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Antioxidant, antimicrobial, cytotoxic activities and biosynthesis of silver & gold nanoparticles using *Syzygium jambos* leaves growing in Egypt

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ABSTRACT

The aims of the current study were to use the leaves of Syzygium jambos (syn. Eugenia jambos L.) for the biosynthesis of silver (AgNPs) and gold (AuNPs) nanoparticles and to evaluate their in vitro antioxidant, antimicrobial, cytotoxic activities as well as their total phenolic content(TPC). The antioxidant activity was evaluated qualitatively using dot-blot and DPPH staining, and quantitatively via phosphomolybdenum assay. Also, the in vitro antimicrobial activity was evaluated via disc agar plate method against five pathogenic microbial strains including Gram +ve and Gram -ve bacteria & fungi. The preliminary cytotoxic activity was evaluated via brine shrimp lethality test (BSLT), and total phenolic content (TPC) was estimated via Folin-Ciocalteu's assay. Silver (AgNPs) and gold (AuNPs) were synthesized and characterized via UV-vis absorbance spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD) analyses. The results indicated that AgNPs and AuNPs can be synthesized using Syzygium jambos leaf extract. The transmission electron microscopy (TEM) analysis showed that the sizes of the synthesized AgNps ranged from 6-23nm, and the synthesized AuNPs also exhibited an average size of 6-23nm. Moreover, the results revealed that TPC of the tested extracts was ranged from 548.85 to 123.30 mg gallic acid equivalent (GAE)/ g dry extract. The total antioxidant capacity (TAC) was ranged from 643.90 to 147.96mg ascorbic acid equivalent (AAE)/g dry extract. Furthermore, there is a promising antimicrobial activity against four strains viz., Pseudomonas aeruginosa with inhibition zones from 9 to 15.5mm; Staphylococcus aureus with inhibition zones from 9 to 14.5mm; Methicillin-resistant Staphylococcus aureus with inhibition zones from 8.5 to 14.5mm and Candida albicans with inhibition zones from 9.5 to 13.5mm; respectively for 85% MeOH, defatted 85% MeOH, pet. ether, CH₂Cl₂, EtOAc, n-BuOH, H₂O, and 85% MeOH of flower part), while the cytotoxic results showed LC_{50} values ranged from 50.11 to 446.68 µg/ml. In, conclusion the leaves of Syzygium jambos showed a noticeable antioxidant, antimicrobial & cytotoxic activities and the ability to produce AgNPs and AuNPs.

Keywords: Syzygium jambos L.; antioxidant; antimicrobial; cytotoxic; TPC; AgNPs; AuNPs; UV-vis; TEM, XRD.

INTRODUCTION

The green biosynthesis of metal nanoparticles attracts an increasing interest due to their novel features and attractive applications in various fields [1]. Medicinal plants have been used for thousands of years to treat health disorders and to prevent diseases including epidemics [2]. The microbial resistance to existing drugs is a serious problem in antimicrobial therapy, therefore there are urgent needs to search for new classes of antimicrobial agents especially that form natural sources that are not based on existing synthetic antimicrobial agents [3]. In addition, human bodies are exposed to exogenous oxidizing agents such as pollutants, different types of chemicals, and smoking, as well as

endogenous factors via metabolic processes. These processes may be followed by creation of chemical substrates that act as oxidizing agents contain reactive oxygen species (ROS) like superoxide anion $(O2^{\bullet})$, and hydroxyl (HO) radicals as well as reactive nitrogen species (RNS) like nitric oxide (NO) radical [4, 5]. Moreover, cancer is the second leading cause of death in the worldwide, and is only preceded by cardiovascular, infectious and parasitic disease. Moreover, cancer is often been regarded principally as a problem of the developed world, more than half of all cancers occur in the developing countries [6, 7]. Brine shrimp (Artemia salina L.) bioassay is considered as a preliminary screening for the presence of antitumor compounds and used to determine the toxicity of plant extracts [8]. The brine shrimp lethality test (BSLT) represents a rapid, inexpensive and simple bioassay for testing plant extracts lethality which in most cases correlated well with cytotoxic and antitumor properties [9]. Several Syzygium species were reported to possess antibacterial [10, 11], antifungal [12], anti- inflammatory [13], anti-allergic [14], antioxidant [15, 16], and antidiabetic [17]. Syzygium jambos (syn. Eugenia jambos L.), commonly known as "Rose apple", which belongs to the family Myrtaceae [18]. It is widely distributed in sub-Saharan Africa, Central America, and some regions in Asia [19]. Some phenolic compounds have been reported from the various parts of the plant [20]. Therefore, the aims of this study were to use the leaves of Syzygium jambos for the biosynthesis of silver (AgNPs) and gold (AuNPs) nanoparticles and to evaluate the antioxidant, cytotoxic, and antimicrobial activities of different fractions of S. jambos growing in Egypt.

