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Biosynthesis, Characterization, Radical Scavenging and Antimicrobial Properties of *Psidium guajava* Linn Coated Silver and Iron Oxide Nanoparticles

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Abstract

The present research describes a rapid, green, and convenient method for bio-stimulated preparation of silver and iron oxide nanoparticles using the aqueous leaf extract of *Psidium guajava* Linn (PG). The physicochemical properties of bio-synthesized nanoparticles were determined through UV/Visible, SEM, EDX, and FTIR spectroscopic techniques. The wavelength maxima (nm) at 435 and 300 depict silver and iron oxide nanoparticles' formation, respectively. Scanning electron microscopic analysis infers the spherically shaped silver (81.4-102 nm) and iron oxide (80.3-99.1 nm) particles. The energy dispersive X-ray technique accomplished elemental mapping. The *in vitro* antioxidant properties of silver and iron oxide nanoparticles were assessed using the DPPH (1,1-diphenyl-2-picryl-hydrazil) method with IC_{50} values of 85.39±0.28 and 78.6±0.63, respectively. The antimicrobial potential was evaluated against selected Gram-positive and Gram-negative bacterial and fungal strains by the agar well diffusion method. The significant zone of inhibition against microbial pathogens reveals the potential industrial and biomedical applications of obtained nanoparticles. The antimicrobial prospective of guava extract-coated iron oxide nanoparticles has been studied for the first time against *Bacillus pumilus*, *Streptococcus faecalis*, *Streptococcus pneumoniae*, and *Candida albican*. The current report envisions the comparative evaluation of these nanoparticles' antimicrobial and antioxidant characteristics for the first time from this plant.

Keywords: Green synthesis; Metallic nanoparticles; *Psidium guajava* Linn.; Radical scavenging property; Antimicrobial activity

1. INTRODUCTION

The biogenic reduction of metal ions into nanoparticles is considered a rapid, non-toxic, low-cost, and eco-benign approach in nano-biotechnology. Metallic nanoparticles (MNPs) exhibit unique chemical and physical properties; therefore, they have found great attention in textile [1], food coatings and cosmetics [2], electronics, engineering, catalysis, optics, and medicines [3]. A vast number of applications are emplaced due to numerous interesting properties including high magnetic permeability, good chemical and colloidal stability, size, surface modifications, dispersion in aqueous media and cost-effectiveness [4-6]. However, nanomaterials' preparation requires energy and time-consuming processes that are complicated and produce harmful effects on the environment and health [7]. In recent years, the plant extract is considered an appealing eco-benign process for nanoparticle formation. The metabolites inherent in plant work as potential stabilizing and reducing agents in bio-stimulated synthesis. These natural constituents also control nanoparticles' sizes and morphologies during the process [8-10]. In the present work, aqueous leaves extract of *Psidium guajava* Linn. (Guava) is used for bio-reduction of silver and iron ions. *Psidium guajava* (PG) belongs to the *Myrtaceae* family and consider as a valuable traditional medicinal plant in Asia and Eastern Europe. The plant (Figure 1) has worth mentioning antioxidant, antimicrobial, anti-diarrheal, anti-malarial, anti-inflammatory, and anti-tumor effects due to numerous bioactive compounds phyto-constituents including alkaloids, terpenoids, flavonoids, polyphenols, polysaccharides, proteins,

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enzymes, and ascorbic acid [11-13]. These biomolecules mentioned above actively coordinate with silver and iron ions to facilitate the formation of stabilized nanoparticles. Therefore, PG can be considered as an efficient chelating and stabilizing agent for nanoparticle synthesis. The lesser availability of literature on iron oxide particles from *Psidium guajava* also reflects current research's significance. The process we report herewith is cost-effective, simple, environment friendly, and rapid for the synthesis of silver and iron oxide nanoparticles. The subsequent nanoparticles were characterized and compared for their optical, morphological, and biological properties. The antioxidant potential of nanoparticles was estimated by the DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging method, and the antimicrobial activities against various pathogenic cultures of Gram-positive and Gram-negative bacteria and a fungus were screened by the disc diffusion method. These particles inhibit pathogenic bacteria's growth by attachment or penetration on bacterial surfaces *via* electrostatic forces through different mechanisms [14]. Therefore, silver and iron oxide nanoparticles obtained from *Psidium guajava* have strong potential for biotechnological applications and can be efficiently utilized against infection-causing bacteria. This systematic comparison introduces the utilization of silver and iron oxide nanoparticles against infection causing microbes, which has not yet been explored from this plant.

