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Original Research Article

Cytotoxic activity of silver nanoparticles prepared from *Psidium guajava* L. (Myrtaceae) and *Lawsonia inermis* L. (Lythraceae) extracts

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Abstract

Purpose: *To biosynthesize silver nanoparticles (AgNPs) using Psidium guajava L. and Lawsonia inermis L. leaf extracts, and investigate their antioxidant and cytotoxic activities.*
Methods: *The aqueous extracts were prepared by maceration in distilled H_2O followed by partitioning with EtOAc. AgNPs were prepared by treating the extracts with 1 mM $AgNO_3$ and then were characterized by UV-vis and FTIR analyses, and transmission electron microscopy (TEM). MTT cytotoxicity and 2,2`-azinobis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) antioxidant assays were used to assess their cytotoxic and antioxidant properties, respectively.*
Results: *AgNPs from P. guajava and L. inermis extracts exhibited good morphological stability and showed moderate antioxidant activity (68.1 and 71.9%, respectively) compared to their extracts. Equipotent cytotoxicity against HCT-116 and MCF-7 cells was observed for AgNPs derived from P. guajava, while AgNPs derived from L. inermis possessed two-fold cytotoxicity compared to their corresponding extracts. Phytochemical analysis of P. guajava afforded pyrogallol, quercetin, quercetin-3-O-β-xylopyranoside, quercetin-3-O-β-arabinopyranoside, and quercetin-3-O-α-arabinofuranoside, while L. inermis afforded lawsone and luteolin.*
Conclusion: *Flavonoids and phenolics play a major role in reducing Ag+ ions, surface coating, antioxidant, and cytotoxic activities of AgNPs. The biocompatible AgNPs produced by L. inermis demonstrate promising cytotoxic activity that could contribute to new cancer treatments.*

Keywords: *Silver nanoparticles, AgNPs, Cytotoxic activity, Psidium guajava, Lawsonia inermis, Phytochemical analysis, Green synthesis*

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INTRODUCTION

Over the past few years, there has been an upsurge in the synthesis of metallic nanoparticles (NPs) due to their powerful applications in different areas, including medicine [1]. Methods used for synthesizing AgNPs include chemical and physical techniques, such as ion-sputtering, thermal production, reduction, inert gas condensation, and sol-gel methods which

unfortunately use high energy requirements, hazardous chemicals, difficult wasteful purification processes, and high cost [2]. So, there is a rising demand to develop environmentally safe processes, such as green syntheses/biological syntheses either by microorganisms or plant extracts [3,4]. The leaves of *Psidium guajava* L., the common guava tree, Myrtaceae, contains flavonoids, which are responsible for many pharmacological activities, including antioxidant, anti-inflammatory, antitumor, and antiviral activities [5]. *Lawsonia inermis* L. (Lythraceae), commonly known as Henna or Mehndi, has many benefits, including cosmetic, psychological, and medicinal applications with lawsone (2-hydroxy-1,4 naphthoquinone) as the main biomolecule, in addition to diverse groups of compounds, such as simple phenolics, coumarins, flavonoids, tannins, naphthalene derivatives, lignans, alkylphenones, triterpenes, steroids, alkaloids, and volatile oils [6].

Breast and colon cancers are common causes of death worldwide [7]. Several cancers respond to chemotherapy initially but finally, develop resistance. Thus, discovering a safe and cost-effective drug for cancer treatment is vital. AgNPs were reported to inhibit human glioblastoma cells' proliferation, impair normal cellular functions, disrupt membrane integrity and induce several apoptotic signaling genes, causing programmed cell death [8][9].

Hence, this study aimed to investigate forty-one plant extracts for their ability to synthesize AgNPs, and also, to evaluate the antioxidant and cytotoxic activities of the latter and to isolate the bioactive molecules.

EXPERIMENTAL

Plant materials

The specified plant parts (Table 1) were either harvested in February 2014 from the farm of Pharmacognosy Dept., Faculty of Pharmacy, Mansoura, or purchased from a specialized herbal store in Mansoura, Egypt. The taxonomical identities of the obtained plants were confirmed by Prof. Farid A. Badria, Professor of Pharmacognosy, Faculty of Pharmacy, Mansoura, and according to Boulos (2005) [10]. The collected plant parts were dried and ground to a fine powder before their extraction. Voucher specimens were deposited in the herbarium of Pharmacognosy Dept., Faculty of Pharmacy, Mansoura, Egypt under the successive codes (from 014-Mansoura-5 to 014-Mansoura-46).

