Mycosynthesis of silver nanoparticles using extract of endophytic fungi, *Penicillium* species of *Glycosmis mauritiana*, and its antioxidant, antimicrobial, anti-inflammatory and tyrokinase inhibitory activity

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Abstract

Silver nanoparticles were synthesized using endophytic fungal species, *Penicillium* species from *Glycosmis mauritiana*. Phytochemicals, namely tannins, saponins, terpenoids and flavonoids, were identified in *Penicillium* species extracts, and act as agents of reducing and capping in the conversion of silver nanoparticles into nanoparticles. Using SEM, UV-spectroscopy and XRD, the *Penicillium* species silver nanoparticles (PsAgNPs) were characterized. The PsAgNPs are shown to be strong antioxidants (DDPH and FRAP), have demonstrated anti-inflammatory properties by three different methods in vitro and strongly inhibited the activity of xanthine oxidase, lipoxygenase and tyrosine kinase. *E. coli* and *P. aeruginosa* bacterial species were strongly inhibited by PsAgNPs activity at maximum levels and SEM picture of *P. aeruginosa* confirms these effects and that they were shrunken due to the toxic effect of PsAgNPs.

Keywords: *Penicillium* species, AgNPs, SEM, XRD, biological activities

Classification numbers: 2.03, 2.04, 4.02

1. Introduction

Nanotechnology plays an important role in manufacturing of new materials at nanolevel [1]. Nanoparticles synthesized from metal have catalytic, optical, electrical and magnetic properties and are used as antibacterial agents. Many reports say that silver nanoparticles have been proven as most efficient in possessing strong antioxidant and antimicrobial properties [2–6]. On the basis of less toxic effects, plant extracts were used for the synthesis of silver nanoparticles. The colloidal state of silver has distinctive chemical stability, conductivity and biological activities [7]. The biosynthesized nanoparticles were proficiently used for unicellular organisms [8] and plant extracts [9–12]. Recently, nanoparticles synthesized by using endophytic fungal species have been reported by Hullikere *et al* [12] and Devi and Joshi [13]. They exhibited strong antibacterial activity. The plant *Glycosmis mauritiana* is a small tree recorded in Hindu medicine. The genus plants were used in traditional medicine to treat various diseases [14]. Elumalai *et al* [15] and Niraimathi *et al* [2] reported that herbal extract nanoparticles have shown
antibacterial and antioxidant properties. The *Glycosmis* (family Rutaceae) species were reported to have furoquino-
line, acridine alkaloids, sulphur-containing amides, quino-
lone, quinazoline, carbazole, coumarins and flavonoids [16–
19]. Many green synthesized nanoparticles have been proven as antimicrobial agents [20–22]. The present investigation
was aimed to isolate fungal endophytes and to synthesize the silver nanoparticles evaluated for biological (antioxidant,
antibacterial, anti-inflammatory and tyrokinase) activity.

2. Experimental

2.1. Chemicals and reagents

Silver nitrate, all the chemicals obtained from Sigma-Aldrich, Merck. The bacterial media were from Hi-Media, Mumbai, India and all are AR grade.

2.2. Plant collection

Bark part of *Glycosmis mauritiana* collected in the Western Ghat of Agumbe Ghats, Shimogga district of Karnataka, India during February 2015 identified and authenticated by Dr K G Bhat, Taxanomist, Udupi.

2.3. Isolation of endophytic fungal species

The bark material was washed several (3–5) times with running tap water and distilled water. Then the sample was sequentially surface sterilised by dipping in mercuric chloride solution (1 mg ml⁻¹), rinsing with distilled water, 70% etha-
nol followed by distilled water. The surface was blotted and the edge was trimmed under sterile condition. The sterilized
pieces were placed on petri plates containing potato dextrose
agar (PDA) and incubated at room temperature 26 ± 2°C for 7–8 days. The incubated plates were monitored every day for
the growth of endophytic fungal colony. The fungi which
grew out from sample segment were isolated and individually
were subcultured in plates containing PDA. The fungal iso-
lates were identified based on its morphology and reproduc-
tive characters with the help of microscopic studies.

