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Isolation and identification of endophytic fungi and its potential as a pharmacological activities

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Abstract

The present investigation deals with the isolation and identification of endophytic fungi from medicinal plants such as *Carica papaya* and *Melia dubia* leaves, the biosynthesis of silver nanoparticles and the pharmacological activities of potential fungi. The global issue of drug-resistant microbes and their impact on public health is a significant concern, necessitating the urgent development of new and more potent antimicrobial treatments. The endophytic fungi like *Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Curvularia* sp., *C. lunata, Fusarium* sp., *F.oxysporum, Helminthosporium sativum, Nigrospora sphaeria, Pencillium janthinellum, Trichoderma* sp. and *T. viride* were recorded from *C. papaya* and *M. dubia* leaves. The maximum number of colonies seen in *A. niger, A. flavus* and *T. viride* was recorded from medicinal plant leaves. The potential endophytic fungi such as *A. niger, A. flavus* and *T. viride* were analyzed for bioactive compounds like alkaloids, aminoacids, carbohydrates, coumarins, flavonoids, phenols, proteins, quinones, saponins, steroids, tannins, terpenoids and triterpenoids. The antioxidant and anti-inflammatory activity of secondary metabolites of different concentrations of endophytic fungi were analyzed. The agar well diffusion method to analyze the UV-Vis spectra of silver nanoparticles and the zone of inhibition in bacterial and fungal organisms from potential endophytic fungi. These findings suggested that different parts of papaya fruits harbour an array of fungal endophytes that could be important agents in attributing the high nutritive status to the fruit and can serve as potent microbial cocktails for developing value-added fermented products of this important fruit.

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Introduction

Endophytic fungi inhabit plant tissue across all plant species, typically residing within living plant tissue. These bacteria exist within the spaces between plant cells and engage in a symbiotic mutualistic relationship with their host plants. This mutualistic endophytic fungi to produce secondary metabolites that closely resemble those of their host plants (Kumala and Poppy, 2006). Based on papaya leaves are rich in secondary metabolites like alkaloids, flavonoids, tannins, saponins and steroids. These compounds have antioxidant properties, the secondary metabolites can be sourced not only from leaf extracts but also from endophytic fungi that live in symbiosis with papaya leaves. It is common for plants with bioactive compounds to host endophytic fungi that possess similar capabilities to their hosts in metabolite production (Strobel and Daisy, 2003). In recent years, there has been a growing focus on enhancing food sources and crops as a result of the rapid population growth (Khalil *et al.*, 2021). This has led to an increased demand for natural products for various purposes including food, medicine, energy and biotechnology (El-Esawi *et al.*, 2020). The excessive use of chemical fertilizers possess several environmental risks such as soil pollution and decreased microbial diversity in soil, compromised food safety and the leaching of minerals into groundwater (Rafi *et al.*, 2019).

In recent times, there is an urgent demand for natural agricultural approaches to supplant the extensive use of chemical fertilizers in order to improve crop productivity and decrease environmental pollution stemming from chemical compounds (Soliman *et al.*, 2020). Endophytes are non-harmful microorganisms residing in plant tissues, establishing a symbiotic relationship with the host plants. This mutualistic association results in the production of bioactive compounds by the endophytes which in turn aid the host plant in enhancing its nutritional status, resistance to pests and diseases and tolerance to physical stress (Mbilu *et al.*, 2018). Numerous individuals residing in rural regions and tribal communities continue to depend on conventional remedies made from non-toxic medicinal plants, despite the advancement of contemporary pharmaceuticals for managing illnesses. Currently, nanotechnology has various applications and herbal treatments facilitated by nanoparticles are gaining increased popularity (Chandra *et al.*, 2022). Treating bacterial and fungal infections with modern medications has become increasingly difficult because of the development of drug resistance in these pathogens against traditional antibiotics (Chandra *et al.*, 2024). Various approaches have been investigated to enhance the quality and quantity of bioactive secondary metabolites linked to medicinal plants (Tsipinana *et al.*, 2023). In the present study, the isolation and characterization of endophytic fungi from medicinal plants like *Carica papaya* and *Melia dubia* leaves, the synthesis of silver nanoparticles, and the evaluation of the pharmacological properties of these fungi.

Materials and methods

Plant material collection

Plant material was collected from the Srinivasapuram, Thanjavur district. A purposive sampling method was utilized to select two zones with healthy.