Plant extraction

The dried powders of the investigated plants (100 g) were extracted by maceration with dist. H_2O for 24 h. (3 x 150 mL) and kept in a refrigerator for further processing. The combined aqueous extracts, in each case, were filtered using a Büchner funnel and partitioned by shaking with EtOAc (3 x 450 mL) in a separating funnel. The combined EtOAc extracts, in each case, were then evaporated to dryness using a rotary evaporator at 45 ˚C and stored at 4 ˚C for further investigation using AgNPs synthesis assay. For phytochemical investigation of the most active extracts, the above extraction procedure was repeated using 1000 g of powdered plant materials.

Synthesis of AgNPs

Aqueous silver nitrate solution ($AgNO_3$, 1 mM) was prepared in a dark brown bottle to be used in the green synthesis of AgNPs [11]. Serial dilutions (0.1, 0.05, 0.025, and 0.0125 mg/mL) of each plant extract were prepared in dist. H_2O to investigate the suitable concentrations that cause rapid synthesis of stable AgNPs. Typically, in a Wassermann tube, 2 mL of 1 mM $AgNO_3$ solution were added dropwise to 1 mL of each plant concentration at 50 ˚C. The change in color in each case was monitored at several time intervals for one hour by measuring the absorbance of the reaction mixture at 450 nm. The produced AgNPs were collected by an ultracentrifuge (MIKRO 220 R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 15,000 r.p.m. for 30 min followed by re-dispersion of the obtained pellets in DMSO.

Characterization of AgNPs

UV-visible spectroscopy

The process of reducing silver ions (Ag^+) from solution to AgNPs was observed by recording the UV-vis spectra of the reaction mixture at 10, 20, 30, and 60 min [12,13]. The spectra were measured at the UV-vis range from 200 to 800 nm using a SHIMADZU, UV-1601PC spectrophotometer. Silver nanoparticles were analyzed using quartz cuvettes (1 cm path) at room temperature. The corresponding dilution of untreated aqueous plant extract was used as the blank.

Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis for each plant extract before and after AgNPs production was achieved to

characterize and compare the diminished peaks of the functional groups of the biomolecules that are possibly involved in the green synthesis process [14]. FTIR measurements were performed by blending the dry synthesized AgNPs with KBr pellets (IR grade) and analyzing with an FTIR spectrometer (Nicolet IS10 of Thermo Fisher Scientific Inc., USA) from 400-4000 cm^{-1}, using OMNIC 8.0.380 software, at a resolution of 8 cm^{-1}, and at room temperature.

Transmission electron microscopy (TEM)

Imaging and analytical identification of the synthesized AgNPs were performed by TEM to evaluate their morphological properties, including shape and size [15]. A JEOL TEM-1230 instrument attached to a CCD camera, at an accelerating voltage of 120 kV was used for this purpose. The sample was prepared by adding a few drops of AgNPs, dispersed in dist. H_2O on a carbon-coated copper grid, and was left to evaporate before imaging.

ABTS antioxidant assay

The antioxidant activity of the investigated materials was assessed using ABTS radical scavenging assay according to the reported data [16]. The inhibition (%) was calculated based on the reduction in absorbance (OD_{734}) of the reaction mixture and according to the equation:

$$H\ (\%) = \{OD_{control} - OD_{test} / OD_{control}\} \times 100 \ldots\ldots\ldots (1)$$

Ascorbic acid (100 µL, 2 mM) was used as a positive control. Blank was prepared from a methanol-phosphate buffer (1:1 v/v) with no ABTS added. Negative control was run with ABTS with no sample added.

MTT cytotoxicity assay

Cell lines

Two human cell lines; the colon cancer (HCT-116) and the breast cancer (MCF-7) cell lines (Holding company for biological products and vaccines, VACSERA, Cairo, Egypt), were used to evaluate the cytotoxic activity.