The isolated endophytic fungi were mass cultured in potato dextrose broth (PDB) containing 250 ml erlenmeyer
flasks incubated at room temperature for 10 days. Then the
grown fungal mats were separated by filtering through
cheesecloth and the mycelial mat was allowed to dry on the
blotting paper to remove the traces of media. Then fungal
mat was ground and centrifuged (6000 rpm, 10 min) and supernatant was incubated in rotary shaker for 24 h.

2.4. Synthesis of endophytic fungi *Penicillium* species-coated silver nanoparticles

80 ml of 3 mM silver nitrate was mixed with 20 ml of *Peni-
cillium* species extract. The colour changes from light yellow to reddish brown and it indicates the formation of of silver nanoparticles. The *Penicillium* species-coated silver nanoparticles (PsAgNPs) were purified from centrifugation at
7000 rpm for 10 min and collected pellets were allowed to dry. The PsAgNPs were used to test proteins and vitamin C and pH was measured.

2.5. Phytochemical screening

The phytochemicals in plant extract and *Penicillium* species were indentified by using standard procedures [23–26].

2.5.1. Test for tannins. 1 ml of endophytic fungal and plant extracts were mixed with 5 ml of distilled water separately and 2–3 drops of 10% ferric chloride. Formations of precipitates and colour change were observed. A bluish-
black or brownish-green colour changes were due to tannins.

2.5.2. Test for saponins. 1 g of both extracts were boiled with 10 ml of distilled water for 10 min and filtered in hot, then were subjected to cool. The plant and endophytic fungal extracts (2.5 ml each) were distilled by 10 ml of distilled water and shaken in 2 min (froths were formed due to saponin). Add 2 drops of olive oil to 2.5 ml of extracts, and then add a suitable amount of distilled water to obtain 10 ml of a mixture. This mixture was shaken for few minutes, and the formation of a fairly stable emulsion was observed.

2.5.3. Test for terpenoids. Two extracts (5 ml) were mixed with chloroform (2 ml) and concentrated H₂SO₄ (3 ml) were added, then the formation of a layer was observed. A precipitate of the reddish brown colour was formed due to terpenoids.

2.5.4. Test for flavonoids. 1 g of the powdered dried plant and endophytic fungal extracts boiled in distilled water (10 ml) for 5 min and filtered. 20% sodium hydroxide (few drops) solution was added to cooled filtrate (1 ml). Yellow colour formed due to addition of acid depicts the flavonoids presence.

2.5.5. Test for cardiac glycosides. The extracts (5 ml) mixed with glacial acetic acid (2 ml) containing one drop of ferric chloride solution and concentrated sulphuric acid (1 ml). Interface formation of brown ring indicates the characteristics of cardenolides and the violet ring formed with acetic acid layer and a greenish ring may be formed.

2.5.6. Test for combined anthraquinones. Each powdered sample (1 ml) was boiled with 10% hydrochloric acid (2 ml) for 5 min and filtered powders were allowed to cool. The filtrates were cooled partitioned with chloroform and transferred into clean test tubes. 10% ammonia solution was mixed with chloroform layer and after mixing allowed to separate. The colour changes was observed in aqueous layer and formation of rose pink indicates the presence of an anthraquinone.

2.5.7. Test for free anthraquinones. Chloroform (5 ml) was mixed with powdered plant (0.5 g) of fungal extract and
shaken well for 5 min for filtration. To the filtrate added equal volume of 10% ammonia solution and observed for colour changes to bright pink.

2.5.8. Test for alkaloids. The powdered sample (1 g) was boiled with water and added 10 ml hydrochloric acid on a water bath and filtered. The pH was adjusted to 6–7 by using ammonia. The reagents (picric acid, 10% tannic, potassium mercuric iodide) were added to 0.5 ml extracts contained in different test tubes, and precipitation or turbidity was observed.

2.6. UV–vis spectra analysis

The reduction of pure silver ions of the reaction medium at 5 h and diluted with distilled water was measured. Analysis was carried out with UV–vis spectrophotometer UV-2450 (Shimadzu).

