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A facile synthesis of green gold nanoparticles from fruit waste peels and their bioactivity evaluation against HT-29 colon cancer cell lines

Lakshmy Venkateswaran [a], Bindhu Radhakrishna Kamath [a], Somanathan Thirunavukkarasu [a,*], Rajkumar Thirunavukkarasu [a], Suresh Dhanaraj [b], Atchudan Raji [c], Rajabhuvaneswari Ariyamuthu [d]

[a] Department of Chemistry, School of Basic Sciences, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Pallavaram, Chennai 600 117, India
[b] Department of Microbiology, School of Life Sciences, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Pallavaram, Chennai 600 117, India
[c] School of Chemical Engineering, Yeungnam University, Gyeongsan 38541, Republic of Korea
[d] Department of Chemistry, Karpaga Vinayaga College of Engineering and Technology, Chengalpattu, India

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ABSTRACT

In this work, we report an eco-friendly green synthesis of gold nanoparticles (GNP) from $HAuCl_4.3H_2O$ using combined fruit peel (BOPA) extract waste as bioreductant. The synthesized Au nanoparticles coordination environment was observed an intensive peak at 525 nm with UV–Visible spectroscopy. The XRD showed highly ordered *fcc* structure. The FTIR showed presence of functional groups responsible for reduction and stabilization of Au^{3+} to Au^0 species. TEM analysis showed the presence of irregular spherical like shaped Au NPs distributed in sizes between 25 and 45 nm. Importantly, the biogenic gold nanoparticles showed considerable cytotoxicity towards HT-29 colon cancer cells, indicating a dose-dependent inhibition and possible pathways for inducing apoptosis. This research emphasizes the dual benefits of utilizing waste materials and advancing nanomedicine, setting the stage for sustainable cancer treatment options. The antibacterial activity was evaluated against E.Coli and S.*typhi.* Conclusively, the extract of BOPA (Banana, Orange, Pomogranate, Apple) rapidly reduces Au^{3+} to Au^0 and improves amalgamation of GNP with inhibiting property against abnormal cell growth and antibacterial activity. The bio-synthesized AuNPs demonstrated higher cytotoxicity than chemically synthesized counterparts, suggesting enhanced biocompatibility and efficacy. This green synthesis approach provides a sustainable and cost-effective method for nanoparticle production, with promising implications in cancer therapeutics.

1. Introduction

Green nanotechnology related to fabrication of nanoparticles using natural plant resources are gaining tremendous interest [1]. Among various kind of nanoparticles (NPs), biocompatible metal nanoparticles like Ag, Au, Zn NPs are being widely investigated [2]. In particular, gold nanoparticles are being comprehensively investigated in view of their biomedical applications [3]. Several literatures related to the multi-step chemical routes have been reported [4–7]. These methodologies openup for the fabrication of nanoparticles like laser ablations, ion implantation, high energy ball milling so on [8,9]. However, the experimental set up design are very costly, requires multi-step protocols, solvents and are potentially eco-unfriendly [10,11]. Several biosynthesis using microbial enzyme has been reported. For Au NPs, many biological substrates such as enzyme [12], fungus [13], and algae [14,15] have proved as a successful sources. The consumption of fruits and vegetables are steadily increasing worldwide [16]. In parallel, huge amount of horticulture waste remains unused and disintegrate into environment [16].

Green synthesis of metal NPs by fruit and vegetable waste as bio-reductant is prospected to be a cost effect and green technology [17–21]. However, only afew reports available related to the synthesis of NPs using fruit peels [22]. The occurrence of secondary metabolites like flavonoids and alkaloids, in crude peel extract is reported to help in bioreduction for generation of nanoparticles [23]. Cancer (Malignancy) caused by the uncontrolled cell growth pose a serious health issues [24]. The cancer burden is expected to increase to about 24 million by 2035. Recently, nano particle therapeutics (nanomedicine) has been attractive for drug therapy research. [25,26]. The mode of cancer treatment is widely through Chemotherapy [27]. Though such treatment is effective for selected cancers, it remains less effective for liver, skin, and breast

* Corresponding author.
E-mail address: soma.sbs@vistas.ac.in (S. Thirunavukkarasu).

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cancers. [28]. Recently, silver nanoparticles are utilized for anticancer treatment against mammalian cancer growth [29–31] such as langryl cancer, breast cancer, colon cancer etc.

In this study, we present a new eco-friendly method for producing gold nanoparticles using fruit peel extract as a reducing agent. This is the first report on the sustainable biosynthesis of gold nanoparticles and their bioactivity assessment against HT-29 colon cancer cell lines. The successful creation of gold nanoparticles has been verified through extensive characterization techniques, including UV–VIS, FTIR, XRD, and TEM analysis. Additionally, the synthesized gold nanoparticles were tested for antibacterial activity against *Escherichia coli* and *Salmonella typhi*.

2. Experimental

2.1. Materials

Raw fruits of Banana (*Musa acuminate)*, Orange (*Citrus sinensis*), Pomegranate (*Punicagranatum*), Apple (*Maluspumila*) peels were collected from Ulavarsandhai, Pallavaram, Chennai, Tamilnadu, India. Chloro auric acid (99 %) was obtained from Sigma Aldrich, USA. Dimethyl sulfoxide (99.9 %) was obtained from SRL, India.

2.2. Extraction of fruit peels

Fruit peels was gathered from koyambedu market, Chennai. A total of 12.5 g of each fruit peel waste was chopped into small pieces, rinsed twice with double distilled water, and then placed in a 250-mL beaker with 100 mL of distilled water. The mixture was stirred thoroughly and boiled for 15 min at a temperature of 50 °C. After one hour, it was filtered and allowed to cool to room temperature. Once filtered, the extract was stored for future synthesis.