Carica papaya and *Melia dubia* trees, with the assistance of local guides. The collected plant leaves materials was placed in properly labeled sterile plastic bags and transported to the laboratory in an icebox where it was stored in a refrigerator at 4°C until processing. The processing of the plant material took place 48 hours after collection.

Isolation of endophytic fungi

The endophytic fungi were isolated following the methods described (Petrini and Fisher, 1986) with minor adjustments. The plant material underwent thorough rinsing with running tap water to eliminate dust, soil particles and debris (Sardul *et al.*, 2014). Surface sterilization was carried out by immersing the plant material in 75% ethanol for 1 minute followed by 12% sodium hypochlorite for 1

minute and then rinsed twice in sterile distilled water. After drying on a sterile filter paper, the plant material was cut into pieces of 3 - 3.5cm with a sterile scalpel. Four pieces of each part were placed on tap water agar plate using a sterile forceps and incubated for 6 days at 26 - 270˚C (Gond *et al.*, 2007). Colony purification was done by further sub culturing the fungal colonies in PDA until pure isolates were acquired.

Identification of endophytic fungi

The fungi were identified through the utilization of various standard manuals including the Manual of Soil Fungi (Gillman, 1957), A Manual of Penicillia (Raper and Thom, 1949), Dematiaceous Hyphomycetes (Ellis, 1971), More Dematiaceous Hyphomycetes (Ellis and Ellis, 1976), Higher Fungi (Kohlmeyer and Kohlmeyer, 1979), Soil Fungi (Domsch *et al.*, 1980). Additionally, the Coelomycetes Fungi Imperfect *Pycnidia acervuli* and Stromata by Brain C. Sutton (1980) from CABI Publishing were consulted. The *Fusarium* Laboratory Manual (John F. Leslie and Brett A. Summerell, 2006) was also referenced.

Bioactive compounds of endophytic fungi

Qualitative bioactive compounds analysis

The potential endophytic fungi such as *Aspergillus niger, A. flavus* and *Trichoderma viride* were tested for qualitative bioactive compounds using standard methods (Harborne, 1973).

Quantitative bioactive compounds analysis

Preliminary bioactive compounds like alkaloids (Boham and Kocipai, 1994), amino acids (Boham and Kocipai, 1994), carbohydrates (Harborne, 1973), coumarins (Boham and Kocipai, 1994), flavonoids (Boham and Kocipai, 1994), phenols (Boham and Kocipai, 1994), protein (Van-Burden and Robinson, 1981), quinones (Boham and Kocipai, 1994), saponins (Van-Burden and Robinson, 1981), steroids (Boham and Kocipai, 1994), tannins (Boham and Kocipai, 1994), terpenoids (Boham and Kocipai, 1994) and triterpenoids (Boham and Kocipai, 1994) were analysed by using standard methods.

In vitro Anti-inflammatory activity (Chandra *et al.*, 2012)

The 5 mL reaction mixture was composed of 0.2 mL of egg albumin (obtained from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of different concentrations of extracts (100, 200, 300, 400 and 500µg/mL respectively). A similar volume of double-distilled water was utilized as a control. Subsequently, the mixtures underwent incubation at (37± 2°C) in an incubator for 15 minutes followed by heating at 70°C for 5 minutes. Upon cooling, the absorbance was determined at 660 nm using the blank. Diclofenac sodium at final concentrations (100-500µg/ mL) was employed as a reference drug and subjected to the same treatment. The percentage inhibition of protein denaturation was then calculated using the formula.

% inhibition = 100 x (Vt / Vc – I)

Where, Vt = absorbance of the test sample, Vc = absorbance of control.

The extracts concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

Antioxidant activity: Hydrogen peroxide (H_2O_2) Radical scavenging activity assay (Singh and Singh, 2008)

According to the Indian Pharmacopoeia 1996 standards, a solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide was prepared. In a 200 ml volumetric flask, 50 ml of potassium dihydrogen phosphate solution was combined with 39.1 ml of 0.2 M sodium hydroxide solution. The volume was then adjusted to 200 ml with distilled water to create a phosphate buffer with a pH of 7.4. Subsequently, 50 ml of the phosphate buffer solution was mixed with an equal amount of hydrogen peroxide to generate free radicals. The resulting solution was left at room temperature for 5 minutes and allowed the reaction. One ml of extracts in distilled water was added with 0.6 ml of hydrogen peroxide solution. The absorbance of the solution was

measured at 230 nm using a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H_2O_2 by the extract was then determined. Ascorbic acid of 0.1 mg/ml was utilized as a standard and test solutions were prepared at the same concentrations.