2.7. Analysis of silver nanoparticles by SEM

Analysis was carried out with Hitachi S-4500 scanning electron microscope (SEM) to analyze morphological character of the sample. Thin film samples were prepared on a carbon coated copper grid by dropping small amount of the sample on the grid, the extra solution was removed using a blotting paper, and films were allowed to dry by subjected under a mercury lamp for 5 min.

2.8. X-ray diffraction studies

The x-ray diffraction (XRD) technique helps to check the formation and quality of PsAgNPs, and the pattern measured by drop coating film on glass substrate was recorded in a wide range of Bragg angles θ at a scanning rate of 2 min⁻¹, carried out on a Philips PW 1830 instrument that was operated at a voltage of 40 kV and current 30 mA with Cu-Kα radiation (1.5405 Å).

2.9. Measurement of particle size of PsAgNPs

Particle size measurement was done with laser diffractrometer using Zeta size nano-series (Malvern) in the range between 0.1 and 10 000 nm.

2.10. Thermo gravimetric differential scanning colorimetre (TG-DSC) analysis

Analysis was carried out using SDT Q600 model, TA instruments, USA. Each samples (5 mg) was added to alumina cup holder and heated to 1000 °C at the rate of 10 °C min⁻¹.

2.11. Antioxidant activity

The antioxidant properties of the PsAgNps was studied by using ferric ion reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays.

2.12. FRAP assay

25 ml of acetate buffer (300 mM, pH 3.6) were mixed with 2.5 ml of 2, 4, 6-tris-(2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM HCl) and 2.5 ml FeCl₃ (20 mM) water solution. Each plant and PsAgNPs sample, 150 μl and 0.5 mg ml⁻¹, was dissolved in methanol, added by freshly prepared FRAP reagent (4.5 ml) and mixed. Absorbance was measured at 593 nm, the FRAP working solution was used as blank [27, 28]. Ferrous sulfate (100 to 1000 μmol l⁻¹) calibration curve was expressed in μmol Fe²⁺/mg dry weight extract. Each sample activity was compared to L-ascorbic acid.

2.13. DPPH radical assay

The effect of PsAgNps extracts was estimated by the method presented in [29]. The samples were freshly prepared by dissolving 24 mg DPPH in 100 ml ethanol and stored at −20 °C. 150 μl of sample solution (10 μl of sample and 140 μl of distilled water) was allowed to react with 2850 μl of agent (190 μl of reagent and 2660 μl of distilled water) for 24 h, the experiment was done in dark condition and the absorbance was measured at 515 nm. The linear standard curve ranged from 25 to 800 μM of ascorbic acid (aa). Additional dilutions of DPPH values were measured over the linear range of the standard curve. 10 ml of stock solution was mixed with 45 ml of methanol to obtain a solution with the absorbance of 1.1 ± 0.02 units at 517 nm using spectrophotometer [30]. All experiments were done in triplicate. The DPPH radical inhibition percentage of each samples were calculated according to standard formula [31]

\[
\text{Inhibition}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100, \quad (1)
\]

where \(A_{\text{control}}\) is the absorbance of the DPPH radical with ethanol, \(A_{\text{sample}}\) is the absorbance of DPPH radical with sample extract/standard.

2.14. Determination of antimicrobial activity

Bacterial standard species of Staphylococcus aureus (gram positive cocci), Escherichia coli and Pseudomonas aeruginosa (gram negative bacilli) were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient broth and tur- mulinosa C. were collected from the Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India and grown in nutrient bro

1 ml of each bacterium was transferred to 1 ml of each concentration of the test compound (PsAgNPs). Using a sterilized bacteriological loop, one loop full of the bacterium plus test compound was inoculated (by streaking) on Mueller Hinton agar plates at 0, 2 and 4 h. The inoculated plate was incubated at 37 °C overnight to assess the action of test compound on growth of bacteria.

