

Research Article

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Punica granatum peel extracts mediated the green synthesis of gold nanoparticles and their detailed *in vivo* biological activities

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Abstract: Requirements for developing new methodologies to biosynthesize nanoparticles are increasing day by day. The typical chemical synthesis of nanoparticles has raised concerns regarding environmental safety and adverse impact on human health. Therefore, there is an urgent need to develop green synthesized nanoparticles that are considered to be safe, ecofriendly, and cost-effective as compared to chemical approaches. Hence, in this study, we synthesized and characterized pomegranate peel extract-based gold nanoparticles (PP-AuNPs) through UV-visible spectroscopy, FT-IR, and AFM microscopy. Furthermore, the biological activities like analgesic, muscle relaxant, and sedative properties of synthesized PP-AuNPs were also determined. The change of color to dark ruby indicates the

formation of AuNPs. The surface plasma resonance (SPR) peak in the absorption spectra was shown at 525 nm by using (UV-Vis) spectroscopy. A single distinctive peak implied the shape of nanoparticles to be spherical. AFM images revealed that the biosynthesized nanoparticles were spherical in shape. Furthermore, the images confirm the uniform distribution of PP-AuNPs with particle sizes ranging from 4 to 16 nm. Different classes of phytochemicals were preliminarily identified in extracts. The analgesic effect of extracts (70.04%) and PP-AuNPs (81.98%) demonstrated a significant ($p < 0.001$) percent reduction in writhing at a dose of 100 and 15 mg·kg⁻¹, respectively. A mild muscle relaxant effect was noted against both the tested samples while a significant sedative effect was observed for both samples; however, PP-AuNPs were more sedative compared to the extract. Pomegranate peel extracts and synthesized PP-AuNPs were found to possess significant analgesic, muscle relaxant, and sedative properties.

Keywords: *Punica granatum* L., AuNPs, analgesic, muscle relaxant and sedative

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1 Introduction

Pomegranate (*Punica granatum* L.), indigenously known as “Anar,” belongs to the family Punicaceae. It is abundantly available in Pakistan, India, Iran, Afghanistan, Arizona, and California [6]. In folk medicine, *P. granatum* has been used for the treatment of several diseases like diarrhea, male infertility, fever, dysentery, and bleeding [1]. Evidently, various parts of pomegranate including peel, arils, flower, leaves, roots, and seeds possess phenolics (ellagic acid, punicalagin, ellagitannins, and punicic acid) that are responsible for its health-promoting benefits against diabetes, obesity, cancer, arthritis, Alzheimer, and cardiovascular diseases [2–5]. Among all these parts, pomegranate peel is an abundant source of ellagitannins, punicalagin, and punicalin. The peel of this fruit contains a

cache of polyphenols and has been utilized in different food, cosmetic, and medicinal formulations [6]. Phytochemical profiling of pomegranate peel has revealed an array of bioactive compounds responsible for its antioxidant and antimicrobial properties [7]. Inorganic compounds (silver and gold) are used for the synthesis of NPs to enhance the drug delivery model, antimicrobial, and antioxidant properties [8].

Nano-biotechnology helps significantly in developing new methodologies that may provide a better understanding regarding interactions among nanomaterials and intracellular structures, the process, and the environment. The synthesis of nanoparticles is known to be a key aim in nano-biotechnology. In this context, research studies carried out nowadays are focused on the bio-fabrication of nanoparticles. The synthesis of NPs is preferred using natural resources to have green and eco-friendly end products as compared to traditionally fabricated NPs from physicochemical synthetic routes [9]. Green nano-technological methodologies are characterized by employing natural sources like biomolecules, plants, fungi, and bacteria for the effective synthesis of nanoparticles [10]. Numerous factors such as pH, temperature, reaction time, and content of plant extract have been reported to influence the synthesis of nanoparticles [11]. Amongst all the materials used for the synthesis of nanoparticles, gold (Au) has attained a significant spot with special reference to its biomedical applications. Hence, the production of gold nanoparticles (AuNPs) is a keen area of interest for different disease therapeutics, specifically cancer, owing to their diversified applications, i.e., target drug delivery, cellular bioimaging, and gene therapy [10,12–14].

As pomegranate peel is an agro-industrial byproduct, therefore, in this study, valorization was achieved via green production of gold nanoparticles to enhance their biomedical applications. Keeping in view the significance of nanoparticles and the pomegranate peel extract, the current study focused on the synthesis of pomegranate peel-based gold nanoparticles (PP-AuNPs). These bio-synthesized PP-AuNPs were characterized using AFM analysis, FTIR (Fourier transform infrared spectroscopy), and UV-visible (UV-Vis) spectroscopy. The conversion of the traditional extract into nanoparticles is the demand of the modern era when scientists are in the search of targeted therapies. Therefore, such chemically modified shapes of extracts might be more fruitful for the treatment of various ailments. In the current study, pomegranate peel extracts and nanoparticles were subjected to various *in vivo* biological and phytochemical studies.