The ability of the extract to scavenge the H_2O_2 radical was calculated using a specific equation.

H_2O_2 scavenging activity (%) = (A0 – AI) / A0 × 100

Where A0 is the absorbance of the control and A1 is the absorbance in the presence of an extract given sample. A standard of ascorbic acid was run using the same concentrations as that of extract. The antioxidant activity of the sample was expressed as a concentration (mg/ml) of the sample that inhibited the formation of H_2O_2 radicals by 50%.

Reducing power assay

The extract reducing power was assessed following the protocol outlined (Raper and Thom, 1949). A 1.0 mL solution containing varying concentrations (100, 200, 300, 400 and 500 µg/mL) of the extract was combined with 5.0 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 5.0 mL of potassium ferricyanide (1.0%). This mixture was then incubated at 50°C for 20 minutes. Subsequently, 5.0 mL of 10% trichloroacetic acid was introduced followed by centrifugation at 980g for 10 minutes at 5°C using a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and the absorbance was measured at 700 nm after the addition of ferric chloride.

DPPH assay (Sardul et al., 2014)

The antioxidant activity of the potential endophytic fungi based on the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined. The extraction solvents were prepared in 100, 200, 300, 400 and 500µg/mL. Five ml of each solution was prepared and the concentration was mixed with 0.5 mL of 1 ml DPPH solution. The test tubes were incubated for 30 min at room temperature and the absorbance was measured at 517nm. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Vitamin C (0.1 mg/ml) was used as a standard and the same concentrations were prepared as the test solutions. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical.

% Scavenged [DPPH] = [(AC – AS)/AC] × 100

Thiobarbituric acid (TBA) assay (Singh and Singh, 2008)

The TBA reagent was prepared by dissolving 57.66 mg of TBA in 100 mL of glacial acetic acid to create a standard solution of 4.0 mm TBA. Samples of fungal species were prepared using 100% glacial acetic acid (AA) and 50% glacial acetic acid with water (AW). The leaf sample extract (1 mL) was mixed with 1 mL TBA reagent and this process was repeated five times (n = 5). A standard solution of Ferric Thiocyanate (0.1 mg/ml) was used, and test solutions were prepared at the same concentrations. The TBARS was calculated using the formula as µM/g of the sample.

TBARS (µµM/g) = (Ac x VV) /WW

Where Ac is the amount determined from the calibration curve and WW is the weight of the sample taken and VV is the volume in mL or dilution factor of the respective fungal cultures.

Antimicrobial activity (Singh et al., 2017)

The four bacterial and fungal strains were utilized and assessed antimicrobial activity. These strains were sourced from the Indian Biotrack Research Institute in Thanjavur.

The agar well-diffusion method was employed to determine antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed with 24-hour culture and 48-hour old-broth culture of the respective bacteria and fungi using sterile cotton swabs. Agar wells with a diameter of 5mm were created in each plate using a sterile cork borer. Aqueous and benzene extracts at concentrations of 25, 50, 75 and 100µg/ml were added to the wells

using sterilized dropping pipettes. The plates were then left for 1 hour to allowed for pre-incubation diffusion and minimize the impact of time variation between the application of different solutions. Subsequently, the plates were incubated upright at 37˚C ± 2˚C for 24 hours for bacteria and at 28˚C ± 2˚C for fungi. The results were recorded based on the presence or absence of an inhibition zone. Triplicates were maintained and the average values were calculated for antimicrobial activity.

Synthesized silver nanoparticles (Ag-NPs)

Ten ml of mushroom extract was added to 150 ml in a conical flask that containing 90 ml of a solution of 1 mM silver nitrate. The mixture was again incubated at 60˚C in the dark while being stirred at different intervals. Over the period of 24 hours, the resulting reduction in silver ions (Ag+) was periodically monitored. The reaction mixture's colour changed from light yellow to pale yellow and finally to dark brown after 4 hours of incubation and indicated the formation of Ag-NPs (Singh *et al*., 2017).