2.3. Synthesis of gold nanoparticles at RT

The collected filtrate was treated with aqueous 0.1 mM $HAuCl_4$ solution to form gold nanoparticles. The yellow to brownish colour transformation further confirms the nanoparticle formations [17]. The results were in agreement with the previous report [18,32]. Finally, the particles was centrifuged at 6000–7000 rpm for 20–30 min. The solid deposits are collected and kept on hot air oven for drying purpose make into a powder in the need of particles characterizations.

2.4. Antibacterial activity of AuNPs

The well diffusion assay was used to test Au NPs against two specific microorganisms *S. typhi*, *E. coli* were harvested from the early stationary phase of their growth and their cultures' concentrations using sterile Sabourad Dextrose broth. The grown bacterial pathogens were swabbed on the MHB, then using cork borer wells were made and different concentrations of AuNPs were loaded separately, the zone of inhibitions was then measured using a zone scale after the plates were incubated at 37 °C for 16 h.

2.5. Characterization techniques of nanoparticles

The UV–VIS-NIR spectrum of effectively obtained NPs was collected with an (Shimadzu UV 5600 plus wide wavelength range 185–3300 nm with low noise 0.00003 abs at 1500 nm, Japan) spectrophotometer [33]. The FTIR spectrometer (Perkin Elmer, Spectrum two FT-IR/Sp10 software, USA) with KBr pellet was used for collecting functional group data in the region of 4000–400 cm-1. XRD measurement of the NPs, where only 5 mg sample was added, was done on a Rigaku smart lab, Japan diffractometer operating at a voltage of 9 kW with Cu-Kα radiation (λ = 1.5406 Å). The XRD spectrum has been examined and acquired with scanning range values of 20° and 80°. The inner morphology of the nanoparticles was studied using a HR-TEM (The Thermo Scientific Talos F200S G2, VeloX software, USA). For descriptive purposes, a 5 mg of the materials were sonicated in ethanol, and a drop of it was cast in a copper grid with a 300-mesh carbon layer by layer for magnetic measurements.

2.6. MTT assay

The standard MTT assay protocol has been followed as prescribed in our previous report [34]. Determination of the Minimum Inhibitory Concentration (MIC) using the Micro Broth Dilution Method combined with the MTT Assay. The test material is dispersed in 10 % of DMSO solution. The primary concentration of the sample was consecutively thinned in a 96 well plate and incorporated with 5 µl of suspension contains about 10^8 CFU ml^{-1} bacterial growth. The plates were incubated at 37 °C for 24 h. The intensity of culture from each well was monitored at 600 nm and the results were compared with control. The MIC of synthesized gold nanoparticles was determined at the least concentration leads to the inhibit the microbial growth.

3. Results and discussion

3.1. Physicochemical characterization

The visual confirmation for the transformation of Au nanoparticle using fruit peel extract was obtained by the rapid colour transformation from yellow to dark brownish was shown in the Fig. 1. The phytoconstituents present in the BOPA extract shows a band at 427 nm (Fig. 2 (a)) and aqueous $HAuCl_4$ shows a band at 306 nm (Fig. 2 (b)). After reduction of Au^{3+}, the formation of AuNPs with the presence of peak at about 525 nm (Fig. 2 (c)) due to surface plasmon resonance of AuNPs (Fig. 2). This result shows that gold metal ions are slowly reduced by an extract of combined peel contains active phytochemicals which is coincide with the previous investigation [31,35]. (See Table 1.)

3.2. X-ray diffraction studies

The XRD pattern of GNP showed crystalline phase with Bragg's reflection (111), (200), (220) and (311) at 2θ = 38.35°, 43.50°, 63.70° and 77.15° (Fig. 3). The peaks indicates the FCC structure of the GNP. The average size of GNP was determined using the Debye–Scherrer's equation [36].

3.2.1. TEM analysis

The Au NPs size and morphology was obtained using TEM analysis. The size of synthesized Au nanoparticles was measured based on the image of TEM (Fig. 4). The size distribution of NPs was about 38 nm. The results of TEM image (Fig. 4a–c) explained that the gold nanoparticles are low polydispersity spherical form with the diameter range of 25–50

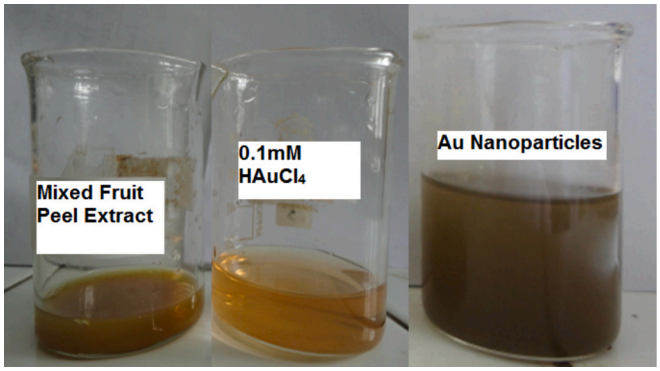

Fig. 1. Colour transformation from yellow to dark brownish of GNP. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