Figure 1. *Psidium guajava* Linn.

2. EXPERIMENTAL SECTION

Reagents and instrumentation

Analytical grade salts (silver nitrate and ferric chloride) were purchased from Sigma Aldrich and thus used as received. Deionized water was used for solution preparation. The biogenic reduction of metallic ions in solution was recorded by UV–visible spectrophotometer (Beckman Coulter, DU-730) in the range of 200 to 800 nm. FT-IR characterizations were adopted in the range of 450 to 4000 cm^{-1} (Shimadzu IR-Prestige-21) by FTIR spectrophotometer, as KBr pellet in a quartz cell with 1 cm path length. The size and morphological characterization of nanoparticles were examined by SEM analysis (Scanning electron microscopy, JEOL from Japan, JSM-6380A; Sample coater model#JFC-1500), and the elemental composition was determined from the different area of the NPs by EDS (Energy-dispersive X-ray spectroscopy, JEOL Japan, Model No: EX-54175IMU, the sample were coated up to 300 ºA with gold).

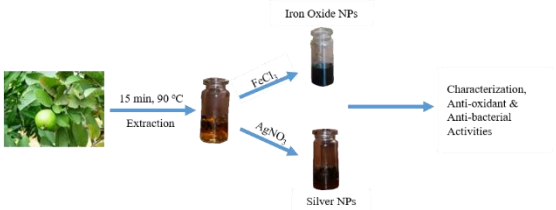
Preparation of Psidium guajava leaf extract

Freshly collected leaves of *Psidium guajava* Linn. were identified by Dr. Muneeba Khan (herbarium voucher number KUH-GH N0.53976, Department of Botany University of Karachi, Pakistan). The leaves were rinsed thrice with distilled water to remove the dirt. They were then thoroughly washed with deionized water, dried, and grounded in powder form. The dried plant powder (20 grams) was mixed with 200 mL of deionized water and allowed to boil at 90 ºC for 15 minutes. The resultant extract was cooled and filtered through Whatman filter paper 1. The yellowish filtrate was kept safely for further characterization.

Bio-mediated synthesis of silver and iron oxide nanoparticles

Five milliliters of PG extract was added in 1mM $AgNO_3$ (15 mL) with vigorous stirring at room temperature (for thirty minutes). The formed silver nanoparticles were visualized by color changes from yellow to dark brown (Scheme 1). The extract-coated silver nanoparticles (PG-AgNPs) were collected by centrifugation (4,000 rpm). These particles were purified with deionized water and acetone to remove unconverted silver ions and stored for further analysis.

The iron oxide nanoparticles were required little drastic conditions [15] and they were synthesized by adding PG extract (5 mL) to a solution of $FeCl_3$ (15 mL, 100 mM) with constant stirring for 30 minutes at 70 ºC. The pH 9 was adjusted by 1N of NaOH. The emergence of black color indicated the formation of nanoparticles, Scheme 1.



Scheme 1: Biosynthesis of silver and iron oxide nanoparticles using the extract of *Psidium guajava* Linn.

In vitro DPPH free radical scavenging activity

The antioxidant activities of the bio-synthesized nanoparticles from *P. guajava* were determined with the help of DPPH (1,1-diphenyl-2-picryl-hydrazil), based on reported methodology with little modifications [16]. In ethanol, 0.3 mM DPPH solution was prepared. Five micro litre sample of different concentration (50 µg - 100 µg) was mixed with 95 µl of DPPH solution. The reaction contents were then incubated in 96 well plate (37 ºC for 30 min). The absorbance was monitored at 517 nm. The result was calculated by using BHA (butylated hydroxyl anisole) as standard.