MATERIALS AND METHODS

Plant materials

Leaves of *Syzygium jambos* L. (Family Myrtaceae) were collected from Zoo Garden, Giza, Egypt in May 2014. The plant was identified by Dr. Threase Labib consultant of plant taxonomy at the Ministry of Agriculture; formerly, the Head of Taxonomist Specialists at the garden, a voucher specimen (No.S7/3/1) was kept at the herbarium of the garden.

Chemicals and equipments

All solvents and reagents used were of analytical grade. 2,2'-diphenyl-1-picraylhydrazyl (DPPH) free radical and Folin-Ciocalteu's reagent (FCR) was purchased from (Sigma-Aldrich Co.). Gold chloride (AuCl₃), silver nitrate (AgNO₃), sodium carbonate, sodium phosphate, ammonium molybdate, ascorbic acid, and gallic acid were purchased from (Merck Chemical Co.). Thin layer chromatography (TLC) was performed over pre-coated silica plates (GF254, Merck). The absorbance measurements for antioxidant activity were recorded using the UV-Vis spectrophotometer Spectronic 601 (Milton Roy, USA).

Extraction and fractionation

The dry powdered leaves (250 g), were soaked in (3000 ml) of 85% methanol for one week at room temperature with shaking day by day followed by filtration and again extraction for four times. The organic solvent was removed in vacuo using rotatory evaporator. The 85% methanolic crude extract (85 g) was defatted by washing several times with petroleum ether (60-80 °C). Eighty grams of the defatted methanol extract was undergoes fractionation using organic solvents *i.e.*, CH₂Cl₂, EtOAc, and *n*-BuOH (4 x 150mlfor each solvent). The yield of each fraction was determined and kept in dark for further analysis.

Determination of total phenolic content (TPC)

The total phenolic content was determined using Folin-Ciocalteu's reagent according to the reported methods [21, 22].

Antioxidant assays

Rapid screening of antioxidant by dot-blot and DPPH staining

The antioxidant by dot-blot and DPPH staining was qualitatively estimated according to reported method [23].

Determination of total antioxidant capacity (TAC)

The antioxidant activity was determined according to phosphomolybdenum assay [24, 25].

Antimicrobial Activity

The antimicrobial activity was evaluated via disc agar plate method according to the reported method [26].

Cytotoxic activity using brine shrimp lethality test (BSLT)

The preliminary cytotoxic activity of all tested extracts/fractions of *S. jambos* was evaluated according to the reported procedures [27].

Preparation of plant leaf extract for biosynthesis of nanoparticles

The fresh green leaves of *S. jambos* were thoroughly washed with distilled water to remove of any odd materials especially soil and dust. Twenty gram of clean leaves were boiled in 50ml distilled water in Erlenmeyer flask of 500-ml volume for 30min and the leaf debris were removed by filtration through Whatman filter paper (No. 1),the extract was evaporated up to 25ml[1].

Biosynthesis of silver nanoparticles (AgNPs)

Fifty milliliter of 5mM silver nitrate solution (AgNO₃) were prepared in stopper conical flask and 0.25ml of the formerly prepared *S. jambos* leaf extract were added and left at room temperature for 12h and the produced reddish brown colour indicate the biosynthesis of silver nanoparticles (AgNPs)[1].

Biosynthesis of gold nanoparticles (AuNPs)

Fifty milliliter of 5mM gold chloride solution (HAuCl.3H₂O) were prepared in stopper conical flask and 0.5ml of the previously prepared *S. jambos* leaf extract were added and left at room temperature for 12h and the produced purple- reddish colour indicate the biosynthesis of gold nanoparticles (AuNPs) [1].