2 Materials and methods

2.1 Procurement of raw materials and chemicals

Pomegranate peels were procured from the local market of Peshawar, Pakistan. *P. granatum* peels were dried under shade followed by grinding to collect the respective powder samples [15]. Chemicals and reagents like sodium chloride, methanol, deionized water, and hydrogen tetrachloroaurate trihydrate [$\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$] used in this study were of analytical grade and were purchased from Merck.

2.2 Extract preparation

Powdered pomegranate peels were soaked in distilled water and methanol for 7 days followed by filtration through Whatman No. 1 filter paper. After filtration, the crude extract was concentrated using a rotary evaporator (45–50°C) [3].

2.3 Preparation of fractions

For the preparation of fractions, the pomegranate peel extract was dissolved in distilled water (100 mL) along with the addition of ethyl acetate (200 mL). Afterwards, the separating funnel was shaken at continuous intervals and was given stay time for the formation of two layers. Ethyl acetate was later evaporated by using a water bath in order to obtain the fractions. A similar procedure was adopted for the collection of chloroform and butanol fractions [16].

2.4 Synthesis of pomegranate peel-based gold nanoparticles (PP-AuNPs)

The pomegranate peel extract (5 mL) was mixed with HAuCl_4 (1 mM) in a titration flask along with continuous stirring at room temperature for 4 h. The formation of PP-AuNPs was confirmed from the color (dark ruby) of the solution [17].

2.5 Characterization of the prepared PP-AuNPs

UV-visible spectrophotometry was used to monitor the synthesis of AuNPs at wavelengths ranging from 300 to 700 nm. AFM (atomic force microscopy) was carried out to characterize the particle size of AuNPs, while FTIR analysis was performed to identify probable functional groups responsible for bioreduction.

2.6 Kinetic studies and stability of PP-AuNPs

Kinetic studies were performed to find the time-dependent synthesis of PP-AuNPs. The prepared samples were taken out from the reaction mixtures at specific time intervals followed by UV analysis. For assessing the stability of the prepared PP-AuNPs under various pH conditions, the samples were subjected to different pH intervals. For adjustment of pH, either 1 M HCl or NaOH was added dropwise to adjust the pH value between 2 and 14 of the prepared PP-AuNPs. After each treatment, the UV-Vis spectrum was obtained.

2.7 Phytochemical screening

For determination of phytochemical screening (secondary metabolites) of the pomegranate peel extract and their subfractions, standard protocols were adopted [18–24].

2.7.1 Alkaloids

For this test, crude extracts and their subfractions (0.5 g) were mixed with H_2SO_4 (2%) and warmed for 2 min. Afterwards, this mixture was cooled and filtered followed by the addition of Dragengroff's solution. The appearance of precipitates (orange red) indicates the presence of alkaloids.

2.7.2 Tannins

For the determination of tannins, crude extracts and their subfractions (0.2 g) were mixed with water and heated in a water bath. Moreover, the mixture was filtered followed by dropwise addition of Ferric chloride in each filtrate.

Conclusively, the appearance of dark green color precipitates indicated the presence of tannins.

2.7.3 Anthraquinones

For this purpose, each extract and its subfraction (0.5 g) was heated with hydrochloric acid (10%) for a few minutes in a water bath. The resultant mixtures were cooled under tap water and filtered. The same volume of CHCl_3 along with 10% NH_3 (a few drops) was mixed in each filtrate. The presence of anthraquinones was indicated by the production of rose-pink color.

2.7.4 Glycosides

To assess the presence of glycosides, crude extracts/subfractions (0.3 g) were hydrolyzed with HCl followed by neutralization in the presence of NaOH. Afterwards, in each mixture, Fehling's solution A and then B were mixed (a few drops). The formation of precipitates (red color) demonstrates the occurrence of glycosides in respective crude extracts/subfractions.

2.7.5 Reducing sugars

For this purpose, crude extracts/subfractions (0.3 g) were thoroughly mixed in distilled water. After filtration, a few drops of Fehling's solution A and B were added to each filtrate and resultant mixtures were subjected to a few minutes of boiling. The formation of precipitates (orange-red) reveals the presence of reducing sugars.

2.7.6 Saponins

Initially, in this test, 0.5 g of each crude extract/subfractions were mixed thoroughly with distilled water. Later, the resultant reaction mixtures were boiled. Froth formation confirms the presence of saponins.