Cytotoxicity assay procedure

The investigated cells were seeded (5 x 10^4 cells/mL) in 96-well plates (100 µL/well). After overnight incubation, different samples (6 concentrations, in triplicates) were added and further incubated at 37°C and in a 5% CO_2 atmosphere for 48 hours. DMSO (0.5%) was utilized as a negative control. Subsequently, aliquots of MTT solution (15 µL, 5 mg/mL in PBS) were added to each treated well and incubated for another 4 hours. The produced formazan metabolite was dissolved by the addition of aliquots of acidified sodium dodecyl sulfate solution, SDS (100 µL, 10% SDS in 0.01 M HCl). The absorbance (OD_{570}) was recorded by a Biotek® microplate reader after incubation for 14 hours [17]. Standard deviation (SD) and IC_{50} were calculated. The anticancer drug, cisplatin was utilized as the positive control.

Phytochemical studies

Purification of the EtOAc extract of Psidium guajava

The EtOAc extract of *P. guajava* leaves (2.2 g), was loaded onto the top of a silica gel chromatographic column, cc. (3 cm i.d. x 35 cm), eluted with CH_2Cl_2-MeOH (gradient), and the effluents were collected in 50 mL-fractions. Fraction F1 (43-47), 260 mg, eluted with CH_2Cl_2-MeOH (96:4 v/v), was re-chromatographed over a Sephadex LH20 cc. (1 cm i.d. x 15 cm) using CH_2Cl_2-MeOH (98:2 v/v, gradient), the effluents were 20 mL, sub-fractions 33 - 36 afforded **G1** (15 mg), while sub-fractions 60-71 afforded **G2** (55 mg). Fraction F2 (72 – 92), 355 mg, eluted with CH_2Cl_2-MeOH (92:8 v/v), was purified using a Sephadex LH20 cc. (1 cm i.d. x 15 cm), using CH_2Cl_2-MeOH (98:2 v/v), and the effluents were 20 mL, sub-fractions 40-45 yielded **G3** (20 mg). Fraction F3 (93 – 165), 425 mg, eluted with CH_2Cl_2-MeOH (9:1) was purified using a Sephadex LH20 cc. (1 cm i.d. x 15 cm), using CH_2Cl_2-MeOH (98:2 v/v, gradient), the effluents were 20 mL, sub-fractions 27 - 30 afforded **G4** (18 mg), while sub-fractions 35 - 41 afforded compound **G5** (20 mg).

Purification of EtOAc extract of Lawsonia inermis

The EtOAc extract of *L. inermis* leaves (2 g), was purified using a silica gel cc. (3 cm i.d. x 35 cm), eluted CH_2Cl_2-MeOH (gradient) and the effluents were 50 mL. Fraction F1 (35 – 50), 100 mg, eluted with CH_2Cl_2-MeOH (98:2 v/v), was further purified using a Sephadex LH20 cc. (1 cm i.d. x 15 cm) using CH_2Cl_2-MeOH (99:1 v/v, gradient), the effluents were 20 mL fraction, sub-fractions 41 - 45 afforded **H1** (22 mg). Fraction F2 (55-78), 95 mg, was purified by a Sephadex LH20 cc. (1 cm i.d. x 15 cm) using CH_2Cl_2-MeOH (95:5 v/v), the effluents were 20 mL, sub-fractions 38 - 45 yielded **H3** (15 mg). Compound **H2** was identified in the EtOAc extract by co-chromatographic TLC with authentic lawsone (its purification and

identification were previously confirmed by Pharmacognosy Dept., Faculty of Pharmacy, Mansoura).

Statistical analysis

Data are presented as mean with SD. Statistical significance was set at $p < 0.05$. Data were subjected to One-Way ANOVA followed by a post-hoc Tukey's test. All statistical analyses were carried out using GraphPad Prism software.

RESULTS

Characteristics of silver nanoparticles

UV/Vis spectra

The color change obtained after adding $AgNO_3$ solution is a quick indicator for monitoring AgNP synthesis [12]. A stable yellowish-brown color was produced due to the excitation of surface plasmon resonance (SPR).