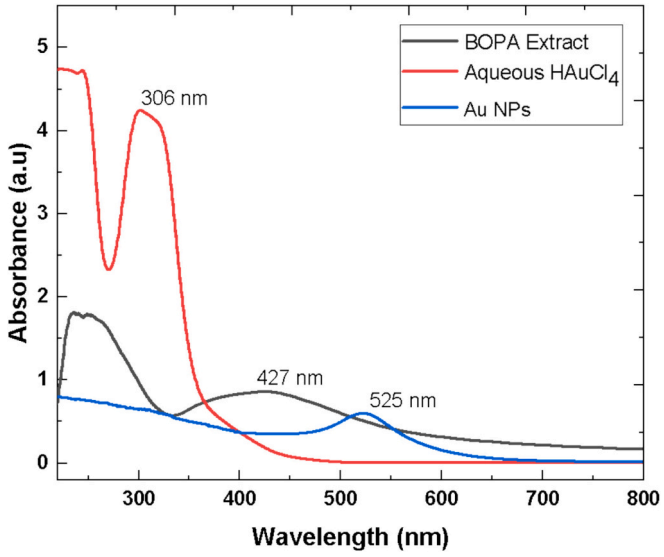

Fig. 2. UV–Visible spectra of (a) BOPA Extract, (b) aqueous $HAuCl_4$ and (c) Au NPs.

nm, influence due to the existence of carbon-based materials that execute the role of surfactant [37]. The interplanar distance (0.216 nm) of the samples synthesized was shown in Fig. 4b, which correspond to the distance from the plane (111) of gold nanopartilces. This result joined to the SAED (Fig. 4d & Fig. 4e) prove the high crystallinity of the AuNPs. The peak which denotes (111) is more strong than the other planes signifying that (111) is the major alignment and the synthesized GNP are crystalline in nature. The result were coincide with reported earlier in GNP [38,39]. Due to the symmetric fcc lattice of Au, the formation of anisotropic Au nanostructures is impossible due to symmetry breaking in fcc metals. The particle size of the GNP was around 38.33 nm (Fig. 4f).

3.2.2. FTIR analysis

FTIR analysis is employed for analysing the biomolecules of combined fruit peel extract represent in Fig. 5.1 which depiction the possible biomolecules present in the fruit extract which is responsible for the reduction of gold ions and its interaction with the AuNPs. The occurrence of flavonoids in the fruit extract which result in reduction of chloroauric acid [40] and the electrostatic repulsion are due to the presence of carbohydrate group [41,42]. The schematic representation of the reduction and stabilization of NPs by the phytochemical present in the fruit extract is shown in Fig. 6.

The key IR peaks observed for BOPA are at [provide wavenumbers, e.g., 3200 cm^{-1} (O—H stretching), 1650 cm^{-1} (C═O stretching), etc.], which correspond to functional groups such as [hydroxyl, carbonyl, etc.]. These peaks confirm the presence of bioactive compounds in the extract (Fig. 5.1).

The IR spectrum of AuNPs (Fig. 5.2) shows intense bands at 3269.24, 2926.01, 1751.36, 1585.49, 1276.88 and 796.70 cm^{-1}. The broad band at 3269.24 cm^{-1} corresponds to the stretching vibrations of —OH group of phenolic compounds. The weaker band at 2926 cm^{-1} decide to asymmetric stretching of —C—H groups. The sharp peaks at 1751.36 cm^{-1} and at 1585.49 cm^{-1} were assigned to the carbonyl stretching vibration (amide I) and a mixed vibration of NH deformation and CN stretch (amide II) in amides respectively. The peaks 1276 cm^{-1} denotes to C—N stretching vibrations of aromatic amines [43]. Flavonoids and polyphenols provide electrons to convert gold ions, while carboxyl and hydroxyl groups help stabilize the resulting nanoparticles, preventing them from clumping together. FTIR analysis verifies the existence of hydroxyl (—OH) and carbonyl (—C═O) groups on the gold nanoparticles (AuNPs), which enhance cellular interactions and boost anticancer effectiveness. The bio-functionalized surface may facilitate the internalization of nanoparticles via endocytosis, activating oxidative stress and apoptosis pathways in cancer cells. The synergistic effect of AuNPs and polyphenols results in greater damage to bacterial membranes, as demonstrated by TEM analysis. (See Fig. 10.)

3.3. Green approach of nanoparticles synthesis

Plants are rich in bioactive compounds like alkaloids, phenols, flavonoids, proteins, and vitamins. These compounds possess antioxidant properties that help reduce metals to their corresponding nanoparticles and act as stabilizing agents for these metal nanoparticles. Additionally, they aid in the functioning of metal nanoparticles within the human body without causing harm. Numerous studies have explored the synthesis of metal nanoparticles, including Pt, Mg, Ag, Zn, and Cu, using natural materials such as seeds, peels, leaves, and root extracts from plants. The findings indicate that noble metal nanoparticles are significant in medical, industrial, and environmental applications.

Peel extract contains large amounts of poly-phenol compounds like flavonoids and Phenolic groups corresponding to the mechanism of metal ion reduction [44]. More over the enzyme protein presentin the combined fruit peel extract get released and bind with Au^{+} ions to form

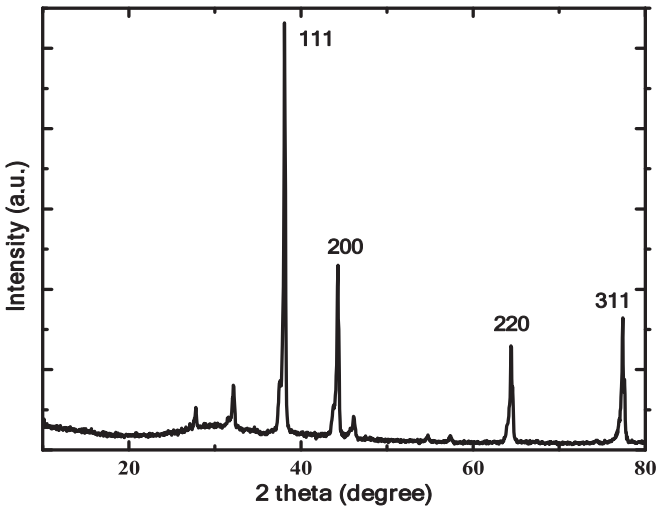

Fig. 3. XRD pattern of GNP.