Antimicrobial activity assay by disc diffusion method

The antimicrobial activities of biosynthesized silver and iron oxide nanoparticles were tested by standard zone of growth inhibition and minimum inhibitory concentration assays against a series of human clinical pathogens; Gram-negative (*Escherichia coli*), Gram-positive (*Bacillus pumilus, Staphylococcus aureus, Streptococcus faecalis* and *Streptococcus pneumoniae)* and a fungi (*Candida albican*) by disc diffusion method **[**17**].** Clinically isolated microorganisms are used in this study which were maintained on tryptic soya agar slants at 4 ºC prior to testing and left to set at room temperature (for 30 minutes). Sterile cork borer was used in a well of 6 mm (diameter) in the center of each seeded plate. Antibacterial plates were incubated at 37 ºC ±1 ºC for 24 hours and antifungal assay plates were incubated at 28 ºC ±2 ºC for 48 hours. Particles were dissolved in dimethylsulfoxide (DMSO) to prepare a concentration of 5 mg/ml. Vernier caliper was used to measure the inhibition zone. All tests were repeated three times to minimize the error. For positive and negative controls, standard ampicilline (10 µg) and amphotericin B (20 µg) were used.

3. RESULTS AND DISCUSSION

UV-visible spectral analysis

The formation and stabilization of metal nanoparticles were monitored by UV-vis absorption spectroscopy. The UV-visible spectrum of *Psidium guajava* (guava) extract, biosynthesized silver, and iron oxide nanoparticles (shown in Figure 2). Ag-NPs usually absorb UV radiation between 400-450 nm. In our study, a sharp absorption band was observed at 435 nm, which confirms the reduction of silver ions into NPs. The characteristic peak in this region may have been due to the SPR (surface plasmon resonance absorbance) band [18-22]. The appearance of dark brown color by adding PG leaf extract (yellow color) to $AgNO_3$ solution also attributes to the formation of silver nanoparticles [23].

Similarly, the reduction of iron was ascertained by a convenient spectroscopic signature of color alteration from yellow to black for the formation of iron oxide nanoparticles. The absorption maxima's appearance at 300 nm is due to the electronic vibration of the conduction band (SPR) of iron oxide nanoparticles. The absorption band at a wavelength of 275-400 nm indicates the formation of iron oxide NPs as reported earlier [15, 24-25].

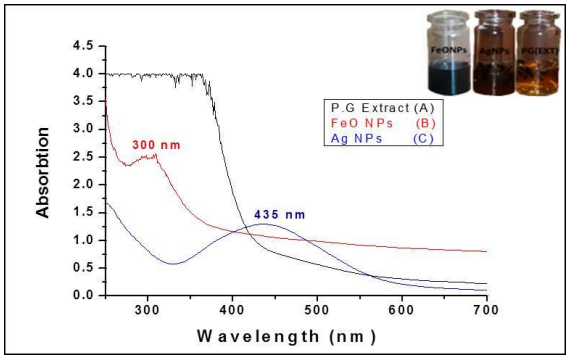


Figure 2. UV/Vis absorption spectra of (A) *Psidium guajava* extract (B) Iron oxide nanoparticles (C) Silver nanoparticles

Size and morphology (SEM) analysis

The surface morphology and size of particles were characterized by Scanning Electron Microscopy analysis. SEM images Figure 3 clearly demonstrated the uniformly distributed spherically shaped silver (81.4-102 nm) and iron oxide (80.3-99.1 nm) nanoparticles that phyto-capping agents stabilized. However, fewer particles with irregular morphologies were also observed due to flocculation during the nucleation and drying process.