Characterization of AgNPs and AuNPs nanoparticles

UV-vis absorbance spectroscopy analysis

The bio-reduction of silver nitrate (AgNO₃) to AgNPs and gold chloride to AuNPs was monitored periodically by UV-vis spectroscopy (Shimazu2401PC) after dilution of the samples with deionized water [28]. A UV-vis spectrograph of AgNPs and AuNPs was recorded by using a quartz cuvette with water as reference. The UV-vis spectrometric readings were recorded at a scanning speed of 190-900 nm [29].

TEM analysis

The suspensions containing AgNPs and AuNPs synthesized by *S. jambos* leaf extract were sampled by TEM analysis using JEOL model 1200 EX electron microscope. TEM samples were prepared by placing a drop of the suspension of AgNP or AuNPs solutions on carbon-coated copper grids and allowing water to evaporate. The samples on the grids were allowed to dry for 4 min. The shape and size of nanoparticles from *S. jambos* were determined from TEM micrographs [30].

X-ray diffraction (XRD)

Measurements XRD of the *S. jambos* reduced silver nanoparticles or gold nanoparticles were carried out on dropcoated films of the respective solutions onto glass substrates by a Phillips PW 1830 instrument operating at a voltage of 40 kV with Cu Kx radiation [1].

Statistical analysis

All data were presented as mean \pm S.D. of triplicates (*n*=3) according to Annegowda et al. 2010 using SPSS 13.0 program (SPSS Inc. USA) [31].

RESULTS AND DISCUSSION

Total phenolic content (TPC)

The results in Table 1 revealed that the total phenolic content of different extracts/fractions of *S. jambos* were arranged in the order defatted 85% MeOH (548.85), 85% MeOH (507.73); *n*-BuOH (384.50)> EtOAc (304.40)> H_2O (223.29) CH_2Cl_2 (156.62)> pet. ether (123.30), in comparison with 85% MeOH of flower part (544.38) mg GAE/g dry extract. These results are in agreement to some extent with those available in the literature. Also, Islam et al. (2012) evaluated the total phenolic content (TPC) of the *S. jambos* leaves; the results revealed that TPC was found to be 161.78 ± 11.78 mg GAE/gm extract [32].

Antioxidant activities

The antioxidant activity of different parts of *S. jambos* is well known [20, 32-34]. The results in (Figure 1) revealed that the most tested fractions showed promising qualitative antiradical activity appeared through their white zones upon the dark purple background. Among them, the *n*-BuOH fraction showed the potent activity followed by the defatted 85% MeOH, 85% MeOH, EtOAc, and H₂O in comparison of two standards quercetin and ascorbic acid. Furthermore, the results in Table 1 revealed that the *n*-BuOH fraction has higher antioxidant capacity (643.90 \pm 0.69), than the defatted 85% MeOH extract (619.51 \pm 0.80), than 85% MeOH (538.20 \pm 0.60), than ethyl acetate fraction (460.15 \pm 1.68), followed by the H₂O fraction (315.44 \pm 0.53) mg ascorbic acid equivalent/g dry extract) in comparison with the 85% MeOH of flower part (560.97 \pm 1.15). The weak activities were recorded with the remaining fractions CH₂Cl₂ (222.76 \pm 1.85), and pet. ether (147.96 \pm 1.35) mg ascorbic acid equivalent/g dry extract). Islam et al. (2012) reported on the antioxidant activity of the ethanol extract of *S. jambos* which was

evaluated via total antioxidant capacity assay, and the results showed that the total antioxidant capacity was 335.70 \pm 65.77 mg AEE/gm of extract [32]. Reviewing the literature revealed that the antioxidant activity *S. jambos* seeds extract was evaluated using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. The results revealed that at 200 µg/ml, the tested sample exhibited 69.04 % inhibition, the EC₅₀ value was 95.21 µg/ml [33].



Fig. 1. Dot-blot qualitative antioxidant assay of different fractions of S. jambos on silica sheet stained with DPPH solution in methanol.

Table 1. Total extractable content, total flavonoid content, total phenolic content, and total antioxidant capacity of 85% methanolic extract of *S. jambos* as well as its sub-fractions.