Table 1
Comparative Studies with previous reported literature.

S·No	Plant name	Part used	Characterization Technique	NPs Shape	NPs Size	References
1	F. cirrhosa	Whole plant	UV–Vis, HR-TEM, XRD, and FTIR	Spherical	40–45	1
2	Dendropanax morbifera	Leaves	UV–Vis, XRD, TEM, DLS	Polygon and hexagon	100–150	2
3	T. polium	Leaves	UV–Vis, XRD, SEM, FTIR	Spherical	70–100	3
4	Scutellaria barbata	Whole plant	UV–Vis, FTIR, TEM, DLS, AFM	Spherical	154	4
5	Licorice	Root	UV–Vis, XRD, FTIR, FE-SEM, HPLC	Spherical	53	5
6	*Datura stramonium*	Whole plant	FT-IR, UV–vis, EDX, SEM	Spherical	75.1–156.5	6
7	Various fruits	Peels	UV–Vis, XRD, FTIR, TEM	Spherical	25–45	Present

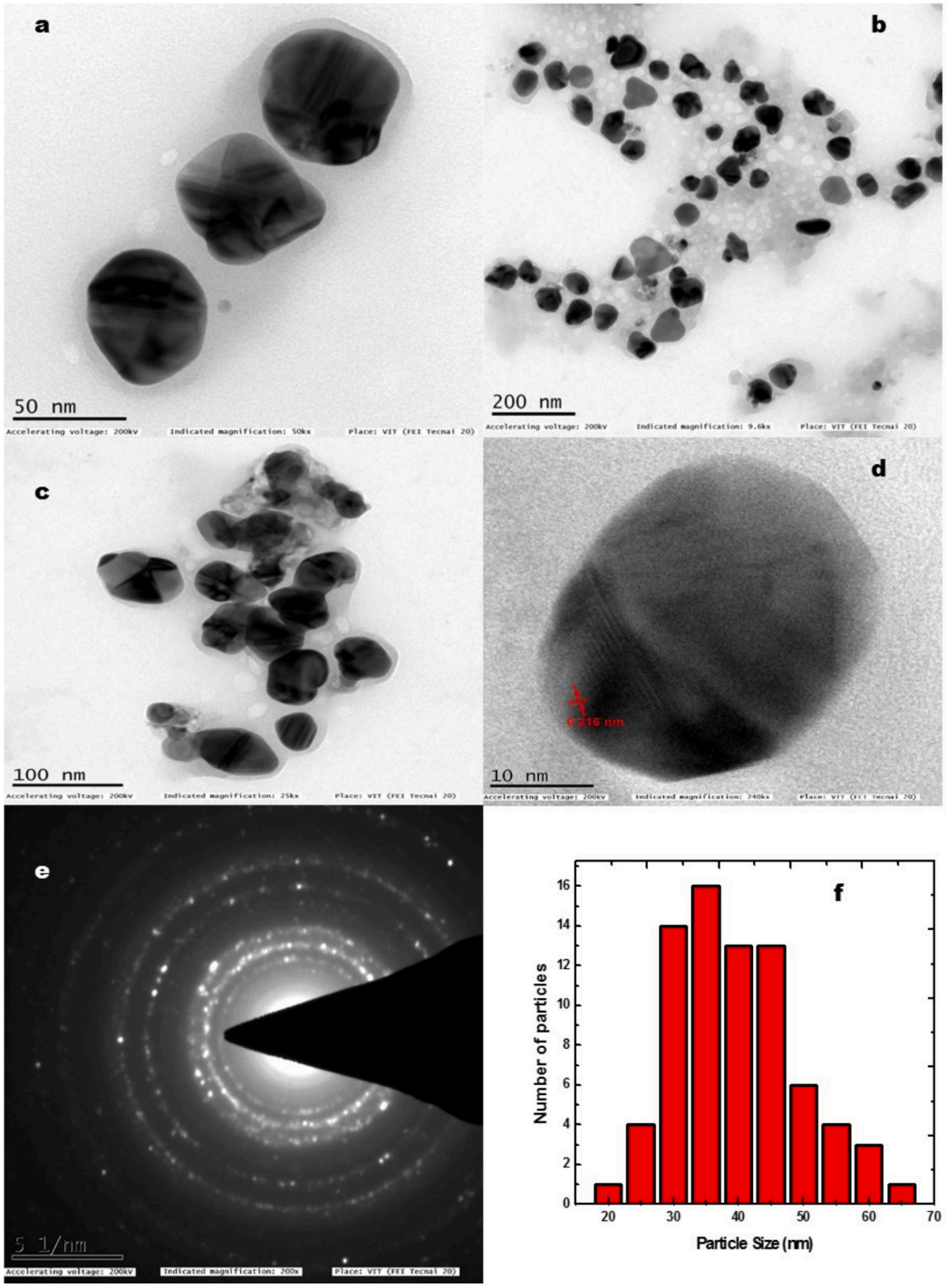

Fig. 4. TEM image of GNP.

protein substrate complex [45]. This enzyme induced the conversion of metal state ions into nanopartice formation with enzyme protein as capping agent.