2.15. Inhibition of albumin denaturation

The experiment was carried out using standard procedure [32, 33]. Each extract was mixed with 1% aqueous solution of...
bovine albumin incubated at 37 °C for 20 min and heated to 51 °C for 20 min. The turbidity of each sample was measured at 660 nm using spectrophotometer. Protein denaturation inhibition was calculated as equation (1), where \( A_{\text{control}} \) is the absorbance of the DPPH radical, \( A_{\text{sample}} \) is the absorbance of DPPH radical with sample extract/standard.

### 2.16. Membrane stabilization test

10 ml of fresh human blood was collected in centrifuge tubes centrifuged at 3000 rpm for 10 min and washed three times with saline. The blood volume reconstituted with 10% v/v saline [33].

### 2.17. Heat induced hemolytic

1 ml of test sample consisted of 10% red blood cells (RBCs) suspension and added control saline was prepared. Aspirin was used as positive control. Reaction mixture was incubated in a water bath at 56 °C for 30 min After incubation, the test tubes were cooled under running tap water. The samples were centrifuged at 2500 rpm for 5 min and the absorbance was measured at 560 nm. The experiment was performed in triplicates for all the test samples. Percentage of membrane stabilization was calculated using standard formula [24].

### 2.18. Protein inhibitory action

The experiment was carried out based on standard methods [32–34]. Reaction mixtures (2 ml) containing 0.06 mg of trypsin and 20 mM of iris HCl buffer were incubated at 37 °C for 5 min and 0.8% (w/v) was added. 70% perchloric acid (2 ml) was added to terminate the reaction and centrifuged. The absorbance of the reactions was measured at 210 nm. The experiment was performed in triplicate. Proteinase inhibitory activity was calculated based on percentage of inhibition.

### 2.19. Anti-lipoxygenase activity

Using linoleic acid as substrate and lipoxidase as enzyme, the activity of antilipoxugenase was studied [35]. 2 M (0.25 ml) borate buffer with pH 9.0 was mixed with 0.25 ml (20 000 U ml\(^{-1}\)) lipoxidase enzyme solution and incubated at 25 °C for 5 min. 1 ml (0.6 mM) linoleic acid solution was added to solutions and well mixed. Absorbance was read at 234 nm. Indomethacin was used as reference standard. The percentage of inhibition was calculated from equation (1). A dose response curve was plotted to determine the IC\(_{50}\) values. IC\(_{50}\) is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

### 2.20. Xanthine oxidase assay

The xanthine oxidase was spectrophotometrically analyzed at 300 nm [36]. 500 μl of solution A (0.1 M phosphate buffer containing 0.4 mM xanthine and 0.24 mM nitro blue tetrazolium chloride (NBT)), 500 μl of solution B (0.1 M phosphate buffer containing 0.0449 units/ml xanthine oxidase) and 50 μl of a solution containing 10% of each solvent extracts were incubated at 37 °C for 20 min The enzyme activity was measured at 300 nm per unit time.

### 2.21. Determination of tyrosinase inhibitory activities

Enzyme tyrosinase transformed the L-tyrosine into L-DOPA by hydroxylation and oxidation. Odopaquinone rapidly transformed to melamins measured at 492 nm. The PsAgNps were used for tyrosinase inhibition assay and its effects were measured in a 96-well reader. The reaction was carried out in a 50 mM potassium phosphate buffer (pH 6.8) containing 20 mM L-tyrosine and 125 U ml\(^{-1}\) mushroom tyrosinase at 30 °C. The reaction mixture was preincubated for 10 min and the enzyme was added. Mixture without the enzyme served as blank. The absorbances was measured at 492 nm [37]. The percentage inhibition of tyrosinase was calculated as equation (1) where \( A_{\text{control}} \) and \( A_{\text{sample}} \) are the absorbance values in the presence and absence of inhibitor.

### 3. Results and discussion

Totally four different endophytic fungal endophytes were identified from bark part of Glycosmis mauritiana. They are *Penicillium* species, *Alternaria* species, one unknown and actinomycete fungi (table 1).