2.7.7 Flavonoids

For the determination of flavonoids, crude extracts/subfractions (0.2 g) were mixed with NaOH (diluted) followed by the addition of HCl (a few drops). On the addition of HCl, the color change from yellowish to colorless indicated the presence of flavonoids.

2.7.8 Phlobatannins

In order to detect phlobatannins, crude extracts/subfractions (0.5 g) were mixed thoroughly with distilled water followed by filtration. Afterwards, the mixture was boiled in the presence of hydrochloride (2%) solution. The appearance of precipitates (red) revealed the presence of phlobatannins.

2.7.9 Steroids

For this purpose, crude extracts/subfractions (0.3 g) in a test tube were added along with acetic acid (a few drops). Later, the mixture was heated on a burner followed by cooling, and then H_2SO_4 was added dropwise. The color change of the mixture to green indicated the occurrence of steroids.

2.7.10 Terpenoids

For this test, crude extracts/subfractions (0.3 g) were mixed with CHCl_3 (2 mL) followed by filtration. Further, the obtained filtrates were again mixed with H_2SO_4 (3 mL), shaken, and allowed to stand for a few minutes. The occurrence of golden-yellow color showed the presence of terpenoids.

2.7.11 Caumarine

About 1 mL of aqueous crude extracts/subfractions were treated with 10% NaOH (3 mL). The appearance of yellow color indicated the presence of caumarine.

2.7.12 Emodin

About 0.5 g of crude extracts/subfractions was treated with NH_4OH (2 mL) followed by the addition of benzene (3 mL). The formation of red color indicated the presence of emodine.

2.7.13 Anthocyanin and betacyanin

Crude extracts/subfractions (0.2 g) were mixed with 2 N NaOH (1 mL) and heated for 5 min at 100°C using a flame burner. The appearance of bluish-green color revealed the presence of anthocyanin, whereas yellow color indicated the presence of betacyanin.

2.7.14 Carbohydrates

For determination of the presence of carbohydrates, crude extracts/subfractions (0.5 g) were mixed in distilled water followed by the addition of a few drops of Molisch's reagent. Afterwards, conc. H_2SO_4 (1 mL) was added to it and after 2 min stay time, distilled water (1 mL) was again added. The production of red/dull violet color in the inter-phase of two layers was noted as a positive test.

2.8 Biological applications of synthesized PP-AuNPs

2.8.1 Analgesic activity

The acetic acid-induced writhing *in vivo* paradigm was used for the assessment of the analgesic potential of extract and PP-AuNPs. Animals were classified into different groups ($n = 8$). Distilled water was used for treating the negative control group ($10 \text{ mL}\cdot\text{kg}^{-1}$, I.P.), the positive control group received Diclofenac sodium ($10 \text{ mL}\cdot\text{kg}^{-1}$, I.P.), and the tested groups were treated with extracts ($10, 25, 50$, and $100 \text{ mg}\cdot\text{kg}^{-1}$, PO), and PP-AuNPs ($2.5, 5, 10$, and $15 \text{ mg}\cdot\text{kg}^{-1}$, PO). All the animals were injected with 1% acetic acid solution (IP) after 30 min of the above treatments. After 10 min of the acetic acid injection, the number of abdominal contractions (writhings) was counted (for 10 min) for each group ($n = 8$) of animals [25].

2.8.2 Muscle relaxant activity

2.8.2.1 Inclined plant test

For the evaluation of fixed oil for muscle co-ordination effect, a plane of two wood was used in such a way that an angle of 65° resulted from the connection. Distilled water was used for treating the negative control group ($10 \text{ mL}\cdot\text{kg}^{-1}$, I.P.), the positive group was injected with diazepam ($1 \text{ mg}\cdot\text{kg}^{-1}$), and the tested groups were administered the extract ($10, 25, 50$, and $100 \text{ mg}\cdot\text{kg}^{-1}$, PO) and PP-AuNPs ($2.5, 5, 10$, and $15 \text{ mg}\cdot\text{kg}^{-1}$, PO). After 30, 60, and 90 min of the above treatment, animals were tested for the muscle coordination effect as it was placed on the upper part of the inclined plane for 30 s to hang or fall. This method is the modified form of our published method [26].

2.8.2.2 Traction test

In this *in vivo* paradigm, the animal was classified and treated as an in the inclined plane model. But, in this case, a metal wire coated with rubber was used, both ends of which were rigidly supported with a stand about 60 cm above the laboratory bench. The animals were exposed to the traction test after 30, 60, and 90 min of treatment. Each animal was hung by its hind legs from the wire and the time of hanging was recorded for 5 s. The failure to hang for less than 5 s was considered as the presence of muscle relaxant activity and *vice versa* [26].