BOPA mediated synthesized nanoparticles were examined to assess their antibacterial action against most pathogenic organisms including *Escherichia coli* and *Salmonella typhi* using disc diffusion method. Assessment of bactericidal properties of these nanoparticles was measured by level of zone of inihibition (Table 2a) and Statistical analysis of MIC values (Table 2b) .The result exposed that synthesized gold nanoparticles were possibly powerful in suppressing the microbial growth with variable strength. The best concentrate impeding microbial development of all tried pathogenic microscopic organisms at 10 μg/ml concentration, *E.coli.*was shown extensive inhibitory effect out of gram negative bacteria *S.typhi*. Since, these procedure were followed to evaluate their minimal inhibitory concentration (MIC) against the most liable bacterial strains (Fig. 7). The MIC effect of *S. typhi* and *E. coli* found at 3.5 μg/ml and 2.3 μg/ml with inhibition zones of 9.7 and 8.1 mm, respectively. We have performed the experiment triplicate to identify MIC effect and also provided the standard deviation graph (Fig. 7a & 7b).

The combined fruit peels waste mediated synthesis of Au NPs have been evaluated against two different type of bacteria which revealed better bactericidal activity. The result could be obtained owing to the ability of Au NPs adher to the bacterial outer layer by basic electrostatic

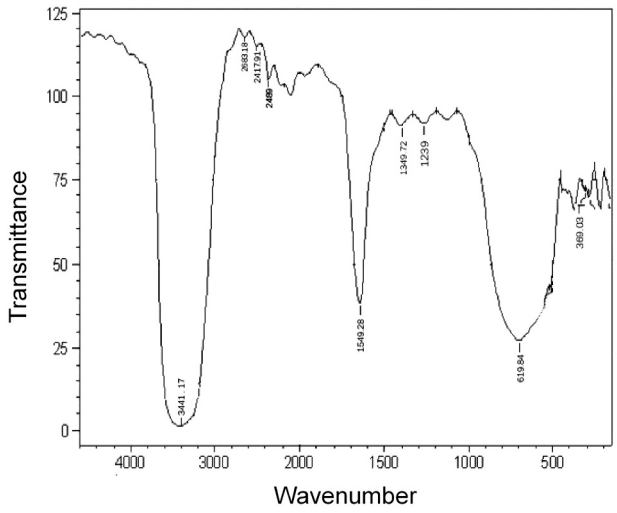

Fig. 5.1. FTIR analysis of peel extract.

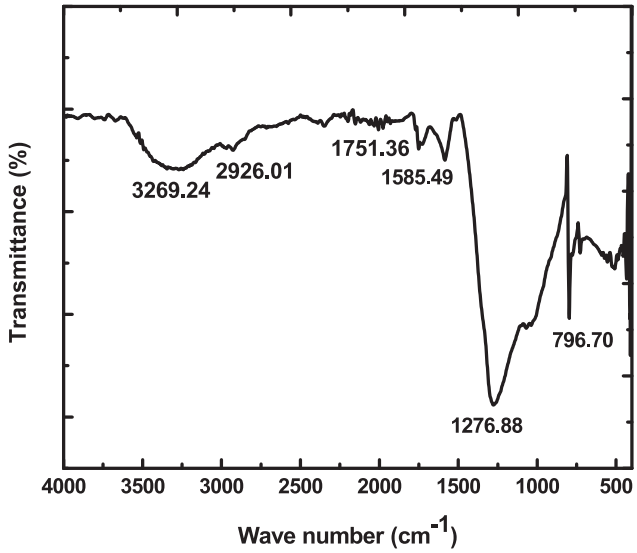

Fig. 5.2. FTIR analysis of GNP.

interaction to rapture the cell completely [46]. More over nanoparticles are tend to stop productions of Adenosine Triphospates (ATPs) in order to inhibit the metabolic pathway [36]. The pathogenicity of Au NPs with the bacterial cell wall to disturb the entire cell contents which can be used in oral drug clinical trials [47]. The free radicals are induced reactive oxidatative stress in order to implement the cell death [48]. The interaction between immoderate size Au NPs and bacteria relatively induce asymmetrical metabolic rate in order to increase intracellular reactive oxygen species (ROS) rapidly that accumulated bacterial death [49–51]. The potential usage of Au NPs in medical science field particulary for cancer treatment has been a subject matter of analysis, due to it's associated with cytotoxicity explored with entire human biomolecules like cells and tissues.

3.4. Cytotoxicity properties of AuNPs against colon cancer cell lines

The cytotoxicity properties of Au nanoparticles against the human colon cancer cells (HT-29) have been shown with cytotoxicity effect (Fig. 8). The cytotoxicity of nanoparticles is entirely depends on the dose treated with cancer cells. The peel mediated synthesized Au NPs expressed comparatively effective nano cytotoxicity against HT29 in concentration dependent manner at 50 µl followed by 40 µl, 30 µl, 20 µl and 15 µl. Several reports has shown the effect of Au NPs functionalized with biocompatible polymers against cancer cells [32, 50.51]. Likewise, silver nanoparticle also been a platform for treatment of several human cancer diseases such as human colon cancer HT 29 cell line [52]. The nano-cytotoxic impact of Au NPs is due to dynamic interation with proteins that are present inside of the cell wall, and also bind with DNA contents [53]. There are number of relevantresearch revealed already that the nano cytotoxic propertiesof metal nanparticles are expressed mainly by blocking tumor inducing proteins. Similarly other nucleic acid based immunization treatment also exhibited a sufficient impact on anti-cancer studies [54]. Fig. 9 represent the antitumour activity of Au NPs against HT 29 cancer cells. The cells were treated with different concentrations of Au NPs of 15 µl, 20 µl, 30 µl, 40 µl and 50 µl, and 0 will be control, respectively. The study shows an effective anti-cancer property of Au nanoparticles in dose dependent manner (Fig. 8).