Table 1. Presence of fungal endophytes in bark part of *Glycosmis mauritiana.*

| Fungal endophytes       | Bark |
|-------------------------|------|
| Unknown                 | +    |
| *Penicillium* species   | +    |
| *Alternaria* species    | +    |
| Actinomycete            | +    |

+ presence, data based on three replicates each.

Figure 1. Incubated parts of plants and pure culture of *Penicillium* species. (A) purified *Penicillium* species in PDA and (B) mass cultures in PDB.
10 min indicates the formation of silver nanoparticles (figure 2). Both Penicillium species and Penicillium species silver nanoparticles (PsAgNPs) extracts show the presence of tannins, saponins, terpenoids, flavonoids and cardiac glycosides, the free anthraquinones and alkaloids were absent in the extracts (table 2). These secondary metabolites posses antioxidant and anti-inflammatory activities in plant and endophytic fungal extracts [38, 39].

The formation and stability of silver nanoparticles were monitored, the absorption spectra of PsAgNPs were recorded. Figure 3 shows the UV–vis spectra of silver nanoparticles formation by constant AgNO₃ concentration at room temperature after 24 h. The pure silver ions reduction confirmed by UV-visible spectra. The maximum absorbance was seen at 435 nm. The solution colour changed to yellowish brown to deep brown depending upon the extract concentration, which indicates silver nanoparticle formation as the colour changes due to excitation of surface plasmon vibration.

SEM analysis shows uniformly distributed silver nanoparticles on the surface of the cells (figure 4). The PsAgNPs were in spherical shape with particle size was observed from two locations at 50 K magnification and they are 65.92 and 64.64 nm. In SEM, the smaller silver particles aggregated to form a larger. Figure 5 shows the XRD patterns of dried silver nanoparticles of endophytic fungi Penicillium species at room temperature. The XRD patterns of PsAgNPs extract indicate that the silver nanoparticles structures are face-centered cubic (fcc) [40]. The XRD peaks were observed at 2θ of 27.79°, 32.20°, 37.94°, 46.08°, 54.48°, 57.48°, 64.61°, 67.41°, 74.26°, and 76.66° attributed to the 111, 200, 220, 222, 311, and 420 planes of crystallography. XRD results show the silver nanoparticles formed due to reduction of Ag⁺ ions by the Penicillium species extract and they are crystalline in nature. The obtained peaks at 2θ = 27.79°, 32.20°, 37.94°, and 46.08° related to crystalline and amorphous organic phases (figure 5). The average size of silver nanoparticles

Table 2. Phytochemical screening of Penicillium species and synthesized silver nanoparticles.

| Phytochemical tests     | Screening agents |
|-------------------------|------------------|
|                         | Penicillium species | PsAgNPs |
| Tannin test             | +                | +       |
| Saponin test            | +                | +       |
| Terpenoid test          | +                | +       |
| Flavonoid test          | +                | +       |
| Cardiac glycosides test | +                | +       |
| Free anthroquinone test | –                | –       |
| Alkaloid test           | –                | –       |

+ presence, – absence; each experiment was repeated three times

Figure 2. (A) Penicillium species extract and (B) synthesized silver nanoparticles from Penicillium species (PsAgNPs) extract.

Figure 3. UV–vis spectrum of synthesized Penicillium species silver nanoparticles.

Figure 4. SEM image of AgNPs synthesized Pencillium species.

Figure 5. XRD pattern of silver nanoparticles biosynthesized by treating silver nitrate with Penicillium species.
concentration of 0.1 mg ml

Figure 6. DPPH scavenging activities of silver nanoparticles synthesized by silver nitrate with *Penicillium* species (aa: standard ascorbic acid).

determined by Debye–Scherrer equation was approximately 18 nm. The obtained results agree with the findings in [41, 42].

The observed total weight loss of 36.2% indicated the metallic core of biomolecule. The silver uncompressed residue was 63.8%. Phase transition temperature of 951 °C was closed to silver melting point (data not shown).