Table 1: Results of screening of forty-one plant extracts for their potential production of AgNPs at a concentration of 1 mg/mL

No.	Plant name	Family	Part used	AgNPs production
1.	*Aralia victoria*	Araliaceae	Leaves	-
2.	*Atropa belladonna*	Solanaceae	Fruits	-
3.	*Atropa belladonna*	Solanaceae	Leaves	-
4.	*Boswellia carteri*	Burseraceae	Resins	-
5.	*Brassica alba*	Brassicaceae	Seeds	++
6.	*Brassica nigra*	Brassicaceae	Seeds	++
7.	*Capsicum annuum*	Solanaceae	Fruits	-
8.	*Carthamus tinctorius*	Asteraceae	Flower	-
9.	*Carum carvi*	Apiaceae	Flower	-
10.	*Centella asiatica*	Apiaceae	Leaves	+
11.	*Ceratonia siliqua*	Fabaceae	Bulbs	-
12.	*Cinchona officinalis*	Rubiaceae	Barks	-
13.	*Citrullus colocynthis*	Cucurbitaceae	Fruits	-
14.	*Chamaemelum nobile*	Asteraceae	Flowers	-
15.	*Coriandrum sativum*	Apiaceae	Fruits	-
16.	*Curcuma longa*	Zingiberaceae	Rhizomes	-
17.	*Datura stramonium*	Solanaceae	Leaves	+
18.	*Digitalis purpurea*	Scrophulariaceae	Leaves	+
19.	*Ferula asafoetida*	Apiaceae	Resins	-
20.	*Foeniculum vulgare*	Apiaceae	Fruits	-
21.	*Fragaria ananassa*	Rosaceae	Leaves	+
22.	*Glossostemon bruguieri*	Malvaceae	Roots	+
23.	*Glycyrrhiza glabra*	Fabaceae	Rhizomes	++
24.	*Hibiscus sabdariffa*	Malvaceae	Flowers	-
25.	*Hyoscyamus muticus*	Solanaceae	Leaves	+
26.	*Lawsonia inermis*	Lythraceae	Leaves	+++
27.	*Melaleuca leucadendra*	Myrtaceae	Leaves	+
28.	*Mentha piperita*	Lamiaceae	Leaves	-
29.	*Morus alba*	Moraceae	Leaves	-
30.	*Nerium oleander*	Apocynaceae	Leaves & flowers	-
31.	*Ocimum sanctum*	Lamiaceae	Leaves	+
32.	*Pimpinella anisum*	Apiaceae	Fruits	-
33.	*Pistacia lentiscus*	Anacardiaceae	Resins	-
34.	*Psidium guajava*	Myrtaceae	Leaves	++++
35.	*Rheum rhabarbarum*	Polygonaceae	Roots	+
36.	*Rosa damascena*	Rosaceae	Flowers	-
37.	*Salvadora persica*	Salvadoraceae	Stems	-
38.	*Syzygium aromaticum*	Myrtaceae	Flowers	+
39.	*Tanacetum cinerariifolium*	Asteraceae	Flowers	+
40.	*Trigonella foenum-graecum*	Fabaceae	Leaves	-
41.	*Zingiber officinale*	Zingiberaceae	Rhizomes	-

*Monitored by measuring absorbance (OD) at I_{450} over a period of 0- 60 min, where OD is represented as (-): <0.5, (+): 0.5 to >1, (++): 1 to >2, (+++): 2 to >3, (++++): ≥ 3

Therefore, the different dilutions for each plant extract were used as blanks to compare the produced color. Forty-one plant extracts were evaluated for their capacity towards the production of AgNPs by measuring the UV-vis absorbance of the reaction mixture at $\lambda_{450,}$ and the results are displayed in Table 1.

Furthermore, the reduction process for the most active extracts (*viz., P. guajava* and *L. inermis* leaves) was monitored by scanning the UV-vis spectra of their reaction mixtures at the wavelength range from 200 - 800 nm, at 0 - 60 min after treatment, Figure 1 (a & b). The recorded spectra showed absorption maxima at 440 to 460 nm, corresponding to the characteristic SPR of the resulting AgNPs [18].