The study evaluates the bioactivity of green gold nanoparticles (GNPs) only against HT-29 colon cancer cells. Testing on additional cancer cell lines and normal cell lines would provide a more comprehensive understanding of their therapeutic potential and cytotoxic selectivity.

The study does not explore the exact molecular mechanisms underlying the cytotoxic effects of GNPs on HT-29 cells, such as apoptosis induction, ROS generation, or specific signaling pathway modulation.

3.5. Statistical analysis and significance of results

All experiments were conducted in triplicate to ensure the reproducibility and reliability of the results. Data are presented as mean values accompanied by standard deviation (mean ± SD). Statistical

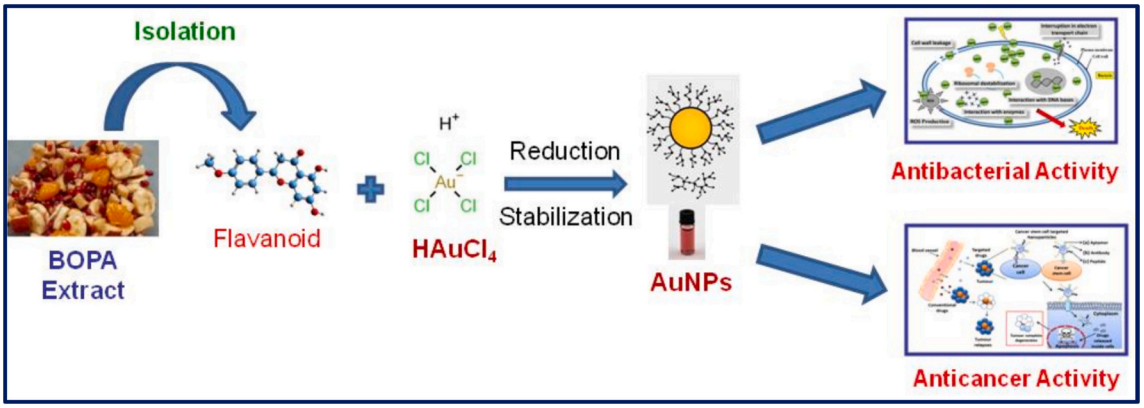

Fig. 6. Schematic representation of GNP synthesis.

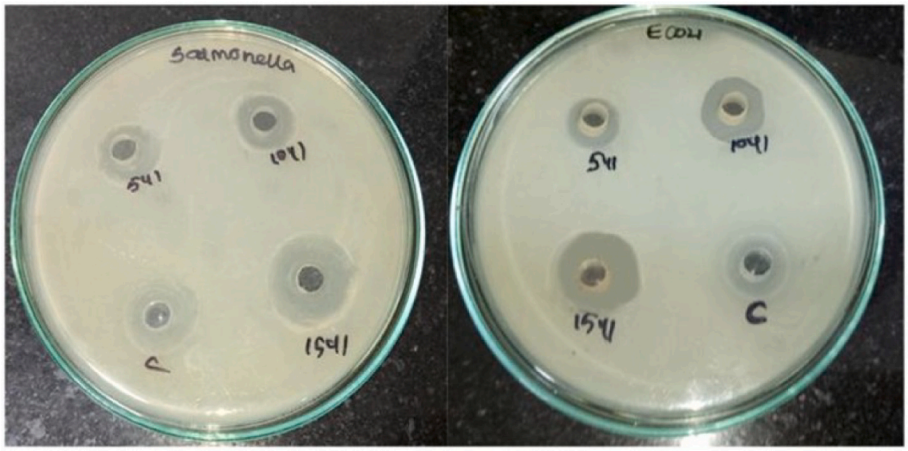

Fig. 7. Antibacterial activity of BOPA-mediated gold nanoparticles (AuNPs) against (a) *Salmonella typhi* and (b) *Escherichia coli* using the well diffusion method. Each well was loaded with different volumes of AuNPs: 5 μL, 10 μL, 15 μL, and control. In the petri plates, the wells are labeled accordingly — "5 μL", "10 μL", and "15 μL" represent the respective AuNP concentrations, and "C" denotes the control well containing standard antibiotic (amikacin). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