Statistical analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

Results and discussion

A total of sixty (60) fungal isolates were isolated from *Warburgia ugandensis* collected from Mount Kenya forest in the year 2017. Twenty (20) isolates were isolated from the leaves, seventeen (17) from stem, fourteen (14) from bark and nine (9) from the root. All the isolates were characterized using phenotypic and microscopic methods. They were preliminary placed in fourteen (14) different genera namely *Nigrospora, Aspergillus, Cladosporium, Fusarium, Phomopsis, Colletotrichum, Alternaria, Cochliobolus, Bionectria, Phyllosticta, Guignardia, Tricharina, Diaporthe* and *Trichoderma*. *Alternaria* sp. was isolated from both the bark and the leaf, *Nigrospora oryzae* and *Bionectria ochroleuca* from leaf, bark and stem, *Aspergillus flavus* from leaf and stem, *Colletotrichum acutatum* from leaf and root, *Fusarium oxysporum* and *Cladosporium* sp., from leaf, root and stem, *Phomopsis* sp. from all the four parts (Mbilu *et al*., 2018). In the present study, totally 13 endophytic fungi were isolated from the leaf segments of *Carica papaya* and *Melia dubia*. Endophytes were mostly recovered from *Carica papaya* (10) followed by *Melia dubia* (12) (Table 1). Out of 13 species, 7 different genera such as *Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Curvularia* sp., *C. lunata, Fusarium* sp., *F. oxysporum, Helminthosporium sativum, Nigrospora sphaeria, Pencillium janthinellam, Trichoderma* sp. and *T. viride* were isolated. The potential endophytic fungi of *A. niger, A. flavus* and *T. viride* were maximum recorded in both of the medicinal plants (Table 1).

Table 1. Isolation and identification of endophytic fungi of *Carica papaya* and *Melia dubia* leaves

Name of the endophytic fungi	No of colonies	
	Carica papaya	*Melia dubia*
Aspergillus niger	5	6
A. flavus	5	5
A. terreus	2	3
A. fumigatus	2	3
Curvularia sp.	3	5
C. lunata	2	4
Fusarium sp.	4	2
F. oxysporum	4	3
Helminthosporium sativum	4	2
Nigrospora sphaeria	2	2
P. janthinellum	2	3
Trichoderma sp.	3	1
T. viride	10	8
Total No. of colonies	48	47
Total No. of species	13	

The methanol mat extract and ethyl acetate extract of endophytic microfungal species of *Cladosporium* and noticed various chemical constituents such as phenolic compounds, saponins, flavonoids, tannins and terpenoids. We observed various bioactive compounds such as flavonoid, saponin, tannin, carbohydrate, phenol, terpenoid, steroids, glycoside, alkaloids and protein in fungal extracts of both the ethyl acetate extract and methanol mat extract. The ethyl acetate and methanol extracts of *C. cladosporioides* contain a high concentration of flavonoid, phenol and protein, whereas a higher level of tannin, carbohydrate, terpenoids, steroids,

glycoside and alkaloids were found to be observed in the methanol mat extracts.

Table 2. Qualitative bioactive compounds analysis of endophytic fungal metabolites from potential endophytic fungi

Bioactive compounds	Different fungi		
	Aspergillus niger	*A. flavus*	*Trichoderma viride*
Alkaloids	+	+	+
Amino acids	+	+	+
Carbohydrate	++	++	++
Coumarins	+	+	+
Flavonoids	-	+	+
Phenol	+	+	+
Protein	++	++	++
Quinone	+	+	+
Saponins	-	-	-
Steroid	+	+	+
Tannins	++	++	++
Terpenoids	+	+	+
Triterpenoids	++	++	++

(++) – Strongly present, (+) – Present, (-) – Absent

Higher level of flavonoid, phenol, terpenoid, and steroids were observed in the ethyl acetate extracts of *Bipolaris australiensis*, while the methanol mat extracts contained the maximal level of tannin, phenol, glycoside, carbohydrate and protein (Soliman *et al.*, 2020). In present investigation, the bioactive compounds in potential endophytic fungi such as *A. niger, A. flavus* and *T. viride* are used. Qualitative bioactive compounds including alkaloids, aminoacids, carbohydrates, coumarins, flavonoids, phenol, protein, quinone, saponins, steroids, tannins, terpenoids and triterpenoids are tested in both potential endophytic fungi (Table 2). Carbohydrate, protein, tannins, triterpenoids were highly presented and alkaloids, aminoacids, coumarins, phenol, quinones, steroids, terpenoids were commonly present in both potential endophytic fungi. Flavonoids were absent in *A. niger* and present in *A. flavus* and *T. viride*. Quantitative analysis of bioactive compounds indicated maximum availability of alkaloids, protein, steroids and tannins in *A. niger* and *A. flavus* respectively. On the other hand, *T. viride* showed higher quantities of carbohydrates and tannins were recorded. The maximum numbers of bioactive compounds were presented at the *A. niger*, respectively (Table 3).