Sample	Yield % Total phenolic		Total antioxidant capacity	
	(TEC) ¹ (mg	gallic acid equivalent/ g extract) ²	2 (mg AAE /g ext.) ³	
Defatted 85% MeOH	H 20.8	548.85 ± 1.53	619.51 ± 0.80	
Pet. ether	1.76	123.30 ± 0.89	147.96 ± 1.35	
CH ₂ Cl ₂	0.25	156.62 ± 1.10	222.76 ± 1.85	
EtOAc	0.58	304.40 ± 1.12	460.15 ± 1.68	
<i>n</i> -BuOH	3.06	384.50 ± 1.78	643.90 ± 0.69	
H ₂ O	2.66	223.29 ± 0.65	315.44 ± 0.53	
Flower 85% MeOH	34.14	544.38 ± 1.62	560.97 ± 1.15	

Results are expressed as mean values \pm *standard deviation* (n = 3).

¹*TEC* (total extractable content).

²TPC (total phenolic content) values are expressed as mg gallic acid equivalent/g extract (mg GAE/g ext.). ³Total antioxidant capacity values are expressed as mg ascorbic acid equivalent/g extract (mg AAE/g ext.).

Antimicrobial activity

The different extracts/fractions of *S. jambos* were tested for their antimicrobial activity against five pathogenic microbial strains including Gram +ve and Gram -ve bacteria and fungi *i.e.*, *Pseudomonas aeruginosa* with inhibition zones (13.5, 13.5, 9.5, 9, 12.5, 14.5, 15.5, and 10.5mm; respectively for 85% MeOH, defatted 85% MeOH, pet. ether, CH₂Cl₂, EtOAc, *n*-BuOH, H₂O, and 85% MeOH of flower part), *Staphylococcus aureus* with

inhibition zones (13.5, 13.5, 9, 11.5, 13.5, 11, 14.5, and 10mm; respectively for 85% MeOH, defatted 85% MeOH, pet. ether, CH₂Cl₂, EtOAc, n-BuOH, H₂O, and 85% MeOH of flower part), Methicillin-resistant Staphylococcus aureus with inhibition zones (11, 10.5, 8.5, 9.5, 11.5, 13.5, 14.5, and 8.5mm; respectively for 85% MeOH, defatted 85% MeOH, pet. ether, CH₂Cl₂, EtOAc, n-BuOH, H₂O, and 85% MeOH of flower part), Candida albicans with inhibition zones (11.5, 13.5, 11.5, 10.5, 12.5, 9.5, 12.5, and 10mm; respectively for 85% MeOH, defatted 85% MeOH, pet. ether, CH₂Cl₂, EtOAc, n-BuOH, H₂O, and 85% MeOH of flower part), and there is no any results were recorded against Aspergillus niger(Table 2). The acetone and aqueous extracts from the bark of S. jambos were tested for antimicrobial activity, and both extracts showed some activity against the tested micro-organisms *i.e.*, Staphylococcus aureus, Yersinia enterocolitica, Staphylococcus hominis, Staphylococcus cohnii, and Staphylococcus warner i[19]. The antimicrobial activity the aqueous and acetone extracts of bark, leaves and seeds of S. jambos were tested against eight different microorganisms i.e. Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi and Vibrio cholera. The results revealed that both extracts showed some activity against the tested microorganisms. Among the three different parts, aqueous extracts of bark have exhibited a minimum inhibitory effect against S. aureus, E. coli and S. typhi, whereas seeds inhibited the growth of P. aeruginosa and V. cholerae, and leaves exhibited inhibitory effect only against S. typhi. Among the acetone extracts, bark was found to be effective against all microorganisms; leaves inhibited only S. aureus, whereas seed extracts failed to exhibit any inhibitory effect against the test organisms [35].

Table 2: Antimicrobial activity of the defatted 85%	6 methanolic extract of S. jambos leaves as we	l as its derived sub-fractions.
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Sample	Clear Inhibition zone (omm)				
	Staphylococcus aureus	MRSA	Pseudomonas aerugino	sa Candida albicans A	spergillus niger
85% MeOH	13.5 ± 0.70	11.0 ± 1.41	13.5 ± 0.70	11.5 ± 0.70	-
Defatted 85% MeOH	13.5 ± 0.70	10.5 ± 0.70	13.5 ± 0.70	13.5 ± 0.70	-
Pet. ether	9.0 ± 1.41	8.5 ± 0.70	9.5 ± 0.70	11.5 ± 0.70	-
CH_2Cl_2	11.5 ± 0.70	9.5 ± 0.70	9.0 ± 1.41	10.5 ± 0.70	-
EtOAc	13.5 ± 0.70	11.5 ± 0.70	12.5 ± 0.70	12.5 ± 0.70	-
n-BuOH	11 ± 1.41	13.5 ± 0.70	14.5 ± 0.70	9.5 ± 0.70	-
H ₂ O	14.5 ± 0.70	14.5 ± 0.70	15.5 ± 0.70	12.5 0.70	-
85% MeOH Flower	10.0 ± 1.41	8.5 ± 0.70	10.5 0.70	10.0 ± 1.41	-

The results of samples against Staphylococcus aureus (G+ve bacteria); Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa; Candida albicans (yeast); Aspergillus niger (fungus); (-); inactive.