2.8.3 Sedative activity

For the evaluation of the sedative effect of the extract and PP-AuNPs, a special box was used. The floor of the box was covered with a white sheet (150 cm diameter) and was divided into 20 squares by black lines. This open field box was placed in a soundproof experimental room. Distilled water was used for treating the negative control group ($10 \text{ mL} \cdot \text{kg}^{-1}$), the positive group was injected with diazepam ($1 \text{ mg} \cdot \text{kg}^{-1}$), and the tested groups were administered the extract (10, 25, 50, and $100 \text{ mg} \cdot \text{kg}^{-1}$, PO) and PP-AuNPs (2.5, 5.0, 10.0, and $15.0 \text{ mg} \cdot \text{kg}^{-1}$, PO). After 30 min of the above administration, each animal was tested for the sedative effect by placing them in the centre of the box, and the number of lines crossed by each animal was counted. The smaller number of lines crossed indicated the sedative effect [27].

2.9 Statistical analysis

All results of the biological screening are shown as the mean \pm standard error of the mean (SEM), and the obtained results were evaluated by one-way analysis of variance (ANOVA). Statistical analysis was performed with the assistance of Dunnett's multiple assessment screening using GraphPad prism 5; the difference was significant at $p \leq 0.05$.

3 Results and discussion

3.1 Selection of Au NPs and the extract ratio

Figure 1 shows the UV-Vis spectrum of various experimented ratios of the gold and extract solution (Au:E). The graph almost showed similar peaks in the range of

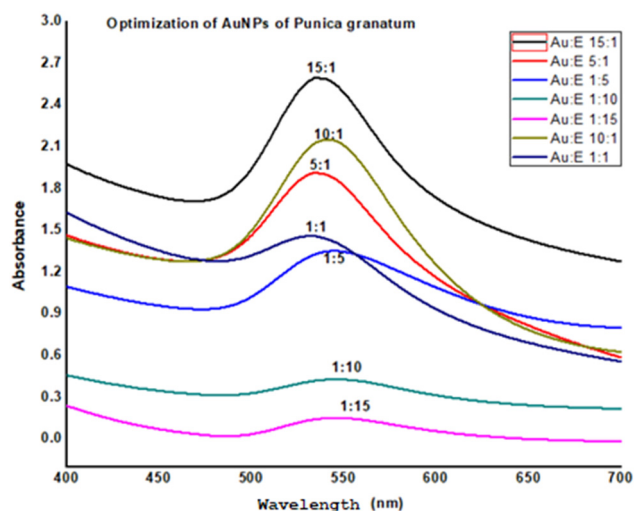


Figure 1: UV-Vis spectra of the synthesized gold nanoparticles of *P. granatum* at various experimental ratios of gold and the extract (Au:E).

540–550 nm. By varying the ratio of gold and the extract, the peak intensity changed. The sharpness of the peaks shows the uniformity of Au NPs. Based on the peak sharpness and intensity, the best ratio of 10:1 was selected due to its uniform peak at 545 nm.

3.2 Kinetic study of PP-AuNPs

In order to analyze the time-dependent synthesis of PP-AuNPs (Au:E = 10:1), a kinetic study was conducted. For this purpose, the prepared samples were taken out from the reaction mixtures at specific time intervals followed by UV analysis. Figure 2 reveals that the uniformity and production of nanoparticles increased with the passage of time. Figure 3 reveals the color change for the prepared PP-AuNPs.

3.3 Stability towards pH

For studying the impact of pH on the stability of PP-AuNPs, the pH of the prepared nanoparticles solution was adjusted (1–14). For this purpose, the PP-AuNP solution was incubated at room temperature for 24 h. Afterwards, data of UV-Vis spectra were analyzed. The results of this trait revealed the stability of PP-AuNPs at pH ranging between 3 and 12, whereas lesser stability was noticed in the pH ranging from 1 to 2 and 13 to 14. The instability of PP-AuNPs observed in highly acidic and basic pH was subjected to the removal of stabilizers (plant extract) from the gold surface resulting in destabilization of nanoparticles. Additionally, very

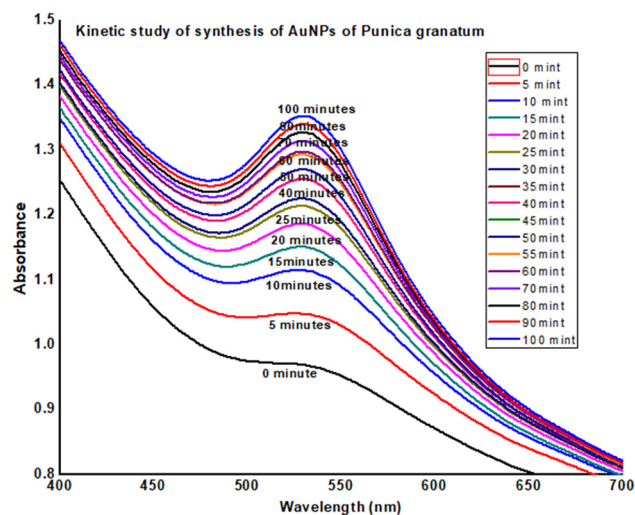


Figure 2: UV-Vis data for the kinetic study of PP-AuNPs.