Table 3. Quantitative bioactive compounds analysis of endophytic fungal metabolites from different fungi

Bioactive compounds	Quantity (µg/g)		
	Aspergillus niger	*A. flavus*	*Trichoderma viride*
Alkaloids	15.02±0.00	14.62±0.01	12.08±0.06
Amino acids	12.00±0.09	11.35±0.03	11.85±0.07
Carbohydrate	18.59±0.24	15.23±0.09	17.66±0.13
Coumarins	09.07±0.00	08.12±0.00	08.17±0.04
Flavonoids	-	10.80±0.17	11.16±0.59
Phenol	10.04±0.18	09.08±0.03	10.58±0.08
Protein	11.24±0.55	16.08±0.70	17.76±0.66
Quinone	08.33±0.21	08.07±0.03	07.45±0.11
Saponins	-	-	-
Steroid	17.69±0.89	16.98±0.11	14.08±0.24
Tannins	17.28±0.16	16.71±0.47	15.29±0.31
Terpenoids	11.37±0.19	08.22±0.09	07.56±0.00
Triterpenoids	10.66±0.60	12.53±0.08	13.78±0.14

The values are expressed in terms of (Mean ± Standard deviation)

The mechanism of the anti-inflammation activity and ability of the selected 8 fungal extracts to denaturated protein was studied. All the fungal extracts and the standard were tested at 10 µl/ml concentration. *Emericella nidulans, Pleospora tarda* and *Penicillium funiculosum* extracts showed higher activities with percentage of inhibition % of protein denaturation reached to 83%, 82.5% and 81.4%, respectively. On the other hand, the extracts of all the 5 *Aspergilli* under the lower activities and inhibited protein denaturation by 65-79.9%.

Standard diclofenac sodium recorded 77.4% inhibition of protein denaturation. Denaturation of proteins is well documented and caused of inflammation and rheumatoid arthritis. Several anti-

inflammatory drugs like salicylic acid have shown dose dependent ability to inhibit thermally induced protein denaturation (Strobel and Daisy, 2003). In the current study, the potential endophytic fungi exhibited a dose-dependent inhibition of protein (bovine serum albumin) denaturation and its standards from different concentrations were recorded. Both potential fungi and diclofenac sodium exhibited concentration-dependent inhibition of protein denaturation in all different concentrations. The increased absorbance in both the potential fungi and the standard drug indicated protein stabilizing activity (denaturation was inhibited) with an increased dose. The concentration 300µg/ml of all potential fungi like *A. niger, A. flavus* and *T. viride* is extremely inhibiting, respectively (Table 4).

Table 4. Anti-inflammatory activity of endophytic fungal metabolites

Different concentration (µl)	% of inhibition			
	Potential endophytic fungi			
	Standard (Diclofenac sodium)	*Aspergillus niger*	*Aspergillus flavus*	*Trichoderma viride*
100	33.71±0.16	31.67±0.06	20.90±0.02	33.02±0.11
200	35.16±0.77	34.16±0.62	25.71±0.23	38.16±0.65
300	39.06±0.81	39.18±0.86	36.20±0.31	41.16±0.09
400	34.97±0.09	32.06±0.11	31.91±0.08	36.85±0.19
500	30.11±0.72	28.28±0.42	26.06±0.66	31.01±0.68

The values are expressed in terms of (Mean ± Standard deviation)

Table 5. Antioxidant activity of Hydrogen peroxide assay from endophytic fungal metabolites

Different concentration (µl)	% of activity			
	Hydrogen peroxide assay (H_2O_2)			
	Standard (Ascorbic acid)	*Aspergillus niger*	*A. flavus*	*Trichoderma viride*
100	15.08±0.02	09.15±0.07	14.61±0.10	14.66±0.01
200	19.25±0.00	11.54±0.56	17.11±0.26	16.12±0.76
300	22.48±0.06	16.78±0.44	20.64±0.63	19.27±0.53
400	25.19±0.03	19.17±0.06	23.68±0.96	21.55±0.53
500	29.74±0.15	21.15±0.09	26.11±0.11	27.83±0.33

The values are expressed in terms of (Mean ± Standard deviation)

Table 6. Antioxidant activity of reducing power assay from endophytic fungal metabolites