Table 1. ***DPPH free radical scavenging activity of PG coated silver and iron oxide nanoparticles.***

Samples	**IC_{50} DPPH(µM)**
PG-AgNPs	85.39±0.28
PG-FeONPs	78.6±0.63
Butylated Hydroxy anisole (standard)	44.2± 0.24

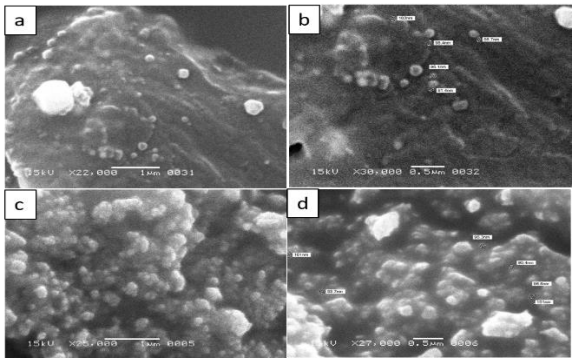


Figure 3. SEM images of nanoparticles at various magnifications: PG-AgNPs (a & b); PG-FeONPs (c & d)

Elemental (EDS) analysis

The elemental profile of PG-AgNPs was monitored by EDS, as illustrated in Figure 4(a). It contains intense signal peaks for silver particles followed by oxygen and carbon in the PG-NPs attributed to the presence of bioactive metabolites bounded to the silver nanoparticles' surface. The peak around 2.98 keV belongs to the binding energy of silver (AgLa). In general, elemental silver gives a signal in this energy region [26-27] while peaks located at binding energies of 0.27, 0.52, and 2.2 keV correspond to Cka, Oka, and ClKa, respectively similar to the reported earlier [28-29]. In the case of iron oxide nanostructure, the EDS spectrum is shown in Figure 4(b). The associated results confirmed the existence of iron, oxygen, carbon, and sulfur elements. The signal around 6.39 keV and 0.52 Kev belongs to Fe and O's binding energy, respectively. Other than these, binding energies of 0.27 and 2.30 Kev attributed Cka and Ska, respectively. The C and S signals are attributed mainly to bioactive metabolites or other sources [15].

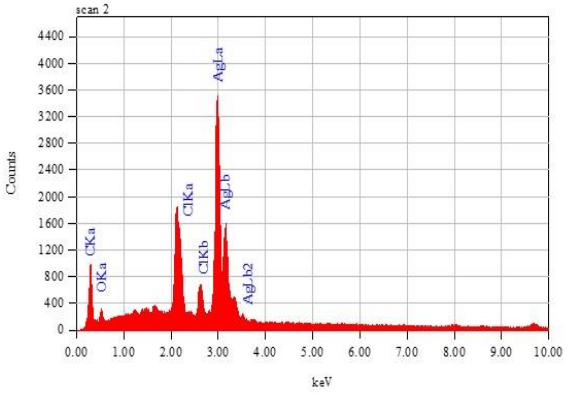


Figure 4 (a). EDX graph of PG-AgNPs

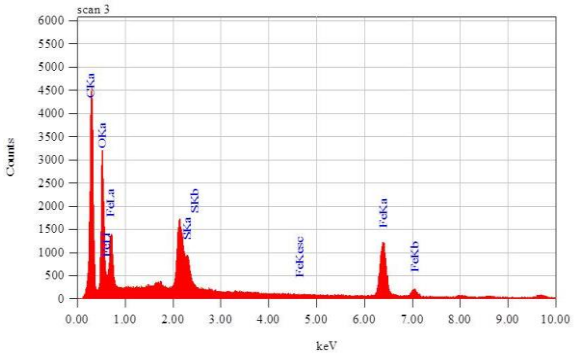


Figure 4 (b). EDX graph of PG-FeONPs.

