preliminary phytochemical screening of medicinal plants, Steroids, Terpenoids, Glycosides, Flavonoids, Sponins. Screening of phytochemical and isolation of endophytic fungi from medicinal plants.

Our study reported that colonizing frequency of endophytic fungi in 15 types of medicinal plants. *Aspergillus flavus* (12.04%), *Aspergillus focus* (23.49%), *Aspergillus fumigates* (9.23%), *Aspergillus niger* (12.08%), *Aspergillus terreus* (4.47%), *Alternaria tenuis* (7.04%), *Rhizopus* (14.00%), *Penicillum* (19.04%), *Helminthosporum* (34.07%), *Helminthosporum oryzae* (23.09%), Fusarium (21.00%).

According to Akanksha *et al*., (2015) phytochemical analysis of fungal extract revealed presence of flavonoids, alkaloids, phenols, saponins, steroids, tennins, terpenoids, and also observed excellent antibacterial activity was obtained bacteria namely, *Bacillus subtilis*, *E.coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*. Our study reports agreed to Srinivas *et al* 2017.Diversity and extracellular enzyme of endophytic fungi associated with *Cymbidium aloifolium L*. The endophytic fungi was to subjected for production of extracellular enzymes namely phosphatase, cellulose amylase, protease, pectinase, lipase and laccase.(80%.60%.70%.63.33%,30%,23.33% and 10%). Our reports agreed to Srinivas 2017, Diversity and extracellular enzymes of endophytic fungi associated with *Cymbidium aloifolium L*

Conclusion

Microbial endophytes reside in plant tissues were stimulate phytochemicals properties of plants with wide variety of clinical application. Hence our research findings could serve as a baseline and producing novel therapeutic compounds from endophytic fungi. The future work is to be pertaining to isolation endophytic fungi from other plants parts, carried out for broad spectrum medicinal plants is to be selected for the study because they come in abundant source, easily available and some of them are already being utilized in traditional medicinal. Further investigation will focus on the stain improvement and genomic variation among these isolates.

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