Different concentration (µl)	% of activity			
	Reducing power assay			
	Standard (Ascorbic acid)	*Aspergillus niger*	*A. flavus*	*Trichoderma viride*
100	21.23±0.10	12.11±0.12	13.60±0.92	19.37±0.84
200	26.58±0.01	15.17±0.71	15.64±0.76	24.76±0.63
300	28.17±0.05	16.68±0.08	17.72±0.20	26.11±0.11
400	33.04±0.11	18.12±0.16	19.68±0.85	32.26±0.18
500	37.52±0.00	20.15±0.05	21.16±0.11	34.11±0.09

The values are expressed in terms of (Mean ± Standard deviation)

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl radical sequestration (DPPH) method. The DPPH solution was prepared at a concentration of 0.06 mmol/L, with methanol protected from exposure to direct light. The assay was performed in 96-well microplates, with 40 µL of extract and the addition of 250 µL of the DPPH solution. For the negative control, 40 µL of 10% DMSO and 250 µL of DPPH solution were added (Tsipinana *et al.*, 2023). In the present study, different concentrations of antioxidant activity such as 100 to 500µg/ml were analyzed from potential endophytic fungi like *A. niger, A. flavus* and *T. viride*. The three standards used in the activity of hydrogen peroxide and reducing power assay corresponding to a standard ascorbic acid, DPPH corresponding to vitamin C and thiobarbituric acid related to ferric thiocyanate were recorded with the percentage of

activity. A reducing power assay was presented at the all concentration for maximum in *A. niger* and *T. viride*, H_2O_2 in *A. flavus*. A reducing power assay was presented for minimum in *A. niger* and *A. flavus*, DPPH in *T. viride* at all concentrations. All methods are standard in same for gradually increased in this activity. In all assays of antioxidant activity, the maximum percentage of activity was observed at the *T. viride* when compared with other potential fungi respected respectively (Table 5, 6, 7 & 8).

Table 7. Antioxidant activity of thiobarbituric acid assay from endophytic fungal metabolites

Different concentration (µl)	% of activity			
	Thiobarbituric acid assay			
	Standard (Ferric cyanide)	*Aspergillus niger*	*A. flavus*	*Trichoderma viride*
100	19.03±0.05	17.91±0.11	10.96±0.52	12.16±0.56
200	21.00±0.33	19.69±0.96	16.49±0.38	16.15±0.56
300	23.89±0.06	21.12±0.08	18.61±0.76	20.76±0.91
400	25.06±0.01	23.17±0.71	23.44±0.29	24.15±0.06
500	31.74±0.09	25.11±0.43	25.96±0.23	29.14±0.71

The values are expressed in terms of (Mean ± Standard deviation)

Table 8. Antioxidant activity of DPPH assay from endophytic fungal metabolites

Different concentration (µl)	% of activity			
	DPPH assay			
	Standard (Vitamin C)	*Aspergillus niger*	*A. flavus*	*Trichoderma viride*
100	14.07±0.26	12.80±0.05	11.27±0.78	11.28±0.05
200	17.52±0.14	14.57±0.66	15.17±0.46	13.27±0.62
300	20.19±0.06	17.13±0.12	18.31±0.41	18.16±0.80
400	24.18±0.10	19.46±0.05	22.03±0.53	22.18±0.92
500	28.16±0.01	21.66±0.64	26.10±0.94	26.83±0.36

The values are expressed in terms of (Mean ± Standard deviation)

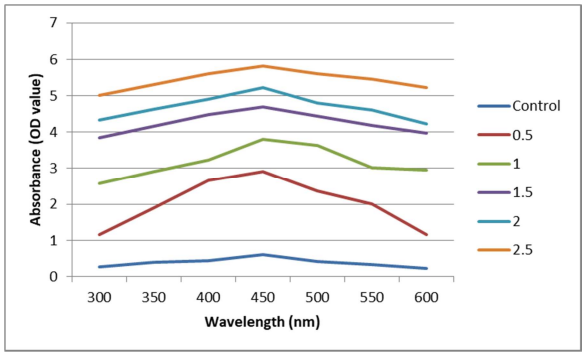

Fig. 1. Bio synthesis of silver nanoparticles from potential *Aspergillus niger*

UV-visible spectra were identified the AgNPs nanoparticles from fungi. It was observed that upon addition of the supernatant of *Alternaria* sp. into $AgNO_3$, change in color from colorless to dark brown was observed within 20 min, indicating the synthesis of AgNPs. Nanostructured metallic particles such as silver and gold have free electrons abundance and they move through conduction and valence band which is responsible for surface plasmon resonance absorption band. The characteristic surface plasmon resonance peak at about 426 nm, which may correspond to spherical AgNPs (Van-Burden and Robinson, 1981). In the current study, the biosynthesis of silver nanoparticles is the colour of the solution changes from yellow to ruby-brown and finally to dark brown for the potential fungi such as *A. niger*, *A. flavus* and *T. viride*, respectively as shown in (Fig. 1).