The disappearance of purple colour after the addition of synthesized PsAgNPs may be due to the presence of antioxidants. The scavenging activity of PsAgNPs reached 74 and it was equal to that of standard ascorbic acid (aa) at the concentration of 0.1 mg ml⁻¹ (figure 6). The FRAP reducing ability potentially reduced by PsAgNPs was 1109.41 where the standard ascorbic acid shows 1648.52 μm Fe(II) (figure 7). The PsAgNPs reduced the TPRZ-Fe(III) complex to TPTZ-Fe(II). The free radical scavenging activity of silver nanoparticles of *Alternanthera sessilis* extract [2] was investigated using two antioxidant methods. The PsAgNPs shows highest activity in both experiments. Three different bacteria were used for the antibacterial studies. The 10 μl culture of each bacterial suspensions were incubated with three different concentrations of PsAgNPs (10 μl, 50 μl, and 100 μl) at three different time intervals (0, 2, and 4 h). The 100 μl of PsAgNPs inhibited the growth of two bacterial species (*Escherichia coli* and *Pseudomonas aeruginosa*) at maximum level except *Staphylococcus aureus* (figures 8 and 9). The endophytic fungi *Fusarium semitectum* and *Penicillium* species synthesized silver nanoparticles found strong antibacterial activity [42–44]. The PsAgNPs-treated bacterial species *Pseudomonas aeruginosa*, was analyzed in SEM, which clearly shows that the bacteria died due to toxicity (figure 10). It indicates how the bacteria growth was inhibited by PsAgNPs, and shrunken bacteria were observed.

The PsAgNPs has inhibited the membrane stabilization (83.63% ± 1.4%) and is near to standard aspirin (85.92% ± 1.2%). Denaturation of proteins leads to inflammation. The PsAgNPs inhibited the denaturation protein in membrane stabilization test. The PsAgNPs effectively induced the hemolysis of anti-inflammatory activity effect which was due to endophytic fungal nanoparticles inhibiting the release of lysosomal of RBC to various levels. The mechanism of anti-inflammation activity due to extract nanoparticle on protein denaturation was studied. More efficiency in inhibiting heat induced albumin denaturation was observed. RBCs membrane stabilization, the PsAgNPs effectively inhibited the heat induced hemolysis. The PsAgNPs inhibited the release of neutrophils lysosomal content at inflammation site. The PsAgNPs inhibited the RBC in heat induced hemolysis (table 3). The highest inhibition was observed with PsAgNPs compared with standard drug aspirin showed 85.92%. Very few reports are available on endophytic fungal silver nanoparticles anti-inflammatory activities, except for the report on the first endophytic fungal silver nanoparticles anti-inflammatory activities. Only reports are available on the anti-inflammatory activities of endophytic fungal species extract [45–48].

The maximum inhibition of xanthine oxidase observed from PsAgNPs (92.65% ± 1.81%) was compared with standard (98.6%). Our results agree with those of Naz et al [48] obtained by synthesizing nanoparticles with 5-amino-β-resorcylic acid hydrochloride dehydrate. The PsAgNPs and standard drug galanthamine extracts significantly inhibited the acetyl cholinesterase activity by 27.38% and 50.00%, respectively, at a concentration of 20 μg ml⁻¹ (table 4) [48]. The maximum lipoxygenase observed with PsAgNPs (68%) was compared with standard aspirin (38%) (figure 11). No reports are available on antilipooxygenase activity of silver nanoparticles extracts. Reports are available on plants extracts on inhibition of lipoxygenase [49–51].

The antityrosinase activity of PsAgNPs was summarized in table 5. The PsAgNPs extract exhibited the potent inhibition (98.86% ± 2.98%) of tyrosinase enzyme when compared to the standard arbutin (99.99% ± 1.28%). No activity was observed in water extract used as negative control. Tyrosinase enzyme is involved in melanin formation and catalyzes the tyrosine oxidation process to dihydroxyphenylalanine (DOPA) and from DOPA to DOPA quinone. Tyrosinase is a metaloenzyme containing copper at an active site and it catalyzed to change the oxidative site of copper atoms [52–54].