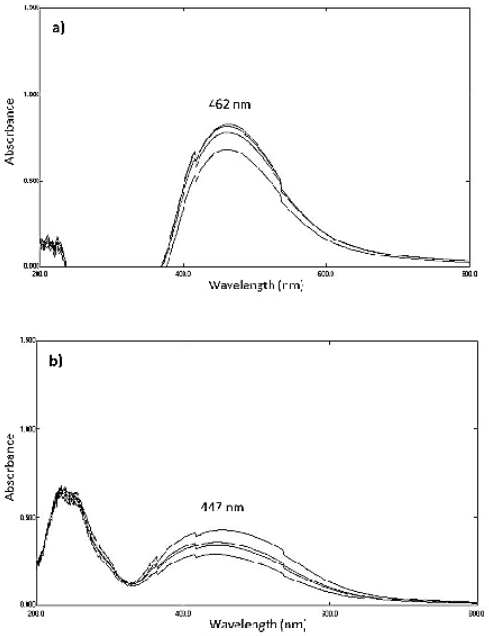

Figure 1: Overlay of the UV–vis absorbance spectra (from λ_{200}-λ_{800} nm) of the reaction mixtures after addition of 1 mM $AgNO_3$ solution for the production of AgNPs, recorded as a function of time; a) *Psidium guajava* leaf extract with a marked peak at λ_{462} nm, and b) *Lawsonia inermis* leaf extract with a marked peak at λl_{447} nm, at 10, 20, 30, and 60 min

Fourier transform infrared (FTIR) spectra

FTIR spectroscopy was used to recognize the potential functional groups of the biomolecules in the active extracts, which may be accountable for the reduction of silver ions and the stabilization of the produced AgNPs [14]. FTIR absorption spectra of *P. guajava* and *L. inermis* leaves extracts, before and after reduction of silver ions, are displayed in Figures 2a & 2b, respectively.

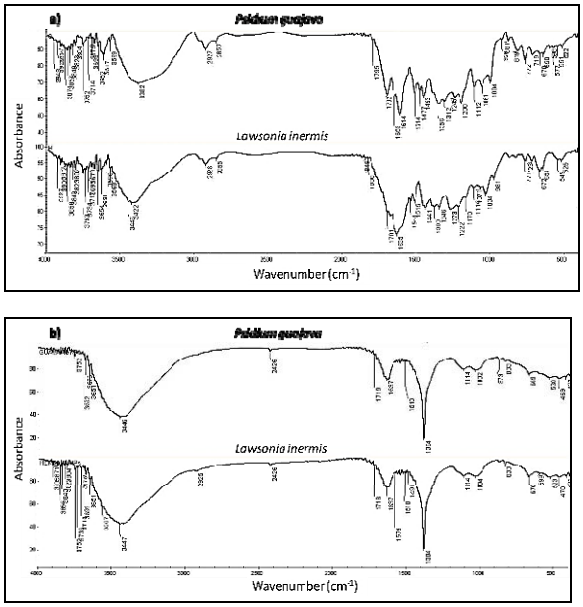

Figure 2: FTIR spectrum of *Psidium guajava* and *Lawsonia inermis* leaf extracts; a) before, and b) after reaction with Ag^+ ions for AgNPs production, showed diminished carbonyl peaks in the region from 1651 - 1680 cm^{-1}

Transmission electron (TEM) micrographs

TEM was used to identify the size and shape of AgNPs formed by the tested plant extracts [15]. Representative TEM micrographs of the developed AgNPs by *P. guajava* and *L. inermis* leaf extracts after 60 min using the optimum concentration in each case is displayed in Figure 3. It revealed the production of nanoparticles of a size range from 5 to 30 nm, mostly spherical, well-dispersed, and capped with an organic matter from the plant constituents.

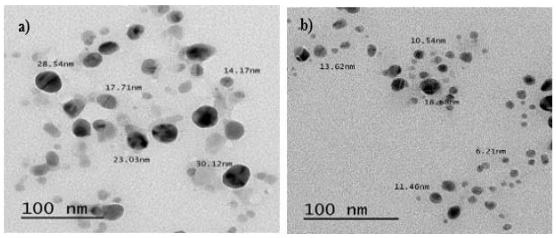

Figure 3: TEM micrographs of AgNPs developed by the leaf extracts of; a) *Psidium guajava*, and b) *Lawsonia inermis*, showing well-dispersed, spherical AgNPs with a size range 5 - 30 nm.

ABTS antioxidant activity

The potential antioxidant activity of AgNPs synthesized and capped by *P. guajava* and *L. inermis* leaves extracts, was evaluated using ABTS radical cation antioxidant assay. Additionally, the antioxidant effect of their

corresponding extracts was tested so it can be compared with the produced AgNPs. The antioxidant capacity is represented as a percentage of inhibition in the color intensity of the preformed blue ABTS radical cation at λ_{734} nm, Table 2.