Comparative FTIR analysis

The stretching vibrations of the extract at 3421, 2926, 1691, 1620, 1454, and 1107 cm^{-1} describe active groups' presence relating to salts' bio-reduction. The band observed at 3421 cm^{-1} indicates the OH group stretching vibrations present in polyphenolic compounds, C-H at 2926, C=O at 1691, and 1620 cm^{-1} in aldehyde, ketone, and amide assigned the presence of phenols, flavonoids, acids, and terpenoids. The IR absorption bands at 1454 cm^{-1}, 1373 $cm^{-1,}$ and 1107 cm^{-1}suggested the presence of other chemical functional groups, including C-C=C, N-O, and C-O. The out-of-plane banding vibrations at 771.5 and 623.0 cm^{-1} show the presence of alkenes and aromatic constituents in the extract [30-32]. The change in the hydroxyl (OH) absorption band and carbonyl (C=O) groups established the association of biomolecules on the surface of the silver and iron oxide nanoparticles shown in Figure 5. The infra-red spectrum of phyto fabricated silver nanoparticles is less complicated as compared with the plant extract itself. The disappearance of a peak at 1691 cm^{-1} may reveal some association of extract constituents with obtained nanoparticles (Figure 5). The change of the intensity and shifting of OH and C=O peaks confirms that the plant's biomolecules are capped on the surface of the particles. These results show that the involvement of some organic compounds of the extract act as reducing and capping agents in biogenic reduction of Ag+ ion to Ag [33]. The FTIR spectrum of FeO-NPs gave similar characteristic peaks as appeared for AgNPs with minor changes. However, the hydroxyl group's peak has been shifted towards the lower wavenumber of 3392.79 cm^{-1}. This shifting of peak reflects the strong association between the particles through H-bonding or electrostatic interactions. The IR peak below 600 cm^{-1} is due to the O-Fe bond's stretching mode, which is also clear evidence of effective immobilization of bioactive functional group on nanoparticles' surface as reported in the literature [34-36].

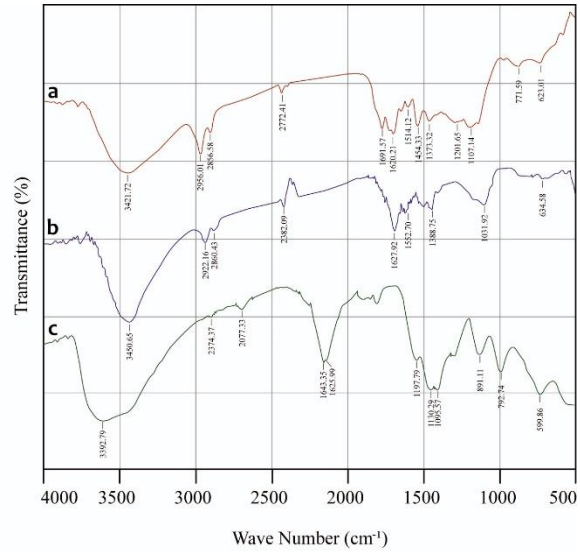


Figure 5. FT-IR spectrum of (a) PG extract (b) AgNPs and (c) FeONPs.

Antioxidant activities of PG-AgNPs and PG-FeONPs

The by the free radical scavenging DPPH reduction reduction activity of the FeONPs was found to be significantly moderate with IC_{50} 78.6±0.63, while the AgNPs showed weak scavenging properties with IC_{50} 85.39±0.28 as compared to BHA with IC_{50} 44.2±0.24 (Table 1). Our results also indicated the presence of phytoconstituents as polyphenolic and flavonoids which are responsible for antioxidant properties. However, some of these are not reduced rather they interacted in stabilization of nanoparticles [37]. Nevertheless, the information concerning the antioxidant comparison of nanoparticles by using *Psidium guajava* leaves extracts is unavailable.