The o-quinone is a highly reactive compound polymerizing to form the pigment melanin and this presents a serious aesthetic problem of human beings [55]. The human body produces reactive oxygen species to induce DNA
Figure 8. Antibacterial activity of AgNPs of Penicillium species on E. coli at (A) 0 h, (B) 2 h and (C) 4 h incubation of PsAgNPs with E. coli.

Figure 9. Antibacterial activity of AgNPs of Penicillium species on Pseudomonas aeruginosa at (A) 0 h, (B) 2 h and (C) 4 h incubation of PsAgNPs with E. coli.

Figure 10. Antibacterial activity of PsAgNPs toxicity on Pseudomonas aeruginosa, (A) live bacteria, (B) shrinken bacteria and (C) dead bacteria were highlighted.

Table 3. Effect of synthesized Penicillium species silver nanoparticles on membrane stabilization, albumin denaturation and proteinase inhibitory activity.

| Test sample | MS (%)  | AD (%)  | PI (%)  |
|-------------|---------|---------|---------|
| PsAgNPs     | 83.63 ± 1.4 | 89.41 ± 0.9 | 87.49 ± 0.9 |
| Aspirin (200 μg ml⁻¹) | 85.92 ± 1.2 | 85.92 ± 0.8 | 92.87 ± 1.3 |

Data based on three replicates each, MS = membrane stabilization, AD = albumin denaturation, and PI = proteinase inhibitory.

Table 4. Inhibition of xanthine oxidase and acetyl cholinesterase activities from PsAgNPs.

| Inhibitors samples activities (%) | Xanthine oxidase IC₅₀ (μg ml⁻¹) | Acetyl cholinesterase |
|----------------------------------|---------------------------------|-----------------------|
| PsAgNPs                         | 92.65 ± 1.81ᵃ                  | 27.38 ± 1.91ᵇ        |
| Allopurinol                     | 98.68 ± 2.31                   | 50.00 ± 1.36ᵇ        |

Data represented the arithmetic mean and standard error of three determinants. According to Duncan’s multiple range test (DMRT) the values provided with different superscripts remains significant at \( p \leq 0.05 \).

Figure 11. Antilipoxygenase activity of Penicillium species silver nanoparticles (aa: standard ascorbic acid).
damage, melanocytes and melanin biosynthesis [56] which result in hyperpigmentation disorders. Melanin production may be reduced in our body by several methods, including inhibition of tyrosinase enzyme and transport of melanosome to the stratum corneum, the supplementation of antioxidants [57] and so on. Skin whitening agents have recently attracted the attention of researchers in order to find the solution for hyperpigmentation disorders. Many tyrosinase inhibitors and antioxidant agents have been tested as a way of preventing overproduction of melanin in epidermal layers [58].

4. Conclusions

We synthesized the silver nanoparticles from *Penicillium* species, an endophytic fungus of *Glycosis mauritiana*. It is a fast, eco-friendly and easy method instead of using plants and is suitable for synthesis of silver nanoparticles within 10 min by changing the colour. Due to surface plasmon resonance, the colour changes in the reaction based on *Penicillium* species extract. It was proved that important phytochemicals were identified in the *Penicillium* species extract. The silver nanoparticles formation from *Penicillium* species was confirmed by XRD, UV–vis spectroscopy and SEM. The PsAgNPs extracts strongly inhibited the growth of *E. coli* and *P. aeruginosa*, as was evident by SEM. The PsAgNPs had shown strong anti-oxidant, anti-inflammatory, antilipoxygenase, xanthine oxidase and tyrosine inhibitory activity at maximum level. The results show that the PsAgNPs have potential applications in different biological activity and can used for further studies.

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### Table 5. Tyrosinase inhibitory activity of PsAgNPs, arbutin and water extracts.

| Types of extract | Inhibition of tyrosinase (%) |
|------------------|-----------------------------|
| PsAgNPs          | 98.86 ± 2.98                |
| Arbutin          | 99.99 ± 1.28                |
| Water            | —                           |

Data based on three replicates each
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