Preliminary cytotoxic activity

The brine shrimp (*Artemia salina* L.) bioassay developed by Vanhaecke *et al.*, as a useful tool for preliminary biological and pharmacological activity [36], it used to determine the toxicity of a wide variety of natural or synthetic products [37]. In the current research work, different extracts/fractions of *S. jambos* were tested as preliminary cytotoxic agent via the brine shrimp lethality test (BSLT) using *Artemia salina* Leach eggs [38]. The results in (Table 3, Figure 2) revealed that the *n*-BuOH extract was the strongest cytotoxic with LC_{50} = 50.11 µg/ mL, followed by 85% methanol (LC_{50} = 70.79), defatted 85% methanol (LC_{50} = 100.0), EtOAc (LC_{50} = 141.25), H₂O (LC_{50} = 199.52) µg/ ml. On the other hand the lowest effect was recorded with CH₂Cl₂ (LC_{50} = 446.65), pet. ether (LC_{50} = 446.65), and 85% methanol of flower part (LC_{50} = 446.68) µg/ ml.

Table 3:	Cytotoxic	activity of	different	fractions	of S.	jambos
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Sample	LC ₅₀ + SE	(CL)
85% Methanol	70.79 <u>+</u> 2.38	(75.55 – 66.03)
Defatted 85% Methano	1100.0 ± 2.80	(105.60 - 94.40)
Petroleum ether	446.65 <u>+</u> 9.77	(466.19 – 427.11)
CH_2Cl_2	446.65 <u>+</u> 10.31	(467.27 - 426.03)
EtOAc	141.25 + 5.24	(151.73 – 130.77)
n-BuOH	50.11 + 3.36	(56.83 – 43.39)
H_2O	199.52 + 8.92	(217.36 - 181.68)
85% Methanol Flower	446.68 + 9.50	(465.68 - 427.68)

Means \pm standard error.

95% confidence limits in parentheses.



Fig. 2. Estimation of LC₅₀ by plot of percent mortality of brine shrimp larvae against different dosage of different extracts of S. jambos.

Biosynthesis of Silver nanoparticles (AgNPs)

S. jambos leaf extract was added to an aqueous solution of silver nitrate (5mM), the colour was changed to reddish brownish colour due to the reduction of silver ions to metallic nano silver (Figure 2). UV/Vis absorbance of the produced measurements of the solution explained appearance of peak at about 460nm (Figure. 3). Leaf extract from Polyalthia longifolia when mixed with aqueous silver nitrate solution a yellowish brown colour has been initiated [39] and this coloured solution exhibited maximum absorbance at 451 and 435nm for 10-3M AgNO₃ at 25 and 60° C, respectively, whereas AgNPs produced form 10-4M AgNO₃, maximum absorbance at 425 and 422nm were produced at 25 and 60°C, respectively. Banerjee et al. (2014) studied the biosynthesis of silver nanoparticles using leaf extracts of three plants, Musa balbisiana (banana), Azadirachta indica (neem), and Ocimum tenuiflorum (black tulsi) [40]. A change in colour has been achieved from colourless to vellowish brown to reddish brown to colloidal brown indicating AgNPs formation. The UV/Vis maximum of the produced AgNPs has been detected in the range 425 to 475nm due to surface plasma resonance. Transmission electron microscopy (TEM) measurement was used to determine the size of silver nanoparticles synthesized by using the leaf extract of S. jambos and it has been found that AgNPs sizes of 6-23nm were synthesized (Figure 4). Figure 5 showing the characteristic peaks of metallic Ag located at 37.8° , 43.3° , and 63.5° corresponding to the crystallographic planes (1 1 1), (0 0 2), and (0 2 2) of silver. respectively establishes a characteristic of crystalline metallic Ag phase [2]. Based on the line width of the peak from crystalline plane (1 1 1), crystallite sizes were found to be around 20 nm for Ag. Silver nanoparticles synthesized by tobacco leaf extract exhibited an average size of 8.43±1.15nm as measured by transmission electron microscopy [41]. On the other hand, AgNPs with a cubic shapes and in the sizes ranged from 35-55nm were produced by using Catharanthus roseus leaf extract [42]. Several investigations studied the XRD of the plant leaf extract biosynthesized AgNPs have been done to study the production of metallic silver (in nano state) and their purity [43, 44].