Table 2: Inhibition (%) of ABTS radical cations by the investigated plant extracts and their corresponding synthesized AgNPs at λ_{734}, calculated at 1 mg/mL

Test sample [a]	Inhibition (%) [b]
Control	0
Psidium guajava extract	89.7 ± 1.65*
Lawsonia inermis extract	90.3 ± 1.3*
G-AgNPs	68.1 ± 2.1*
H-AgNPs	71.9 ± 1.3*
L-Ascorbic acid (2 mM)	90.7 ± 0.7*

[a]G = *Psidium guajava* and H = *Lawsonia inermis,* and ascorbic acid was used as a standard. [b] Data presented as mean ± standard deviation (SD), n = 3; *Compared to control and statistically significant at $p < 0.05$

Cytotoxic activity of AgNPs

Cell-based *in vitro* MTT colorimetric assay is a very important and simple method for the evaluation of cytotoxicity [17]. In our study, two human cancer cells; HCT-116, the colorectal carcinoma, and MCF-7, the breast cancer cells, were used to evaluate the cytotoxic activity of the synthesized AgNPs and their corresponding extracts by MTT assay. Cisplatin (nM) was used as a positive control and IC_{50} for the investigated samples was calculated in mg/mL, Table 3.

Table 3: Cytotoxic activity (MTT) of *Psidium guajava* and *Lawsonia inermis* leaf extracts and their green synthesized AgNPs

Test sample[a]	IC_{50} (µg/mL)[b]	
	MCF-7	*HCT-116*
Psidium guajava extract	60 ± 5.8	70 ± 0.4
Lawsonia inermis extract	>100 ± 4.1	80 ± 4.6
G-AgNPs	60 ± 6.9	70 ± 1.28
H-AgNPs	55 ± 2.6*	56.6 ± 0.47*
Cisplatin (nM)	3 ± 0.18	1.5 ± 0.07

[a]G = *Psidium guajava* and H = *Lawsonia inermis.* Cisplatin was used as a positive control. [b]IC_{50} values are represented as mean ± SD, n = 3. *Compared to their corresponding extract and at statistical significance, $p < 0.05$

Phytochemical profile

In an attempt to identify the bioactive components of the most active extracts of *Psidium guajava* (**G**), and *Lawsonia inermis* (**H**), column chromatographic investigations were conducted, and the isolated structures were identified using NMR spectroscopy and in comparison with previously published spectral data [19]. The isolated compounds from *P. guajava* were identified as pyrogallol **G1** [20, 21], quercetin **G2** [19], quercetin-3-*O*-β-xylopyranoside **G3** [19], quercetin-3-*O*-β-arabinopyranoside **G4** [19], and quercetin-3-*O*-α-arabinofuranoside **G5** [19]. Whereas, *p*-coumaric acid **H1**, lawsone **H2**, and luteolin **H3** were identified from *L. inermis* [22], Figure 4.

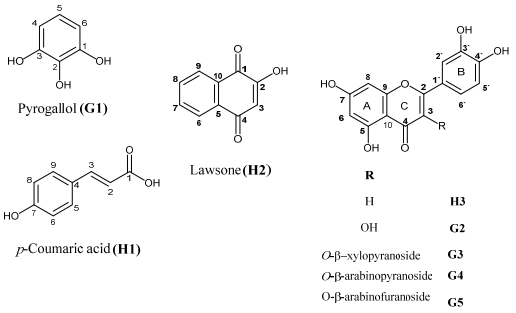

Figure 4: Chemical structures of isolated and identified compounds from *Psidium guajava* (**G1-G5**) and *Lawsonia inermis* (**H1-H3**)

AgNPs synthetic capacity of the isolated compounds

To evaluate the green synthetic capacity of AgNPs by the isolated compounds, the UV-vis absorption spectral patterns after treatment with $AgNO_3$ solution were studied. The obtained results are presented in Table 4.

Table 4: AgNPs synthetic activity of the isolated compounds from the active extracts of *Psidium guajava* and *Lawsonia inermis* using UV-vis spectroscopy

Plant name	Isolated compound	AgNPs activity*
Psidium guajava (Guava)	Pyrogallol (**G1**)	++++
	Quercetin (**G2**)	+++
	Quercetin-3-*O*-β-xylopyranoside (**G3**)	+++
	Quercetin-3-*O*-β-arabinopyranoside (**G4**)	+++
	Quercetin-3-*O*-α-arabinofuranoside (**G5**)	
Lawsonia inermis (Henna)	Lawson (**H2**)	+++
	Luteolin (**H3**)	++
	p-Coumaric acid (**H1**)	-