low pH resulted in re-oxidation of neutral PP-AuNPs. Furthermore, the change in pH also altered the color of AuNPs, which is due to the removal of the stabilizer (Figure 4). However, enhanced stability was observed at alkaline pH, i.e., 8–10. Moderate stability of the Au NPs at other pH values, i.e., 3–4, where the nanoparticles showed redshift and peak broadening.

3.4 Characterization of PP-AuNPs

3.4.1 UV-Vis spectroscopic analysis

UV-Vis spectroscopic analysis of PP-AuNPs revealed the surface plasma resonance (SPR) in the wavelength range

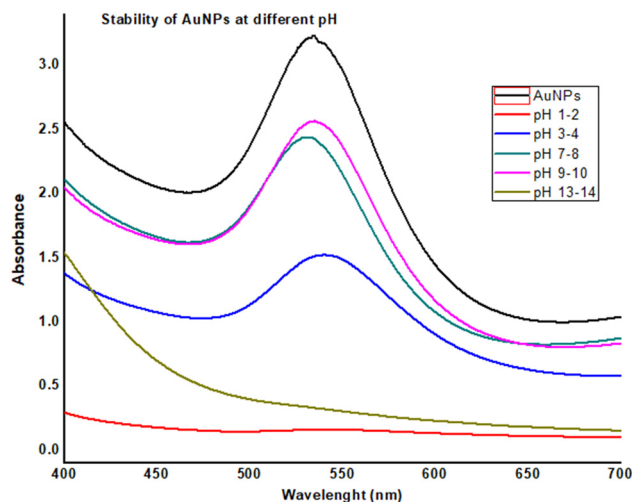


Figure 4: UV data showing the stability of PP-AuNPs (pomegranate peel extract-based gold nanoparticles) at different pH values.

of 400–600 nm, while a maximum characteristic peak was noticed at 545 nm, as shown in Figure 1.

3.4.2 FT-IR analysis

The crude extract of *P. granatum* showed a broad peak in the range $3,200\text{--}3,400\text{ cm}^{-1}$, which shows the presence of alcoholic and phenolic groups, and a sharp peak at $3,000\text{ cm}^{-1}$ corresponds to the C–H stretching frequency. A characteristic peak at $1,000\text{ cm}^{-1}$ shows the presence of C–F stretching frequency. In the case of Au nanoparticles, the OH peak intensity further increased, while the peak at $3,000\text{ cm}^{-1}$ completely disappeared, which



Figure 3: Change in color of the prepared PP-AuNPs.

showed that O–H and C–H olefinic are involved in the formation of nanoparticles. Also, a broadband at 500 cm^{-1} shows the presence of nanoparticles. The FTIR spectra are shown in the inset of Figure 5.

3.4.3 AFM analysis

Atomic force microscopic analysis was conducted to assess the shape of PP-AuNPs. AFM images reveal that biosynthesized nanoparticles are spherical in shape (Figure 6). Furthermore, the images confirm a uniform distribution of PP-AuNPs with particle sizes ranging from 4 to 16 nm.

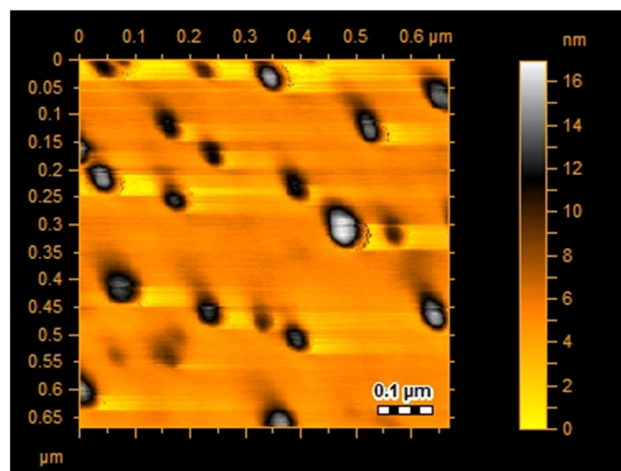


Figure 6: AFM image of AuNPs of *P. granatum*.

3.5 Phytochemical screening

The phytochemical screening test of ethanolic and aqueous extract of *P. granatum* is presented in Table 1. The ethanolic extract indicated the presence of tannins, flavonoides, emodins, terpenoids, cardiac glycosides, caumarine, and soluble starch. The aqueous extract showed the presence of tannins, flavonoides, terpenoids, emodins, cardiac glycosides, caumarine, carbohydrates, and soluble starch.

