Research Article

Dai Hai Nguyen, Thanh Nguyet Nguyen Vo, Ngoc Thuy Trang Le, Dieu Phuong Nguyen Thi, and Thai Thanh Hoang Thi*

Evaluation of saponin-rich/poor leaf extract-mediated silver nanoparticles and their antifungal capacity

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Abstract: One-pot green synthesis of silver nanoparticles (AgNPs) has attracted much attention due to its simplicity, high feasibility in scaling up production, abundantly renewable sources, and environmental friendliness. Herein, *Ocimum tenuiflorum* and *Phyllanthus urinaria* leaf extracts (OT-ext and P.uri.ext, respectively) were chosen as reacting agents with rich and poor saponins to fabricate two biogenic AgNPs and characterize them. OT-AgNPs were simply and successfully generated by OT-ext. Ultraviolet-visible spectra showed the peak centered at 434 nm, which confirmed the presence of AgNPs after an 8-h reaction. FT-IR showed the organic functional groups (OH, C=O, C=C, CH, and COC) capping the surface of OT-AgNPs, which agreed with energy-dispersive X-ray spectroscopy analysis exhibiting the composition containing C, O, and Ag. Transmission electron microscopy micrographs revealed that OT-AgNPs possess spherical morphology, with a size range of 5–61 nm, and the majority having a small size within that range. In comparison, P.uri.AgNPs formed by P.uri.ext had a size distribution in a

similar range, but the P.uri.AgNP diameter shifted toward larger sizes. Further, OT-AgNPs and P.uri.AgNPs showed an effective antifungal ability against *Fusarium oxysporum*, *Aspergillus niger*, and *Aspergillus flavus*. Overall, it was found that the rich saponins in the extracts lead to the formation of smaller AgNPs, but all extract-mediated AgNPs with a size less than 100 nm can act as a fungicide for various applications.

Keywords: silver nanoparticles, plant extract, antifungi, saponins, phytoconstituent screening

1 Introduction

In recent years, bioinspired silver nanoparticles (AgNPs) have been a research focus due to the increasing need for AgNPs and the utmost advantages of green synthesis. AgNPs have been applied extensively in many fields, such as biomedical, medicine, cosmetic, food preservation, catalysis, sunscreen, and textile industries [1–4]. Green synthesis has gained more attention than physical and chemical methods due to a number of reasons: the use of clean and nontoxic chemicals and renewable materials; the use of environmentally benign solvents as the aqueous media of the reaction; simplicity; and low cost [5–7]. Recently, common green methods have been reported to utilize microbes, algae, natural polymers, and aqueous plant extracts to fabricate AgNPs [8], as well as other metallic nanoparticles, such as those of Pd and CuO [9–15]. Among these, green synthesis using plant extracts has been considered a rapid and simple method, utilizing abundant and easily found material sources, with highly scalable production [5,16]. As a result, a large number of studies have been reported on AgNPs formed by aqueous herbal plant extracts [5,17–21], which contain many secondary metabolites including alkaloids, phenolics, tannins, saponins, flavonoids, glycosides, terpenoids, and steroids. Understanding the role of each phytoconstituent could help to manipulate the AgNP reaction. The general

* **Corresponding author: Thai Thanh Hoang Thi**, Biomaterials and Nanotechnology Research Group, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, 700000, Vietnam, e-mail: hoangthithaithanh@tdtu.edu.vn

Dai Hai Nguyen: Institute of Applied Materials Science, Vietnam Academy of Science and Technology, 01 TL29 District 12, Ho Chi Minh City, 700000, Vietnam; Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi 100000, Vietnam

Thanh Nguyet Nguyen Vo: Institute of Applied Materials Science, Vietnam Academy of Science and Technology, 01 TL29 District 12, Ho Chi Minh City, 700000, Vietnam; Biomaterials and Nanotechnology Research Group, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, 700000, Vietnam

Ngoc Thuy Trang Le: Institute of Research and Development, Duy Tan University, Danang 550000, Vietnam

Dieu Phuong Nguyen Thi: Biomaterials and Nanotechnology Research Group, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, 700000, Vietnam

mechanism of plant extract-mediated AgNPs has been explained through the reducing roles of compounds bearing phenol groups, reducing sugars, aldehydes, and enol structures. After a reduction step, the phytoconstituents and/or postreaction products containing hydroxyl, amino, and carboxyl functional groups acting as capping agents form coordination linkages with silver that support stabilization of the nanoparticles [8].