* The reaction mixture is monitored by measuring the absorbance (OD) at λ_{450} over a period of time from 0-60 min, where OD is represented as (-): <0.5, (++): 1 to >2, (+++): 2 to >3, (++++): ≥ 3

DISCUSSION

The current study investigated the capacity of several plant extracts towards the green synthesis of AgNPs. Also, it aimed at the characterization and cytotoxic evaluation of the produced nanoparticles by the most active plant extracts. In addition, identification of the biomolecules and related functional groups responsible for the green synthesis process. The size-based optical characteristics of the produced AgNPs is a fast indicator, and is initially indicated by the color change of the reaction mixture [12, 18]. UV-vis absorbance at λ_{450} indicated that only two extracts *viz*., *P. guajava* and *L. inermis* leaf extracts, out of forty-one investigated plant extracts, showed high AgNPs synthetic activity, Table 1. The optimum concentration for producing AgNPs by *P. guajava* and *L. inermis* leaf extracts was 0.05 mg/mL. The obtained absorbance was stable and increased by time (from 0 - 60 min after adding $AgNO_3$ solution), where typical absorption maxima were obtained in the case of *P. guajava* and *L. inermis* leaf extracts at λ_{462} and λ_{447}, respectively.

FTIR spectral evaluation showed absorption bands at 1034 - 1061 cm^{-1} (C-O stretching), while the presence of absorption bands at 382 - 3421 cm^{-1} (O-H stretching) indicated phenolic/alcoholic groups that are dominant in tannins and flavonoids. On the other hand, peaks that arose around 1651 - 1680 cm^{-1} (C=O) indicated the presence of carbonyl groups which may be ketones as in flavonoids, or carboxylic groups as in tannins. Clearly, the FTIR spectra of the extracts of *P. guajava* and *L. inermis* leaves before and after reduction of Ag^+ to Ag° (Figure 2a & 2b, respectively) showed that the carbonyl peaks were considerably attenuated, indicating its contribution in the AgNPs-synthesis process. Such compounds were participating in both synthesis and capping (coating) of the produced silver nanoparticles [23,24].

The TEM micrographs of AgNPs formed by the leaves extracts of *P. guajava* and *L. inermis*, Figure 3a & 3b, respectively, showed typical morphological characteristics including, the shape and size of the prepared AgNPs, indicating a good stabilization effect of the investigated plant extracts. This was further confirmed by monitoring their physical properties, where no visible changes were observed over the period of a few months [15].

The antioxidant activity of photosynthesized AgNPs by DPPH assay was previously reported, and it was concluded that AgNPs can be utilized as potential free radical scavengers [14]. In the current study, the synthesized AgNPs by *P. guajava* and *L. inermis* showed moderate antioxidant activity with 68.1 and 71.9% inhibition, respectively (Table 2). However, significant antioxidant activities ($p < 0.05$) were obtained for *P. guajava* and *L. inermis* extracts (89.7 and 90.3%, respectively) which is comparable to L-ascorbic acid (90.7% inhibition). Therefore, it is worth noting that the obtained antioxidant activity in the first case is mostly due to AgNPs and is not attributable to the capping compounds, as the antioxidant polyphenolics were involved in the reduction of Ag^+ to Ag^o and become deactivated. This further explains the role of polyphenolics in the green synthesis of AgNPs, and why the antioxidant activity of the synthesized AgNP is lower than that of the extract. These results are in full agreement with the previously published review that correlated the antioxidant activity of green synthesized AgNPs to both the surface coating material as well as the silver nano-sized particles [25].

Previous studies reported the potential use of silver nanoparticles as cytotoxic agents [8,9]. The cytotoxic activity of *P. guajava*-synthesized AgNPs showed no significant difference from that of the corresponding plant extract ($p < 0.05$). However, *L. inermis*-synthesized AgNPs showed a significant increase ($p < 0.05$) in the cytotoxic activity towards both MCF-7 and HCT-116 (IC_{50} 55 and 56.6 µg/mL, respectively) compared to the leaves extract of this plant which showed higher IC_{50} values against the investigated cell lines (>100 and 80 µg/mL, respectively). Consequently, the above findings suggested that the cytotoxic activity is attributed to both the AgNPs and the surface capping material formed by the plant phytoconstituents. In other words, the obtained cytotoxic activity is a result of a synergetic action between AgNPs and the plant phytoconstituents, as in the case of *L. inermis*.