3.6 Analgesic effect

The analgesic effects of extracts and PP-AuNPs is shown in Table 2. In both of the tested samples, a uniform dose-dependent analgesic effect was observed. In the case of extract, the significant ($p < 0.001$) attenuation in induced writhing was noted at a higher dose of $100\text{ mg}\cdot\text{kg}^{-1}$ (70.04%), while PP-AuNPs demonstrated a significant ($p < 0.001$) percent reduction (81.98) at a dose of $15\text{ mg}\cdot\text{kg}^{-1}$.

3.7 Muscle relaxant effect

The muscle co-ordination potential of extracts and PP-AuNPs was tested in two muscle co-ordination models as given in Table 3. A mild muscle relaxant effect was noted after 30 min of sample administration and the effect was improved after 90 min but was not statistically significant. So, in both experimental paradigm, a non-significant effect was noted against the extract and PP-AuNPs.

3.8 Sedative effect

The sedative effect of both tested samples is presented in Table 4. In the case of extract, a significant ($p < 0.05$) effect was observed at a tested dose of $100\text{ mg}\cdot\text{kg}^{-1}$. The sedative effect of PP-AuNPs was more significant ($p < 0.01$) at a dose of $15\text{ mg}\cdot\text{kg}^{-1}$. The sedative effect of PP-AuNPs is better than that of the extract.

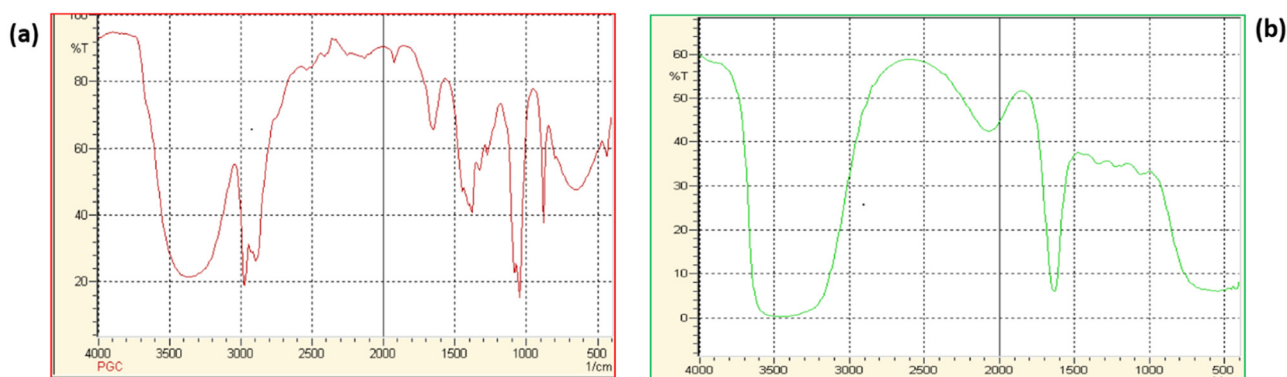


Figure 5: FTIR spectra of the crude extract (a) and AuNPs (b) of the *P. granatum* peel extract.

Table 1: Phytochemical assortment of the *P. granatum* (peel) extract

Chemical constituents	Hexane	Chloroform	Ethyl acetate	Ethanol extract	Aqueous extract
Alkaloids	–	–	–	–	–
Tannins	–	–	+	+	+
Anthraquinones	–	–	–	–	–
Glycosides	–	–	–	–	–
Reducing sugars	+	+	+	–	+
Saponins	–	–	–	–	–
Flavonoids	–	+	+	+	+
Phlobatannins	–	–	–	–	–
Steroids	–	–	–	–	–
Terpenoids	+	+	+	+	+
Cardiac glycoside	+	+	+	+	+
Caumarine	–	–	+	+	+
Emodines	–	–	+	+	+
Anthocyanin and betacyanin	–	–	–	–	–
Carbohydrates	+	+	+	–	+
Monosaccharides	–	–	–	–	–
Reducing sugar	–	–	–	–	–
Combined reducing sugar	–	+	+	+	–
Soluble starch	–	+	+	+	+

+: present; –: absent.