Fig. 2.A visible colour change in color during silver nanoparticle formation.



Fig. 3. UV-vis absorption spectrum of silver nanoparticles biosynthesized by S. jambos leaf extract.



Fig.4. TEM micrographs of silver nanoparticles solution formed by incubation of AgNO₃ solution containing S. jambos leaf extract.



Fig. 5. X-ray diffraction (XRD) pattern of silver nanoparticles biosynthesized by S. jambos leaf extract.

Biosynthesis of gold nanoparticles (AuNPs)

S. jambos leaf extract, when mixed to gold salt (HAuCl₄), a change of colour form yellow to purple (violet) was produced due to the surface plasma resonance phenomenon (Figure 6). Spectrophotometric studies (UV/Vis) explained that the biosynthesizes nano gold solution had maximum absorbance at 550nm as measured by Shimadzu 2401PC (Figure7).Transmission electron microscopy measurements of the biosynthesized AuNPs exhibited an average size of 6 to 23nm (Figure 8). The structural properties of Au-NPs were investigated using the XRD technique. The XRD diffraction pattern of Au nanoparticles, Figure 9 represented the AuNPs acquired in existence of AuCl₄-analogous diffraction peaks are allocated to metallic Au phase with the characteristic peaks at 38.4°, 44.5° and 64.3° attributed to the crystallographic planes (1 1 1), (2 0 0), and (2 2 0), respectively. Eclipta prostate leaf extract was used for the biological synthesis of gold nanoparticles (AuNPs) and the produced AuNPs exhibited a ruby-red colour and had maximum spectral absorbance at 534nm [45]. A violet color was originated as an evident of the formation of Au metal when Au ions were treated with *Elettaria cardamonum* (ELAICHI) aqueous extract [46]. The formed AuNPs showed maximum absorbance at 540, 550 and 540nm according to the ratio of Au solution and plant extract. Transmission electron microscopy (TEM) measurements of the synthesized AuNPs exhibited an average size of 5.8 to 8.84nm (Figure 8). Spherical AuNPs with average size ranging from 3 to 35nm were produced through their biosynthesis using Terminalia catappa plant leaf extract [47]. TEM image for gold nanoparticles synthesized using aqueous extract of Bauhinia tomentosa leaves was done. The synthesized gold nanoparticles were near spherical and polydisperse with an average diameter of 31.32 nm. The AuNP's were encapsulated [48]. The structural properties of Au-NPs were investigated using the XRD technique. The XRD diffraction pattern of Au nanoparticles, figure9 represented the Au NPs acquired in existence of AuCl₄-analogous diffraction peaks are allocated to metallic Au phase with the characteristic peaks at 38.4°, 44.5°, and 64.3° attributed to the crystallographic planes (1 1 1), (2 0 0) and (2 2 0), respectively. Several XRD studies have been done to determine the purity and presence of AuNPs [49, 50].



Fig. 6.A visible colour change in color during gold nanoparticle formation.



Fig. 7.UV-vis absorption spectrum of gold nanoparticles biosynthesized by S. jambos leaf extract.



Fig. 8.TEM micrographs of gold nanoparticles solution formed by incubation of HAuCl4 solution containing S. jambos leaf extract.



Fig. 9.X-ray diffraction (XRD) pattern of silver nanoparticles biosynthesized by S. jambos leaf extract.

CONCLUSION

The current study revealed that silver and gold nanoparticles can be synthesized using *S. jambos* leaves extract. The TEM analysis showed that the sizes of the synthesized AgNps and AuNPs were similarly ranged from 6 to 23 nm. Also, the most tested extracts/fractions of *S. jambos* showed strong qualitative and quantitative antioxidant activities. Moreover, these fractions showed strong *in vitro* antimicrobial against four pathogenic microbial starins namely; *Staphylococcus aureus, MRSA, Pseudomonas aeruginosa*, and *Candida albicans*. Furthermore, the cytotoxic results showed LC₅₀ values ranged from 50.11 to 446.68 μ g/ ml. This finding provides an insight into the usage of the *S. jambos* leaves as good source for the naturally occurring antioxidant, antimicrobial, and cytotoxic agents.