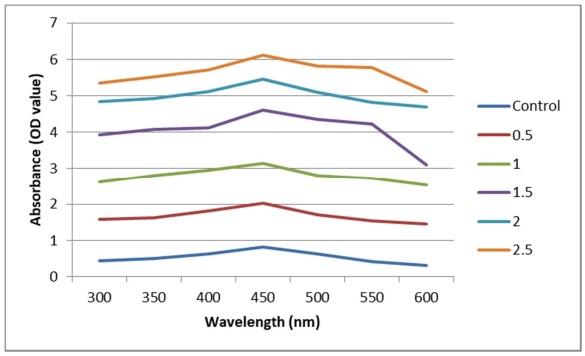

Fig. 2. Bio synthesis of silver nanoparticles from potential *A. flavus*

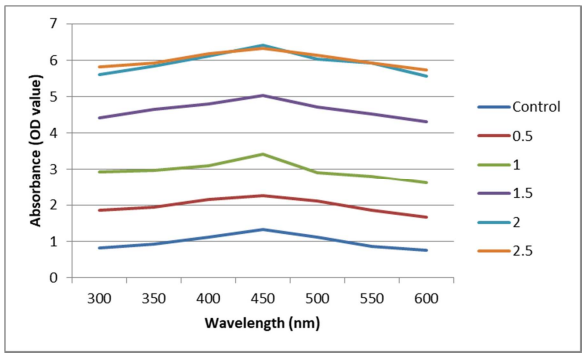

Fig. 3. Bio synthesis of silver nanoparticles from potential *Trichoderma viride*

The three potential endophytic fungi are constant and after addition of five concentration of silver nitrate are used including control (without silver nitrate). It was observed that the color of the solution turned from dark brown after 48 h of the reaction which indicated the formation of silver nanoparticles. Using UV-vis spectrophotometer analysis, the formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored. The UV-vis spectra showed absorbance at 300-600 nm, they showed that increased absorbance in various concentration and the silver nanoparticles surface Plasmon resonance where the peaks were detected. The concentration 2.5mM highest peak in all wavelength at absorbance process were analyzed.

The spectra also clearly showed the increase in silver solution intensity with time, which is a sign that there are increasingly Ag-NPs forming in the solution. The maximum absorption peak was observed in the UV-Vis spectra of silver nanoparticles at 450 nm in all potential endophytic fungi (Fig. 1, 2 & 3).

Table 9. Antibacterial activity of endophytic fungal metabolites against clinical bacteria

Name of the bacteria	Zone of inhibition (mm)											
	Aspergillus flavus				*Aspergillus niger*				*Trichoderma viride*			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
E. coli	12.4± 0.22	23.3± 0.76	26.0± 0.66	10.5± 0.20	10.5± 0.20	20.4± 0.30	23.2± 0.42	25.2± 0.10	16.6± 0.53	22.6± 0.53	18.3± 0.12	24.6± 0.22
K. pneumoniae	21.0± 0.23	24.6± 0.22	24.0± 0.21	30.2± 0.02	18.0± 0.19	23.2± 0.20	19.3± 0.32	25.2± 0.12	11.6± 0.86	14.3± 0.76	20.6± 0.86	22.6± 0.53
Enterococcus sp.	13.3± 0.43	24.0± 0.21	23.6± 0.86	24.6± 0.22	11.4± 0.23	20.1± 0.21	16.6± 0.52	24.3± 0.12	10.6± 0.53	17.2± 0.66	20.6± 0.86	22.6± 0.53
S. aureus	21.6± 0.20	19.0± 0.33	23.2± 0.66	23.3± 0.76	19.3± 0.21	17.2± 0.23	20.2± 0.42	23.0± 0.13	11.6± 0.86	16.0± 0.23	27.0± 0.23	27.6± 0.20

The values are expressed in terms of (Mean ± Standard deviation)