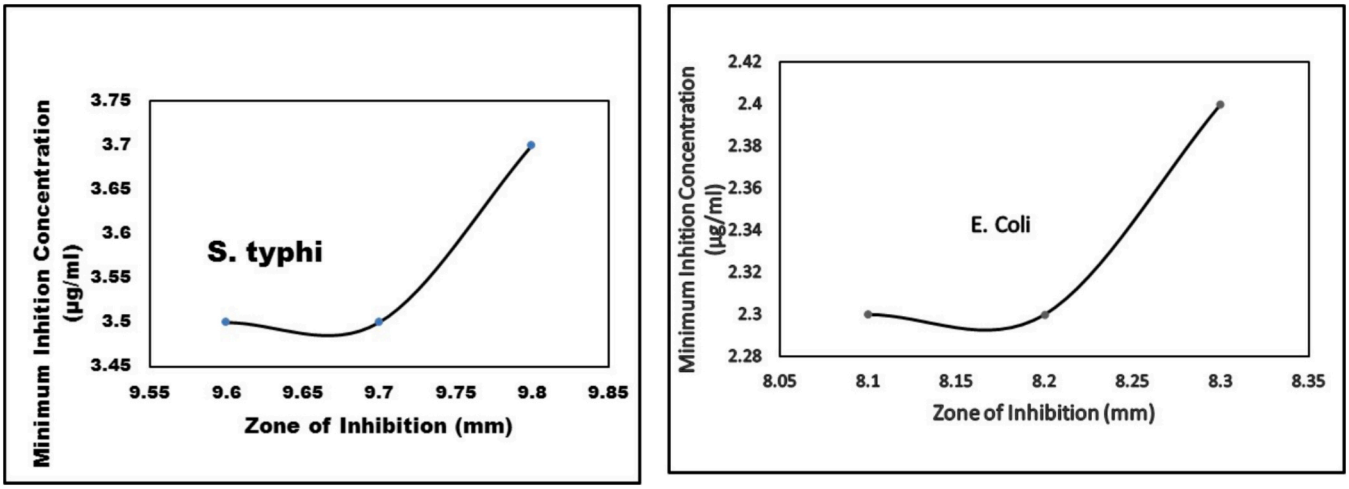

Fig. 8. (a) Standard deviation graph of *S.typhi* and (b) Standard deviation graph of *E.coli.*

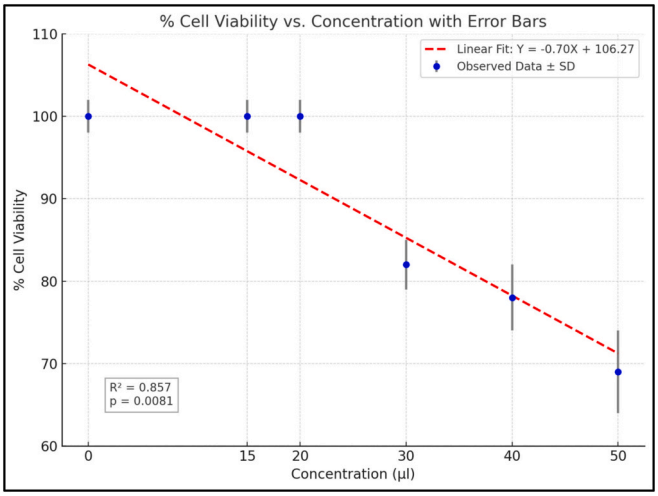

Fig. 9. Effect of concentration on % cell viability.

significance for both cytotoxic and antibacterial assays was assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A *p*-value less than 0.05 were considered statistically significant. All statistical analyses were performed using **SPSS version 26.0** (IBM Corp., Armonk, NY, USA).

For the MTT assay, cell viability percentages were calculated, and the IC_{50} values indicating the concentration required to inhibit 50 % of cell growth—were obtained through nonlinear regression analysis in SPSS. The data revealed a concentration-dependent cytotoxic effect of green-synthesized gold nanoparticles (GNPs) on HT-29 colon cancer cells, with statistically significant differences observed across increasing doses ($p < 0.05$). In the antibacterial assay, minimum inhibitory concentrations (MICs) were determined by identifying the lowest GNP concentration that effectively inhibited bacterial growth. Zones of inhibition (measured in mm) were analyzed using ANOVA to compare the antimicrobial effects of GNPs against *Escherichia coli* and *Salmonella typhi*. The statistical results confirmed significant antibacterial activity ($p < 0.05$), with *E. coli* showing a greater zone of inhibition compared to *S. typhi*. These analyses support the biological effectiveness and potential therapeutic applications of the green-synthesized gold nanoparticles.

Table 3 compares our findings with previous research on green-synthesized gold nanoparticles (GNPs), focusing on variations in