Antimicrobial protocol of PG-AgNPs and PG-FeONPs from plant extract

The Agar Well-Diffusion (AWD) method was examined for testing the antimicrobial activity of guava extract coated nanoparticles (silver and iron oxide) against four Gram-positive, one Gram-negative bacteria, and a fungal strain (shown in Figure 6 and Table 2). The current study reveals the first time comparative assessment of antimicrobial potential of silver and iron oxide particles by the extract of *P. guajava* Linn. Silver nanoparticles were found to be more efficient than iron oxide nanoparticles at a concentration of 5mg/mL against the selected human pathogenic microbial strains. The zone of silver nanoparticles' inhibition was 24.6, 24.1, 19.0, and 15.8 mm against Gram-positive bacterial strains of *B. pumilus*, S. aureus, *St. faecalis*, and *St. pneumoniae*, respectively. These particles showed19.6mm Zone of inhibition (ZOI) against *E.coli* (Gram-negative bacteria strain); similarly, inhibition of 19.5 mm was observed against the fungus *C. albican.* For iron oxide nanoparticles, the ZOI was 14.5, 13.8, and 12.3 mm against S. aureus, *St. faecalis, and St. pneumoniae*, respectively, *whereas B. pumilus* did not show any inhibitory effect. The zone of inhibition for *E. coli* and *C. albican* was observed as 15.5 and 11.8 mm. The standard antibacterial Ampicillin drug (10 µg/disc) showed 30.5, 30, 29.5, 29.3, and 29.0 mm inhibitions against *E. coli*, *B. pumilus*, *St. faecalis*, S. aureus, and *St. pneumoniae*, whereas the fungal standard Amphotericin B drug (20 µg/disc) inhibited the *C. Albican* up to 28 mm. The cell membrane of tested bacterial and fungal pathogens disrupts when nanoparticles adhere to the cell surface and cause structural changes. Silver NPs can reach the cytoplasm more easily due to a greater extent of membrane damage than iron oxide NPs. According to the type and composition of micro-organism, the nanoparticles bind easily with S-containing proteins or nucleic acid and interfere with DNA replication leading to cell death [38, 39].

Table 2. ***Antimicrobial evaluation of PG coated silver and iron oxide nanoparticles.***

Microbial strains	**Zone of inhibition(mm)**				
	PG.AgNPs	PG.FeONPs	Extract	Ampicilline	Amphotericin
Gram-positive bacteria					
Staphylococcu saureus	24.1	14.5	ND	29.3	NA
Streptococcus pneumoni	15.8	12.3	15.1	29.0	NA
Streptococcus feacalis	19.0	13.8	10.0	29.5	NA
Bacillus pumilus	24.6	ND	10.5	30.0	NA
***Gram-negative* bacteria**					
Escherichia coli	19.6	15.5	ND	30.5	NA
Fungus					
Candida albican	19.5	11.8	9.1	NA	28.0

ND: Not detected, NA: Not applicable

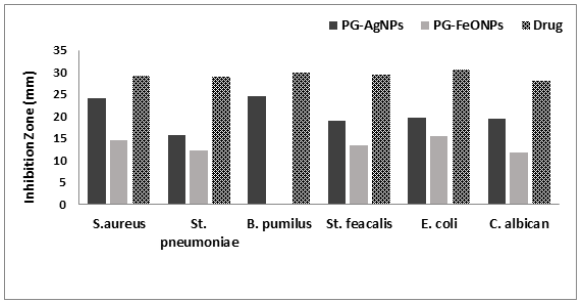


Figure 6. The antimicrobial assay of plant coated nanoparticles against selected pathogens

4. CONCLUSION

The present study describes the biosynthesis of silver and iron oxide nanoparticles from *Psidium guajava* plant extract leaves. These nanoparticles have been characterized by UV–vis, FTIR, SEM, and EDX analyses. We also compared the antioxidant and antimicrobial potential of these particles for the first time. Silver nanoparticles are found to possess weaker DPPH radical scavenging and higher antimicrobial properties as compared to iron oxide nanoparticles. In conclusion, this inexpensive and highly efficient single-step plant-mediated nanoparticle synthesis has promising applications for treating diseases caused by free radicals. It is also concluded that the synthesized nanoparticles may be utilized as low-cost, efficient coating materials in biomedical devices with applications in water purifications and as a remedy in various human ailments.

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