Table 2: Analgesic effect of the extract and nanoparticles

Treatment	Dose	Percent inhibition of writhing
Normal saline	10 mL.kg ⁻¹	–
Diclofenac sodium	10 mg.kg ⁻¹	85.87 ± 0.65***
Extract	10 mg.kg ⁻¹	18.43 ± 1.23
	25 mg.kg ⁻¹	37.98 ± 1.65
	50 mg.kg ⁻¹	56.09 ± 1.43**
	100 mg.kg ⁻¹	70.04 ± 1.09****
PP-AuNPs	2.5 mg.kg ⁻¹	38.20 ± 1.43
	5 mg.kg ⁻¹	53.54 ± 1.00**
	10 mg.kg ⁻¹	67.09 ± 1.43***
	15 mg.kg ⁻¹	81.98 ± 1.23

The data collected are denoted as the mean ± S.D. for all animals; tolerance to thermal stimuli in seconds; the level of significance was identified by ANOVA following the Dunnett's screening model; ** $p < 0.05$; *** $p < 0.01$; **** $p < 0.001$.

4 Discussion

In recent times, the significance of nanotechnology in the field of medicine, optics, and electronics has increased rapidly [28]. The role of nanotechnology in medical sciences has attracted the attention of scientists and researchers owing to its beneficial impact on humans, animals, and plant health. Nanoparticles (NPs) are considered a promising system for drug delivery. Nowadays, various biological, physical, and chemical approaches are used for the

synthesis of nanoparticles [29]. Among these, biologically synthesized NPs are preferred owing to their effectiveness, safe, and cost-efficient nature as compared to chemically and physically synthesized NPs [8]. The green synthesis of plant extract-based NPs has acquired a key position in the medical field due to their efficient and less toxic nature [30,31].

The characteristic peak of SPR confirms the synthesis of gold nanoparticles [31]. A single distinctive peak implies the shape of nanoparticles to be spherical [32]. Phytochemicals present in the pomegranate peel extract (terpenoids, flavones, etc.) are considered to be responsible for the bioreduction of Au⁺ (ion) to Au⁰ (metal) [33]. Our results of UV-Vis spectroscopy are in accordance with the findings of Biao et al. [34]. They also observed a distinct peak by noticing the SPR at 530 nm. FT-IR analysis was performed to determine the probable phytoconstituents present in pomegranate peel extracts that resulted in the efficient stabilization of PP-AuNPs. Signal stretching and vibration bending of peaks could be due to the presence of biomolecules like alkaloids, terpenoids, and flavonoids present in pomegranate peel extracts. Stabilization and reduction of AuNPs may also be due to the presence of these phytomolecules. Pomegranate peel extracts were successfully utilized for the synthesis of gold nanoparticles. The pomegranate peel extract acted as a stabilizing and reducing agent for the formation of Au⁺ to gold nanoparticles. The results of our study were similar to those reported in the literature [33,34]. The results of AFM

Table 3: Muscle relaxant activity of the extract and nanoparticles

Group	Dose	Inclined plant test			Traction test		
		Percent effect			Percent effect		
		30 min	60 min	90 min	30 min	60 min	90 min
Distilled water	10 mL.kg ⁻¹	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Diazepam	1 mg.kg ⁻¹	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Crude extract	10 mg.kg ⁻¹	16.22 ± 1.23	21.86 ± 1.09	25.94 ± 1.17	16.34 ± 1.01	17.34 ± 1.23	16.91 ± 1.33
	25 mg.kg ⁻¹	21.77 ± 1.65	26.98 ± 1.34	31.09 ± 1.13	21.97 ± 1.03	22.77 ± 1.39	21.99 ± 1.23
	50 mg.kg ⁻¹	26.34 ± 1.09	31.90 ± 1.34	32.45 ± 1.10	26.44 ± 1.06	27.34 ± 1.65	28.00 ± 1.88
PP-AuNPs	100 mg.kg ⁻¹	31.32 ± 1.54	35.34 ± 1.66	39.45 ± 1.63	31.34 ± 1.23	32.98 ± 1.09	31.99 ± 1.23
	2.5 mg.kg ⁻¹	21.34 ± 1.43	26.34 ± 1.42	27.34 ± 1.38	21.50 ± 1.09	22.43 ± 1.23	21.90 ± 1.32
	5 mg.kg ⁻¹	26.09 ± 1.07	30.98 ± 1.09	31.34 ± 1.99	26.98 ± 1.23	27.98 ± 1.32	26.87 ± 1.23
	10 mg.kg ⁻¹	31.09 ± 1.87	34.09 ± 1.89	39.09 ± 1.90	31.01 ± 1.65	32.87 ± 1.34	31.67 ± 1.35
	15 mg.kg ⁻¹	36.21 ± 1.43	40.21 ± 1.32	42.09 ± 1.11	36.22 ± 1.00	37.09 ± 1.35	36.00 ± 1.67

The data collected are shown as the mean ± S.D. for all animals; tolerance to thermal stimuli in seconds; and the level of significance was identified by ANOVA following the Dunnett's screening model.