Table 10. Antifungal activity of endophytic fungal metabolites against clinical fungi

Name of the fungi	Zone of inhibition (mm)											
	Aspergillus flavus				*Aspergillus niger*				*Trichoderma viride*			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
Aspergillus niger	5.55± 0.83	10.3± 0.43	11.6± 0.86	13.0± 0.33	10.2± 0.43	13.0± 0.45	18.3± 0.56	10.0± 0.12	13.0± 0.10	16.2± 0.42	19.3± 0.43	20.4± 0.24
A. terreus	6.33± 0.01	7.60± 0.53	9.62± 0.57	12.0± 0.11	12.3± 0.10	16.6± 0.52	19.6± 0.25	11.0± 0.14	18.3± 0.23	17.0± 0.26	12.4± 0.32	19.6± 0.43
Penicillium sp.	5.54± 0.66	7.22± 0.33	8.66± 0.86	9.22± 0.21	20.5± 0.43	22.2± 0.26	32.4± 0.25	06.2± 0.20	20.3± 0.34	10.6± 0.41	18.2± 0.30	21.3± 0.46
Pencillium chrysogenum	3.56± 0.16	4.50± 0.52	7.66± 0.53	8.00± 0.66	23.4± 0.12	24.4± 0.63	21.7± 0.24	09.0± 0.18	23.0± 0.52	14.4± 0.22	13.4± 0.42	22.5± 0.52

The antimicrobial activity of the synthesized AgNPs against the tested human pathogens was assessed on the basis of the zone of inhibition. It has been observed that the biogenic AgNPs exhibited relatively high antibacterial activity against Gram-positive and Gram-negative bacteria as compared to that of controls (Fungal supernatant and $AgNO_3$). This relatively high antibacterial activity can be attributed to the size and the high surface area of the AgNPs which enabled them to reach easily the nuclear content of bacteria and fungi (Zeb and Ullah, 2016). In the present study, the four concentrations are used in the antimicrobial activity such as 25µl, 50µl, 75µl and 100µl. The concentrations of 100µl are the highest zones of inhibition and 25µl

are the lowest zones of inhibition in all microorganisms. The Ag-NPs showed antibacterial activity against bacteria (*E. coli, Klebsiella pneumoniae, Enterococcus* sp. and *Staphylococcus aureus*) and antifungal activity against fungi (*A. niger*, *A. terreus, Penicillium* sp. and *Penicillium chrysogenum*) using the well diffusion method. The highest antibacterial effect on *K. pneumoniae* in *A. niger* and *A. flavus*, *S. aureus* in *T. viride* was found with zone of inhibition. The highest antifungal effect on *A. niger* in *A. niger* and *A. flavus*, *Penicillium* sp. in *A. niger* and *Penicillium chrysogenum* in *T. viride* was found with zone of inhibition. The synthesized Ag-NPs, maximum effect on microorganisms was recorded at *S. aureus* and *Penicillium chrysogenum* and their minimum effect was at *Enterococcus* sp. and *A. niger*. Results were summarized in (Table 9 & 10). Due to their antibacterial properties, biologically significant nanoparticles could be extremely useful in the medical field.

Conclusion

This study is an attempt to isolate the endophytic fungi from the *Melia dubia* and *Carica papaya* plant and explore the pharmacological activities of those fungi along with the plant part. This plant is a plethora of endophytes that are capable of exerting many antioxidants and antimicrobial as well as cytotoxic activities. Plant parts are also bioactive. Thus, this approach may be a way of acquiring novel metabolites having a diverse range of biological activities. However, these endophytic fungi may contain biologically active compounds.

Further research can be conducted to isolate the compound and investigate its pharmacological activities. Synthesis of metallic nanoparticles in a biological way had proven to be a method where the process is effective and less toxic. The AgNPs also showed their effect on both gram-positive and gram-negative bacteria. Thus, the results conclude that isolated potential fungus *A. niger, A. flavus* and *T. viride* is a distinguished producer of differently shaped silver nanoparticles having effective antibacterial and antioxidant activities, which can further accomplish biomedical and industrial purposes. The obtained data reveal the potentiality of endophytic fungi isolated from the medicinal plant as a source for the synthesis of different bioactive compounds, which can be incorporated into biomedical applications, especially against human pathogenic microbes. Additionally, this work emphasizes the importance of endophytic fungi in agricultural sectors as eco-friendly biofertilizers to improve the plant growth performance or defense, enhance plant production, and improve soil quality and fertility.

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