Among phytochemical classes, saponins have recently gained significant attention due to their amphiphilic nature and biological activities in pharmaceutical applications [22]. Saponin structures include two parts: the hydrophilic parts of sugar units are attached covalently to the hydrophobic parts of the triterpenoid or steroid [22]. In addition, saponins have been applied as biosurfactants for environmental applications [23]. Herein, for the purpose of AgNP synthesis, saponins were discovered to provide surfactant functions for capping nanoparticles. In fact, capping agents play an important role in AgNP synthesis and can impact the synthesis effectiveness, the final nanoparticle morphology, and/or the initial nucleation [24]. The common stabilizers include natural polymers (gum, agar, collagen, κ -carrageen, chitosan, etc.) [24], synthetic polymers (polyvinyl pyrrolidone, polyethylene glycol, poly(*N*-isopropyl acrylamide, etc.) [5,25], cationic surfactants (dodecyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, etc.) [26], and citrate [27]. However, the use of drugs modified by these synthetic polymers can lead to the generation of antipolymer antibodies in humans and animals that causes the accelerated blood clearance phenomenon of further treatment using drugs modified by these synthetic polymers [28]. Surfactants can be sources of environmental pollution and, thus, threaten the global ecosystem [29,30]. Other stabilizers may not cause severe toxicity, but a complicated preparation and second step are needed after silver nuclei formation. Thus, saponins inherently present in plant extracts without harmful properties could have the potential as an alternative stabilizer in one-pot AgNP synthesis. Indeed, Zhu *et al.* confirmed the stabilizing role of tea saponins, whose behavior was similar to or better than Tween 80 and medium-chain triglyceride oil under most conditions [31]. In the current study, to continue the development of the simple green method of AgNP synthesis and to evaluate the roles of saponin-rich/poor extracts, *Ocimum tenuiflorum* and *Phyllanthus urinaria* leaf extracts were chosen.

Ocimum tenuiflorum (*O. tenuiflorum*, also called *Ocimum sanctum*), with common names of holy basil or tulsi, is a member of the Lamiaceae (Labiatae) family [32]. It is a tropical herb originating from the Southeast Asia [33]. Many of the reported scientific findings associated with *O. tenuiflorum* relate to natural compound isolation and

identification, and pharmacological functions of fresh *O. tenuiflorum* and its extracts/phytoconstituents [32,34]. Its major constituents were reported to contain glycosides, alkaloids, tannins, saponins, and phenolic compounds [34]. Considering the specific compounds reported [34], aqueous *O. tenuiflorum* leaf extract has been recognized to have high reducing power and rich saponins.

Another traditional herbal plant well-known for its high antioxidant activity is *Phyllanthus urinaria* (*P. urinaria*), commonly called leafflower, belonging to the family of Phyllanthaceae. Phenolics and tannins identified include gallic acid, brevifolin, ferulic acid, protocatechuic acid, gentisic acid, *p*-hydroxybenzaldehyde, brevifolincarboxylic acid, ellagic acid, repandinin A, repandinin B, furosin, repandusinic acid A, mallotin, acetonylgeraniin D, corilagin, isostrictinin, chebulagic acid, phyllanthusiin C, phyllanthusiin E, and excoecarianin [35]. In addition, flavonoids, lignans, and terpenoids have been reported to be present in *P. urinaria*, while saponins have not been mentioned [35]. A recent study reported that the *P. urinaria* leaf extract was successfully used to form P.uri.AgNPs [2]. The aim of the current study was to show the effect of saponins on green AgNP synthesis in one-pot reactions. Thus, the *P. urinaria* leaf extract was chosen as a poor-saponin material, and the properties of P.uri.AgNPs were identified and compared. Phytoconstituent screening was performed for both *O. tenuiflorum* and *P. urinaria* leaf extracts to confirm the major components. Green AgNPs, named OT-AgNPs, were formed by the *O. tenuiflorum* leaf extract under similar experimental conditions with P.uri.AgNPs. OT-AgNPs were characterized by ultraviolet visible spectrophotometer (UV-vis), Fourier-transform infrared (FT-IR) spectroscopy, energy-dispersive X-ray spectroscopy (EDS), and transmission electron microscopy (TEM) to identify their functional groups on the nanoparticle corona, to observe their morphology, and to calculate their dimensions. Furthermore, the antifungal properties of OT-AgNPs were tested against *Aspergillus niger*, *Fusarium oxysporum*, and *Aspergillus flavus*. This study aimed to provide not only a sustainable method for synthesizing AgNPs using other plant extract sources of *O. tenuiflorum* leaf but also valuable information on the natural saponin influence as a stabilizer.