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REFERENCES

[1]M.S.Abdel-Aziz, M.S. Shaheen, A.A. El-Nekeety, M.A. Abdel-Wahhab, J. Saudi. Chem. Soc., 2014, 18, 356-363.
[2]M.A. Ghareeb, A.R. Laila, M.S. Amal, S.O. Nadia, S.A. Mohamed, A.E. Maha, A.S. Mohamed, J. Appl. Pharm. Sci., 2015a, 5, 045-049.

[3]S. Bagde, K. Mayuri, K.P. Rakesh, S. Vinod, J. Pharm. Phytochem., 2013, 1, 127-143.

[4]T.R. Golden, D.A. Hinerfeld, S. Melov, Aging Cell, 2002, 1, 117-123.

[5]H.A. Shoeb, M.F.M. Hassan, A.R. Laila, A.M. Mona, M.S. Amal, M.A. Ghareeb, Br. J. Pharm. Res., 2014, 4, 125-144.

[6]C.D. Mathers, C. Boschi-Pinto, A.D. Lopez, C.J.L. Murray, Cancer incidence, mortality and survival by site for 14 regions of the world, Global Programme on Evidence for Health Policy Discussion Paper No. 13, World Health Organization, 2001, 1-47.

[7] M.A. Ghareeb, A.S. Hussein, M.F.M. Hassan, A.R. Laila, A.M. Mona, M.S. Amal, Global J. Pharm., 2014a, 8, 87-97.

[8]M.A. Ghareeb, A.R. Laila, M.S. Amal, S.O. Nadia, A.E. Maha, S.M. Asmaa, *Int. J. Pharm. Pharm. Sci.*, **2015b**, 7, 507-509.

[9]J.L. Mclaughlin, Crown gall tumors on potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation. In: Hostettmann K. ed. Methods in Plant Biochemistry. London Academic Press; **1991**,pp. 1-31.

[10]T.M. Tsakala, O. Penge, K. John, Ann. Pharm. Fr., 1996, 54, 276-279.

[11]S.S. Gowri, K. Vasantha, Int. J. PharmTech. Res., 2010, 2, 1569-1573.

[12]M. Chandrasekaran, V. Venkatesalu, J. Ethnopharmacol., 2004, 91, 105-108.

[13]S. Muruganandan, K. Srinivasan, S. Chandra, S.K. Tandan, J. Lal, V. Raviprakash, Fitoterapia, 2001, 72, 369-375.

[14]F.A. Brito, L.A. Lima, M.F.S. Ramos, M.J. Nakamura, S.C. Cavalher-Machado, A.C. Siani, M.G. Henriques, A.L. Sampaio, *Braz. J. Med. Biol. Res.*, 2007, 40, 105-115.

[15]R.C. Savitha, S. Padmavathy, A. Sundhararajan, *Ancient Sci.Life*, **2001**, 30, 110-113.

[16]W.H. Lee, I.S. Ismail, Int. J. Med. Arom. Plants, 2012, 2, 219-228.

[17]G. Saravanan, P. Leelavinothan, Int. J. Endocrinol. Metab., 2006, 4, 96-105.

[18]P.P. Rao, P. Subramanian, P.R. Reddy, H.C. Gupta, Indian J. Res. Homoeopathy, 2011, 5.

[19]C.D. Djipa, D. Michel, Q. Joëlle, J. Ethnopharmacol., 2000, 71, 307-313.

[20]U.L.B. Jayasinghe, R.M.S. Ratnayake, M.M.W.S. Medawala, Y. Fujimoto, Nat. Prod. Res., 2007, 21, 551-554.

[21]K.S. Kumar, K. Ganesan, P.V. Rao, Food Chem., 2008, 107, 289-295.

[22]M.M. El-Sayed, M.E. Maher, A.E. Eman, M.A. Ghareeb, *Pharmacologyonline*, **2009**, 3, 590-602.

[23]M.A. Ghareeb, A.S. Hussein, M.F.M. Hassan, A.R. Laila, A.M. Mona, M.S. Amal, Int. J. Phytopharmacol.,2014b, 5, 143-157.