Table 4: Sedative effect of the extract and nanoparticles in open field screening (locomotive activity)

Treatment	Dose	No. of lines crossed in 10 min
Distilled water	10 mL.kg ⁻¹	124.22 ± 1.16
Diazepam	0.5 mg.kg ⁻¹	6.34 ± 1.01
Crude extract	10 mg.kg ⁻¹	118.43 ± 2.11
	25 mg.kg ⁻¹	107.32 ± 2.01
	50 mg.kg ⁻¹	97.11 ± 2.98
	100 mg.kg ⁻¹	86.23 ± 1.87*
PP-AuNPs	2.5 mg.kg ⁻¹	99.54 ± 1.66
	5 mg.kg ⁻¹	88.54 ± 1.40*
	10 mg.kg ⁻¹	76.76 ± 1.65**
	15 mg.kg ⁻¹	64.98 ± 1.20**

The data collected are shown as the mean ± S.D. for all animals; tolerance to thermal stimuli in seconds; the level of significance was identified by ANOVA following the Dunnett's screening model; * $p < 0.05$; ** $p < 0.01$.

analysis are in agreement with the earlier findings of Uz-Zaman *et al.* [35]. The synthesized PP-AuNPs demonstrated a significant ($p < 0.001$) percent inhibition (81.98%) of writhing at a dose of 15 mg.kg⁻¹ revealing its analgesic properties. Further, PP-AuNPs possessed significant sedative potential as compared to the PP extract. Similar results regarding analgesic and sedative properties of plant (saffron) extract-based gold nanoparticles (SS-AuNPs) have been shown by Alhumaydhi *et al.* [36]. They revealed that saffron stigma-based gold nanoparticles had the highest analgesic (84.98%) at 10 mg.kg⁻¹, while all the experimented doses of SS-AuNPs showed a sedative effect.

Natural products are the best source of various classes of phytochemicals. The extracts demonstrated the presence

of flavonoids, tannins, and potential classes of phytochemicals. These phytochemicals are responsible for various pharmacologic activities. The preliminary biological screening along with the identification of chemical classes is the way to new drug discovery from natural products. Based on the preliminary evidence, the current pharmaceutical market is supplemented with a lot of natural products like silymarin that is a very good hepatoprotective agent [37]. Green nanotechnology significantly reduces the potential risks associated with human health and the environment and minimizes the production cost of nanomaterials. For the preparation of nanomaterials, amongst all, plant-based materials are in limelight nowadays [38]. Various phytochemicals like terpenes, phenolics, alkaloids, and saponins act as capping agents. The isolated plant phytoconstituents aid in enhancing the reproducibility of shape and size-controlled nanoparticles. Plant phytochemical-based nanoparticles are biologically active and therefore possess various pharmaceutical applications [39].

The analgesic potential of extracts and PP-AuNPs of *P. granatum* peel strongly supports the antipyretic and anti-arthritis folklore of this plant. The algnesia is mostly attributed to the production of prostaglandins (PGs) through the cyclo-oxygenase (COX) pathway. Different PGs exhibit various good or bad biological actions. These PGs are the source of pyrexia, algnesia, and inflammation. So, it is possible that the chemical constituents of the *P. granatum* peel might be COX inhibitors. The COX inhibitory potential is meant for the analgesic effect. It is also possible that chemical moieties of the *P. granatum* peel might block the sensory receptors as a possible analgesic mechanism.

Traditionally, the *P. granatum* peels are the best constipating agent and locally the powder of *P. granatum* peel is used in watery diarrhea. In the current study, a mild muscle relaxant effect was observed. This muscle relaxant effect is adjuvant to the analgesic action because, in many painful situations, analgesic and muscle relaxation are required. One other aspect of the muscle relaxant effect is also related to the curing of diarrhea if our tested samples are also smooth muscle (SM) relaxants. If they also relax the SM, it means they are the best curing candidate in the treatment of diarrhea. The antidiarrheal action is also linked with the inhibition of COX. Because the PGs are also responsible for the contraction of SM and this contraction will lead to diarrhea. The sedative effect of the *P. granatum* peel is also worth mentioning. The sedative action suggests some further studies for the central analgesic effect. This sedative effect is a good adjuvant pharmacological aspect with analgesia, muscle relaxation, and even as antidiarrheal.

5 Conclusion

Nowadays, the importance of plant extract-based nanoparticles is increasing significantly owing to their effective, safe, and environmentally friendly nature. In the present study, the pomegranate peel extract-based gold nanoparticles (PP-AuNPs) were biosynthesized and evaluated for their analgesic, muscle relaxant, and sedative properties. Keeping in mind the above facts, we concluded that PP-AuNPs could be used for analgesic, muscle relaxation, and sedative effects. All these parameters support the folklore of the *P. granatum* peel.

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