2 Materials and methods

2.1 Materials

O. tenuiflorum and *P. urinaria* leaves were collected from the medicinal plant garden in Tra Vinh University

(Tra Vinh province, Vietnam). Silver nitrate (AgNO_3 , ACS reagent, $\geq 99.0\%$, solid), potato dextrose agar (PDA, powder), sulfuric acid (H_2SO_4 , ACS reagent, 95–97%, liquid), ferric chloride (FeCl_3 , reagent grade, 97%, solid), potassium iodide (KI, ACS reagent, $\geq 99.0\%$, solid), potassium bromide (KBr, ACS reagent, $\geq 99.0\%$, solid), iodine (I_2 , ACS reagent, $\geq 99.8\%$, solid), ethanol (99.8%), *n*-butanol ($\geq 99.5\%$), sodium chloride (NaCl, ACS reagent, $\geq 99.0\%$, solid), and diethyl ether (ACS reagent, $\geq 99.0\%$) were supplied by Sigma-Aldrich (Merck, Darmstadt, Germany). Acetic anhydride ($(\text{CH}_3\text{CO})_2\text{O}$, liquid) was obtained from Labkem (Casablanca, Morocco). *F. oxysporum*, *A. niger*, and *A. flavus* were isolated by the Institute of Applied Materials Science, Vietnam Academy of Science and Technology (Ho Chi Minh city, Vietnam). Deionized water (DIW) was produced by Milli-Q HX 7150 machine (Merck Millipore, France).

Aqueous extract preparation: Fresh leaves of *O. tenuiflorum* were carefully chosen to achieve the same quality and similar leaf lifetime. They were washed by DIW to remove the dust completely, dried at 40°C in an oven, and cut into small pieces. A leaf amount of 2 g was added to an Erlenmeyer flask containing DIW of 100 mL. This flask was heated to 60°C for 1 h. The aqueous broth of *O. tenuiflorum* leaves was then filtered by Whatman No. 1 filter paper and symbolized as OT-ext (Figure 1a). Similarly, the P.uri.ext broth was prepared from the *P. urinaria* leaves as described specifically in a previous study [2]. These extracts were stored in a brown bottle at 4°C for further experiments.

2.2 Identification of phytoconstituents

The Wagner test was used to identify alkaloids. The extract (3 mL) was acidified with 3 mL of concentrated H_2SO_4 , and a few drops of Wagner's reagent (2.5 g I_2 in 250 mL KI solution (5 wt%)) were added. Orange precipitate would appear if alkaloids were present.

The foam test was used to identify saponins. A test tube containing extract of 3 mL was shaken, and the persistence of the produced foam for 10 min was used to confirm the presence of saponins. For quantitative determination of the saponin content [36], 10 g of cut leaves were immersed in 100 mL DIW at 60°C for 1 h. After filtration, 80 mL of aqueous extract was mixed with 20 mL of ethanol. This mixture was heated at 90°C until its volume was concentrated to 40 mL. Diethyl ether (20 mL) was added to the concentrated extract in a separator funnel. After vigorously shaking, the diethyl

ether layer was removed. This step was repeated two times. Then, *n*-butanol (60 mL) was added to extract total saponins. The extraction process using *n*-butanol was carried out two times. The combined *n*-butanol extracts were washed with 5% sodium chloride. The *n*-butanol extract was evaporated in a hot water bath and dried in an oven. The dried residue was weighed to record the saponin content. The percentage of saponin was equal to the ratio between the weight of saponin and the weight of leaves.

The ferric chloride test was used to identify tannins and phenolic compounds. The extract (3 mL) was added to a test tube followed by dropping FeCl_3 (5 wt%) of 1 mL. A bluish-black color was produced because of the phenolic nucleus [37].

The Lieberman-Burchard test was used to identify steroids and triterpenes. In a test tube, 3 mL of extract was mixed with a few drops of acetic anhydride. The concentrated sulfuric acid of 1 mL was added from the side of the test tube. The appearance of a green color indicates the steroid presence. If the tested solution turned pink, the presence of triterpenoids was confirmed.