Phytochemical investigation of the active extracts of *P. guajava* and *L. inermis* afforded eight known compounds categorized as simple phenols (**G1**, **H1**, and **H2**) and flavonoids (**G2**-**G5**, and **H-3**), Figure 4. Generally, pyrogallol **G1** was more active than flavonoids (**G2**-**G5**, and **H-3**) in the green synthesis of AgNPs, Table 4. For pyrogallol, it can be concluded that the occurrence of *ortho*-dihydroxy phenolic function is accountable for reducing Ag^+ and stabilizing the synthesized AgNPs according to the outlined mechanism, Figure 5.

Meanwhile, for flavonoids, the 3-OH is important for the activity, as in the flavonol **G2** (quercetin) that showed higher activity compared to the

flavone **H3** (luteolin), Table 4. However, luteolin is still active due to the presence of 3`,4`-*ortho* dihydroxyl groups of B-ring, indicating their contribution to the activity. The glycosidic linkage present in the 3-*O*-pentosides series did not influence the activity, as deduced from the results of compounds **G3 - G5** that exhibited nearly the same activity as their aglycone, quercetin **G2**. The suggested mechanism of reduction of Ag^+ and stabilization of the produced AgNPs by biomolecules having the flavonoid structure is demonstrated in Figure 6. Regarding lawsone **H2**, it almost has the same activity as flavonoids. Regarding the aromatic acid, *p*-coumaric acid **H1**, it showed no significant activity due to the lack of the *ortho*-dihydroxyl groups, which is shown to be important for the AgNPs synthesis activity.

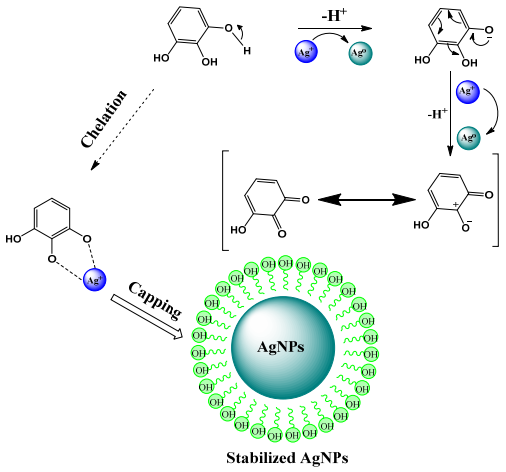

Figure 5: Proposed mechanism for synthesis of stabilized AgNPs by pyrogallol **G1**

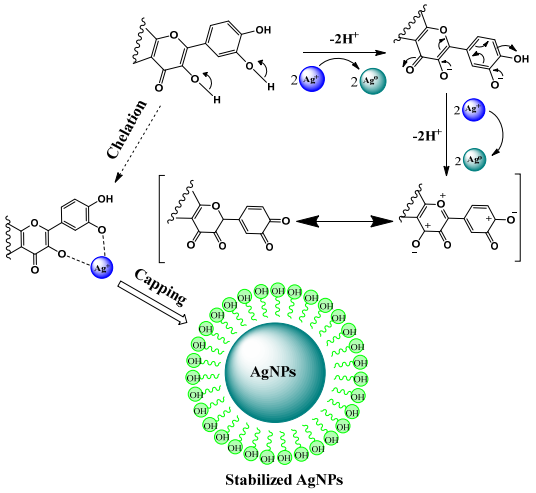

Figure 6: Proposed mechanism for synthesis of stabilized AgNPs by flavonoids

CONCLUSION

AgNPs with good morphological stability have been prepared from the leaf extracts of *Psidium guajava* and *Lawsonia inermis*. Furthermore, the extracts afforded seven compounds and revealed that simple phenols and flavonoids, especially *ortho*-dihydroxy bearing structures, may have a major role in the reduction of Ag^+ and coating of the synthesized AgNPs. The findings further reveal that while the synthesized AgNPs forthrightly contributed to the antioxidant effect, their cytotoxic activity may involve both AgNPs and the surface capping material formed by the plant phytoconstituents. Therefore, more research is needed to elucidate the anticancer mechanism of AgNPs synthesized by these natural products.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors contributed to the research concept and design, critical revision, and final approval of this article.

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