[24]M.M. El-Sayed, A.M. Maher, A.E. Hanan, A.E. Sayed, A.E. Eman, M.A. Ghareeb, *Pharmacologyonline*, **2010**, 3, 317-332.

[25]M.A. Ghareeb, A.S. Hussein, M.F.M. Hassan, A.R. Laila, A.M. Mona, M.S. Amal, *Global J. Pharmacol.*,2013, 7, 486-497.

[26]A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Trucks, Am. J. Clin. Pathol., 1966, 45, 493-496.

[27]J. Ipsen, P. Feigi, Bancroft's Introduction to Biostatistics. 2nd ed., Harper and Row, New York, 1970, pp. 15.

[28] R.W. Raut, N.S. Kolekar, J.R. Lakkakula, V.D. Mendhulkar, S.B. Kashid, Curr. Nanosci., 2009, 5, 117-122.

[29]A. Leela, M. Vivekanandan, Afr. J. Biotechnol., 2008, 7, 3162-3165.

[30]T. Elavazhagan, K.D. Arunachalam, Int. J. Nanomed., 2011, 6, 1265-1278.

[31]H.V. Annegowda, C.W. Nee, M.N. Mordi, S. Ramanathan, S.M. Mansor, Asian J. Plant Sci., 2010, 9, 479-485.

[32]M.R. Islam, M.S. Parvin, M.E. Islam, Drug Discov. Ther., 2012, 6, 205-211.

[33]Z. Ni, W. Zhaoyu, C. Feng, L. Jingming, Afr. J. Pharm. Pharm., 2011, 5, 2411-2419.

[34]B. Gabriela, R.B. Paula, S.B. Karine, S.S. Priscila, B.J. Letícia, S.P. Aline, A. Boligon, M.L. Athayde, T.L. Gonçalves, M.B. Moretto, *Molecules*, **2013**, 18, 9179-9194.

[35]S. Murugan, P.U. Devi, N.K. Parameswari, K.R. Mani, Int. J. Pharm. Pharm. Sci., 2011, 3, 44-47.

[36]P. Vanhaecke, G. Persoone, C. Claus, P. Sorgeloos, *Ecotoxicol. Environ. Saf.*, 1981, 5, 382-387.

[37]T.W. Sam, Toxicty testing using the brine shrimp: *Artemia salina*. In: Bioactive natural product: Detection, Isolation and Structure determination, 1sted. Boca Rotan FL, USA CRC Press,**1993**, p p. 441-456.

[38]B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin, *J. Planta Med.*, **1982**, 45, 31-34.

[39]S. Kaviya, J. Santhanalakshmi, B. Viswanathan, J. Muthumary, K. Srinivasan, Spectrochim. Acta A Mol. Biomol. Spectrosc., 2011, 79, 594-598.

[40]P. Banerjee, M. Satapathy, A. Mukhopahayay, P. Das, Biopro., 2014, 1, 3.

[41]K.S. Prasad, D. Pathak, A. Patel, P. Dalwadi, R. Prasad, P. Patel, K. Selvara, Afr. J. Biotechnol., 2011, 10, 8122-8130.

[42]S. Ponarulselvam, C. Panneerselvam, K. Murugan, N. Aarthi, K. Kalimuthu, S. Thangamani, *Asian Pac. J. Trop. Biomed.*, **2012**, 2, 574-580.

[43]M. Rashidipour, R. Heydari, J. Nanostruct. Chem., 2014, 4, 112.

[44]K. Roy, C.K. Sarkar, C.K. Ghosh, Appl. Nanosci., 2015, 5, 945-951.

[45]G. Rajakumar, T. Gomathi, A. Rahuman, M. Thiruvengadam, G. Mydhili, S-H Kim, T-J Lee, I-M Chung, Appl. Sci., 2016, 6, 1-13.

[46]M. Pattanayak, P.L. Nayak, World J. Nano. Sci. Technol., 2013, 2, 01-05.

[47]B. Ankamwar, E-J. Chem., 2010, 7, 1334-1339.

[48]D. Mukundan, R. Mohankumar, R. Vasanthakumari, IJIRSE (Online),2015, 2347-3207.

[49]E.H. Ismail, M.M.H. Khalil, F.A. Al Seif, F. El-Magdoub, Prog. Nanotechnol. Nanomater., 2014, 3, 1-12.

[50]K.J. Rao, S. Paria, RSC. Adv., 2014, 4, 28645-28652.