2.3 Preparation of biosynthesized AgNPs

Aqueous AgNO_3 solution was prepared at a concentration of 1 mM. The AgNO_3 solution of 0.8 mL was dropped into OT-ext of 8 mL. This mixture was stirred at room temperature for 8 h to completely form AgNPs, named as OT-AgNPs. After completing the reaction, the OT-AgNPs were collected using a centrifuge machine. Adding DIW into OT-AgNPs and shaking for 1 min to remove all the unreacted compounds from OT-AgNPs, centrifugation was applied to obtain OT-AgNPs. This step was repeated 3 times. The OT-AgNPs were lyophilized and stored in a dark desiccator. The biosynthesized AgNPs prepared from the *P. urinaria* leaf broth (named P.uri.AgNPs) [2] were used for comparison.

2.4 Characterization of biosynthesized AgNPs

After 8 h, the reacted mixture (i.e., OT-ext broth) was diluted five-fold with DIW. The UV-vis spectrum of each of these samples was recorded by UV-vis spectrophotometer (UV-1800, Shimadzu, US). The wavelength range was 300–800 nm.

Figure 1: Preparation procedure of the *O. tenuiflorum* leaf extract (OT-ext) (a) and biosynthesized AgNPs (OT-AgNPs) fabricated by OT-ext (b) (rpm: rings per minute).

For FT-IR analysis, the lyophilized OT-AgNPs were homogeneously mixed with KBr at a ratio of 1:10 (w/w). Then, the mixture was crushed into dust and pelleted. The KBr pellets of OT-AgNPs were analyzed with a FT-IR spectrophotometer (Frontier mid-infrared (MIR), far infrared (FIR), PerkinElmer, US).

The morphology of OT-AgNPs was observed by TEM (JEOL model JEM-1400, Japan). The size was estimated by ImageJ software. The elemental composition of AgNPs was recorded by EDS instrument (Horiba H-7593, UK).

2.5 Antifungal activity

To evaluate the antifungal effect of OT-AgNPs, three fungal strains, namely, *Fusarium oxysporum*, *Aspergillus niger*, and *Aspergillus flavus*, were chosen. The PDA solution was prepared by dissolving 39 g of PDA powder into 1 L of DIW. The PDA solution was autoclaved for 20 min at 120°C before use. Various OT-AgNP amounts of 10, 20, 30, 40, and 50 ppm were mixed in the PDA solution to make the culturing agar dishes that were, respectively, symbolized as OT-AgNP10, OT-AgNP20, OT-AgNP30, OT-AgNP40, and OT-AgNP50. The OT-ext (8 mL) was lyophilized and dissolved again in the PDA

solution to make the OT-ext dish for testing the extract activity. The pure PDA was utilized as the control dish. After preparing various agar dishes, the fungal strains were spotted in the center of each dish. The growing zone of fungi was measured every 24 h for 4 days at room temperature. The data were expressed as mean $\pm$ SD.

3 Results and discussion

3.1 Preliminary tests of phytoconstituents in *O. tenuiflorum* and *P. urinaria* leaf extracts

In previous studies, green AgNPs have been successfully synthesized by plant extracts due to the reducing power of tannins, glycosides, alkaloids, phytosterol, chalcone, anthraquinone, and coumarin [38,39]. In the current study, to understand the effect of each phytoconstituent category on green synthesis of AgNPs, first, we carried out phytochemical screening. Figure 1 and Table 1 show the main biochemicals presented in the aqueous extracts of *O. tenuiflorum* and *P. urinaria* leaves (OT-ext and

Table 1: Comparison of the phytoconstituents between OT-ext and P.uri.ext broths

Tests	OT-ext	P.uri.ext	Phytoconstituents
Color	Light brown	Light yellow	
Wagner’s test	+	–	Alkaloids
Foam test	++++	+	Saponins
FeCl ₃ test	+++	+++	Tannins and phenolics
Lieberman-Burchard test	–	–	Steroids and triterpenes

(–): absence, (+): presence.

Figure 2: Qualitative test of phytoconstituents in OT-ext (left) and P.uri.ext broths (right): color of aqueous extracts (a), Wagner’s test (b), foam test (c), FeCl₃ test (d), and Lieberman-Burchard test (e).