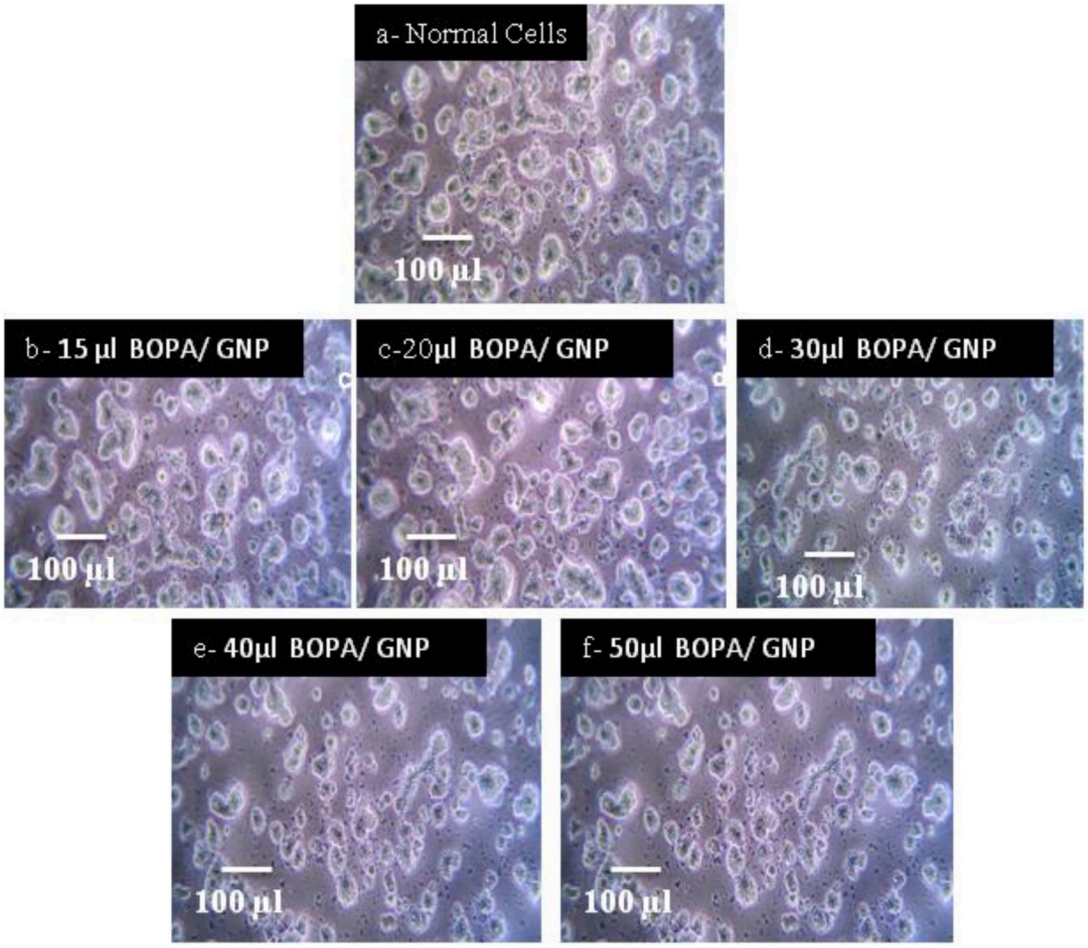

Fig. 10. Antitumour activity of MPE mediated GNP against HT 29 Cancer cell lines (a) normal cell and treated cells at different concentrations (b) 15 µl of BOPA mediated GNP, (c) 20 µl of BOPA mediated GNP, (d) 30 µl of BOPA mediated GNP, (e) 40 µl of BOPA mediated GNP and (f) 50 µl of BOPA mediated GNP.

Table 2a
Antibacterial activity of gold nanoparticles against selected bacteria through MIC. Values are expressed as mean ± standard deviation ($n = 3$).

	MIC values of synthesized GNP against *S.typhi* and *E.coli*			
Organisms	5 µl of AuNP	10 µl of AuNP	15 µl of AuNP	Control Amikacin
S. typhi	9.6 ± 0.12	9.7 ± 0.09	9.8 ± 0.15	10 ± 0.00
E. coli	8.1 ± 0.10	8.2 ± 0.11	8.3 ± 0.13	10.1 ± 0.00

Table 2
(b) Statistical analysis of MIC values for *S. typhi* and *E. coli*. Results are presented as mean ± standard deviation from three independent experiments.

	Mean Value + SD	Maximum Value	Minimum Value	Variance
S. typhi	60.32 ± 8.25	32.00	64.00	79.35
E. coli	61.44 ± 9.65	32.00	64.00	78.51

synthesis methods, particle size, stabilizing agents, and applications. Like other studies, our research highlights the eco-friendly benefits of green synthesis, particularly through the use of plant extracts and other biogenic sources for nanoparticle stabilization. This comparison emphasizes the increasing potential of green-synthesized GNPs across diverse applications, offering a more sustainable alternative to traditional chemical synthesis methods.

4. Conclusion

Green synthesis of biocompatible Au NPs using natural resource are most attractive in biomedical applications. We have shown that waste fruit peel can be used as bioreductant to reduce $HAuCl_4$ to Au NPs. The synthesized Au nanoparticles coordination environment was observed an intensive peak at 525 nm with UV–Visible spectroscopy. The XRD showed highly ordered *fcc* structure. The FTIR showed presence of functional groups responsible for reduction and stabilization of Au^{3+} to Au^0 species. TEM analysis showed the presence of irregular spherical like shaped Au NPs distributed in sizes between 25 and 45 nm. The characterization revealed that natural peel extract may act both as capping and reducing agent. Au NPs showed antibacterial effect with MIC effect of *S. typhi* and *E. coli* found at 3.5 µg/ml and 2.3 µg/ml with inhibition zones of 9.7 and 8.1 mm, respectively. The study showed an effective anti-cancer property of Au NPs in dose dependent manner. Au NPs fabrication mediated by plant products have advantage over the other chemical and physical based techniques. The reported synthesis protocol is simple and eco-friendly, that can be applied to different metal nanoparticles, thereby opening entirely new avenues for various applications. Further experimental evaluation is needed in in vivo studies.

CRediT authorship contribution statement

Lakshmy Venkatesan: Methodology, Investigation, Data curation. **Bindhu Radhakrishna Kamath:** Methodology, Formal analysis. **Rajkumar Thirunavukkarasu:** Validation, Data curation. **Suresh Dhanaraj:** Validation, Formal analysis. **Atchudan Raji:** Writing – review &

Table 3
Comparative summary of our findings against prior studies on green-synthesized GNPs.

Study	Sources	SPR Peak (nm)	Size (nm)	Antibacterial Activity (MIC, μg/mL)	Cytotoxicity (IC_{50}, μg/mL)
This Study	Banana, Orange, Pomegranate, Apple (BOPA)	525	25–45	*E. coli*: 2.3, *S. typhi*: 3.5	~30
Vijayakumar et al. (2019) [1]	Fruit Extract (*Aegle marmelos*, *Eugenia jambolana and soursop* extracts)	530	30–50	*E. coli*: 3.8, *S. typhi*: 5.0	35–40
Yang et al. (2014) [2]	Mango Peel	540	50–100	4.5 (average)	40+
Hamelian et al. (2018) [3]	Thyme	535	45–60	*E. coli*: 5.0, *S. typhi*: 6.2	~50
Ullah et al. (2016) [4]	Blackberry/Turmeric	532	30–55	*E. coli*: 6.2	N/A

editing. **Rajabhuvaneswari Aryamuthu:** Methodology, Data curation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data that has been used is confidential.

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