P.uri.ext). In the OT-ext broth (Figure 2b–e, left tubes), the precipitation appeared in Wagner’s test, the froth stood for over 10 min, a bluish-black color was exhibited when adding FeCl₃, and no phenomenon was shown in the Lieberman-Burchard test. Through these phenomena, it was concluded that trace alkaloids, a high content of saponins, and rich tannins and phenolic compounds were present in OT-ext, while steroids and triterpenes were absent. Alkaloids include numerous kinds of compounds, most of which are insoluble in water. However, in aqueous OT-ext, alkaloid salts were present. In case of P.uri.ext, the presence of poor saponins, rich tannins, and phenolics was detected. In comparison, OT-ext and P.uri.ext had similar tannins and phenolics, while OT-ext was rich in saponins and P.uri.ext was poor in saponins, and only OT-ext contained trace alkaloids. Saponin quantities in OT-ext (1.20%) were significantly higher than the saponin content of P.uri.ext (0.25%). Indeed, saponins were not reported in the

phytochemistry of *P. urinaria* [40], while abundant saponins have been found in *O. tenuiflorum* [34].

3.2 The formation of biosynthesized AgNPs (OT-AgNPs)

The OT-ext had a light brown color which turned to reddish brown after 8 h of reaction with AgNO₃ solution. This color change demonstrated the AgNP formation. Indeed, the UV-vis spectra of the reacted solution (Figure 3a, solid line) indicated the peak centered at 434 nm, which represents the absorption of AgNPs due to surface plasmon resonance [41]. In addition, to the left of the 434- nm peak, there was another peak of 278 nm which was similar to the UV-vis spectrum of OT-ext broth (Figure 3a, dashed line). The 278 nm peak was assigned to the electronic transitions of π -type molecular

Figure 3: UV-vis spectra of the OT-ext (dashed line) and the OT-AgNPs (solid line) (a); the FT-IR spectra (b) and EDS analysis (c) of the OT-AgNPs.

orbitals of phenolic rings. This result was also consistent with phytoconstituent screening, which reported high phenolic contents in OT-ext (Table 1). In addition, *O. tenuiflorum* leaves have been reported to contain a greater amount of ascorbic acid and reducing sugar [42]. These compositions played a reducing role to turn silver ions into AgNPs. The formed AgNPs interacted with other compositions in the extracts to create the protecting layer for nanoparticle stabilization.

To demonstrate the organic layer on the nanoparticle surface, the FT-IR spectra of OT-AgNPs were recorded

(Figure 3b). The troughs at 3,407, 2,923, 1,634, 1,384, and 1,076 cm⁻¹ were assigned to the O–H stretching, C–H stretching, C=C or C=O stretching, O–H bending, and C–O stretching vibrations. These results suggested that the OT-AgNPs were capped by phytochemical compounds of OT-ext broth. Moreover, the EDS analysis indicated the presence of carbon and oxygen elements in OT-AgNPs (Figure 3c), which were the same elements found in the FT-IR spectra. In addition, the EDS showed the Ag peak at 3 keV which confirmed the AgNP core. The undesirable peak of copper was found in EDS spectra from the copper grid for sample preparation. In comparison with the UV-vis spectra, the surface functionalization and the element composition of OT-AgNPs were similar to those of P.uri.AgNPs [2], and only the specific compound structures on the nanoparticles' surface were different to the different fingerprint region in the FT-IR spectra. Overall, the OT-AgNPs and P.uri.AgNPs were formed by the reducing agents including phenolic compounds, ascorbic acid, and reducing sugars in extracts, followed by capping with saponins and oxygen-containing phytoconstituents to stabilize these biological AgNPs in the one-pot reaction (Figure 4).

3.3 Size and surface morphology of AgNPs

Figure 5 shows the TEM micrograph of OT-AgNPs and P.uri.AgNPs. Both AgNPs exhibited diversified morphology, but the spherical shape dominated. The dimensions of OT-AgNPs were estimated by ImageJ software as ranging from 5 to 61 nm (Figure 5b); of these, the size of 40% of OT-AgNPs was 5 nm, a further 40% were 19 nm in size, and the last 20% were larger (33–61 nm). Through TEM images (Figure 5a), these large nanoparticles had triangle, oval, cylindrical, and hexagonal morphologies. In the case of P.uri.AgNPs, spherical nanoparticles were mainly observed (Figure 5c), with a few oval nanoparticles observed. The proportions of 18%, 38%, and 44% of P.uri.AgNPs, respectively, had diameters of 4, 16, and 28–52 nm. As a result, P.uri.AgNPs had half as many 5 nm nanoparticles as OT-AgNPs, although the distribution of these two AgNPs was not significantly different. Examining the phytochemical screening of the two herbal plants (Table 1), it was realized that saponins were the cause of these differences. The OT-ext with rich saponins formed abundant 5 nm nanoparticles, while P.uri.ext, with poor saponins, created a majority of 16 nm nanoparticles. Saponins are a natural nonionic surfactant, including the hydrophilic part of sugar groups and the hydrophobic part of the steroids aglycon or triterpene [43]. Thus, saponins can act as capping agents to control the anisotropic growth of silver

Figure 4: The reaction mechanism to form biogenic AgNPs and the possible linkages between AgNPs and natural compounds for AgNP stabilization.

seeds. However, in plant extracts, not only saponins but also other compounds containing oxygen (tannins, phenolics, and their oxidizing forms after reaction) have a capping function for stabilizing nanoparticles. Thus, the OT-AgNPs and P.uri.AgNPs were stabilized, although P.uri.ext had poor saponins as well as less capping agent. Based on the literature [44], sodium alginate, glycol chitosan, and polyvinyl alcohol, which possess many hydroxyl groups similar to saponins, were demonstrated to be effective stabilizers for nanoparticles around 5 nm.

3.4 Antifungal activity

To evaluate the antifungal effect of OT-AgNPs, three fungal strains, namely, *F. oxysporum*, *A. niger*, and *A. flavus* were

cultured in PDA media with and without OT-AgNPs. The proliferation of each fungal strain was followed every day (24 h) until 4 days (96 h) when the fungus was spread to the whole dish surface (Figure 6). All fungal strains were compact on the agar surface, but each dish showed a different fungal zone. For the three strains (*A. niger*, *A. flavus*, and *F. oxysporum*), the fully fungal zone was observed in both PDA and OT-ext, implying no toxicity caused by OT-ext at the working concentration. The mycelial zones on OT-AgNP10, OT-AgNP20, OT-AgNP30, OT-AgNP40, and OT-AgNP50 were decreased gradually and were significantly smaller than those of PDA. Thus, the growth of these fungi was inhibited by OT-AgNPs as a function of their concentration.

For more quantitative analysis, the growing diameter of each fungus as a function of time was recorded (Table 2). After 1 day (24 h), *A. niger* spread by about

Figure 5: TEM image (a) and size distribution (b) of OT-AgNPs; TEM images (c) and size distribution (d).

Figure 6: Growth zone of *A. niger*, *A. flavus*, and *F. oxysporum* in different media including pure PDA, PDA mixed with OT-ext (abbreviated as OT-ext), and PDA mixed with 10, 20, 30, 40, and 50 ppm of OT-AgNPs (abbreviated as OT-AgNP10, OT-AgNP20, OT-AgNP30, OT-AgNP40, and OT-AgNP50, respectively) after 96 h.

Table 2: Mycelial zone (mm) of *A. niger*, *A. flavus*, and *F. oxysporum* cultured in PDA media without and with various OT-AgNP concentrations for 96 h

Media	Incubation time			
	24 h	48 h	72 h	96 h
<i>A. niger</i>				
OT-ext	24.2 ± 0.3	44.2 ± 0.3	63.8 ± 0.8	88.8 ± 1.6
PDA	24.5 ± 0.5	44.7 ± 0.6	65.2 ± 0.3	89.3 ± 1.2
OT-AgNP10	21.5 ± 0.5	34.2 ± 0.3	51.3 ± 0.6	72.2 ± 0.4
OT-AgNP20	19.2 ± 0.3	30.0 ± 0.5	46.0 ± 0.9	65.8 ± 1.9
OT-AgNP30	17.5 ± 0.5	26.5 ± 0.5	41.3 ± 0.3	63.3 ± 0.6
OT-AgNP40	15.5 ± 0.5	21.5 ± 0.5	35.5 ± 0.5	55.3 ± 0.6
OT-AgNP50	13.3 ± 0.6	19.5 ± 0.5	33.5 ± 0.5	49.2 ± 0.3
<i>A. flavus</i>				
OT-ext	19.7 ± 0.6	32.2 ± 0.3	56.5 ± 0.5	87.8 ± 1.0
PDA	20.7 ± 0.3	32.8 ± 0.3	57.5 ± 0.5	89.3 ± 0.5
OT-AgNP10	16.5 ± 0.5	28.3 ± 0.6	50.0 ± 1.0	66.6 ± 1.0
OT-AgNP20	15.5 ± 0.9	24.3 ± 0.6	45.3 ± 0.5	58.1 ± 0.2
OT-AgNP30	13.7 ± 0.6	22.2 ± 0.3	38.1 ± 0.7	52.0 ± 1.0
OT-AgNP40	12.5 ± 0.5	18.8 ± 0.3	31.5 ± 0.5	48.0 ± 0.5
OT-AgNP50	11.7 ± 0.6	16.5 ± 0.5	26.0 ± 1.0	42.3 ± 1.4
<i>F. oxysporum</i>				
OT-ext	10.5 ± 0.5	26.1 ± 0.2	50.8 ± 0.2	70.8 ± 1.9
PDA	10.8 ± 0.2	26.1 ± 0.7	51.0 ± 0.5	70.5 ± 0.5
OT-AgNP10	8.5 ± 0.5	19.3 ± 1.2	38.5 ± 0.5	55.8 ± 0.7
OT-AgNP20	7.3 ± 0.2	15.8 ± 1.6	32.8 ± 0.2	45.3 ± 0.2
OT-AgNP30	6.5 ± 0.5	15.1 ± 0.7	29.5 ± 0.5	41.0 ± 0.8
OT-AgNP40	6.3 ± 0.2	14.1 ± 1.0	24.1 ± 1.0	31.0 ± 0.8
OT-AgNP50	5.8 ± 0.2	12.8 ± 0.2	20.0 ± 0.5	24.5 ± 0.5

24.2 and 24.5 mm on PDA and OT-ext, respectively. Its diameter growth on these two dishes increased every day and achieved 88.8 and 89.3 mm, respectively, on day 4. These data indicated that the OT-ext did not influence the proliferation of *A. niger*. On the AgNP10, AgNP20, AgNP30, AgNP40, and AgNP50 dishes, the *A. niger* growth zones were 21.5, 19.2, 17.5, 15.5, and 13.3 mm, respectively, after 1-day inoculation, and 72.2, 65.8, 63.3, 55.3, and 49.2 mm, respectively, after 4 days (96 h). These results demonstrated that the *A. niger* spreading ability was controlled by AgNPs and decreased 1.5-fold when increasing the AgNP concentration from 10 to 50 ppm.

In the case of *A. flavus* and *F. oxysporum*, the same phenomena as for *A. niger* were observed. The mycelial diameters on PDA and OT-ext were approximated together. *A. flavus* and *F. oxysporum* were not impacted by OT-ext at the working concentration. However, the OT-AgNPs caused a reduction of the growth zone, and higher inhibition was achieved by increasing the OT-AgNP concentration. These results revealed that OT-AgNPs showed effective antifungal ability against three fungal strains. Compared to the antifungal capacity of

the P.uri.AgNPs [2], the OT-AgNPs exhibited a similar effect, although their size distribution was significantly different. This was because both OT-AgNPs and P.uri.AgNPs achieved nanoscales of less than 100 nm, which can easily penetrate fungal membranes and inhibit fungal DNAase [45–47].

4 Conclusions

In conclusion, biogenic AgNPs (OT-AgNPs) were simply and successfully synthesized using OT-ext. UV-vis spectra of OT-AgNPs showed a peak centered at 434 nm. FT-IR revealed that OT-AgNPs possessed many organic functional groups, including O–H, C–H, C=C, C=O, and C–O. EDS analysis confirmed that OT-AgNPs included C, O, and Ag elements. The spherical morphology of OT-AgNPs was observed by TEM technique, and their size distribution was calculated as 5–61 nm, with a predominance of small sizes. In addition, the OT-AgNPs formed by OT-ext were compared with P.uri.AgNPs formed by the *P. urinaria* leaf extract (P.uri.ext). Phytoconstituent screening of OT-ext and P.uri.ext showed that their phenolic contents were similar, but OT-ext had rich saponins and P.uri.ext had poor saponins. This difference might be the reason for OT-AgNPs forming with much smaller nanoparticles than those of P.uri.AgNPs. Finally, OT-AgNPs expressed an antifungal effect against *A. niger*, *F. oxysporum*, and *A. flavus*, similar to the ability of P.uri.AgNPs, because of nanoscales less than 100 nm. These results suggest that although plant extracts with rich saponins can generate smaller nanoparticles, they can be used in the eco-friendly synthesis of AgNPs possessing similar antifungal effects for agricultural applications.

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