Research Article

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Synthesis, characterization, biological activities, and catalytic applications of alcoholic extract of saffron (*Crocus sativus*) flower stigma-based gold nanoparticles

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Abstract: Currently, nanotechnology is gaining massive attention compared to conventional methods as the bio-synthesis of plant-based nanoparticles is considered safe, effective, and ecofriendly. Therefore, keeping in view the importance of nanotechnology, the present study was designed to synthesize, characterize, and evaluate the biological effectiveness of saffron stigma-based gold nanoparticles (SS-AuNPs) for their *in vitro* and *in vivo* biological properties. These gold nanoparticles were characterized by UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). The highest antibacterial effect was observed by the saffron extract against *Escherichia coli* (22 mm). SS-AuNPs significantly inhibited the activity of enzyme urease (54.98%) and CA-II (64.29%). However, the nonsignificant inhibitory effect was observed in the case of α -chymotrypsin. Maximum analgesic (84.98%) and antiinflammatory (88.98%) effects were observed for SS-AuNPs

(10 mg/kg). Similarly, SS-AuNPs demonstrated a significant ($P < 0.01$) sedative effect at all tested doses.

Keywords: gold nanoparticles, saffron (*Crocus sativus*) flower, stability of AuNPs, catalytic degradation of dyes

1 Introduction

In the last few decades, nanotechnology has proven to be of significant importance owing to its application in optical, catalytic, electronic, and medicinal fields [1]. The application of nanotechnology in medicinal science is of most importance due to its beneficial impact on human as well as animal and plant health. Control drug delivery, tissue engineering, tumor detection and destruction, electroluminescent, drug and disease sensors, and diagnosis of cancer through MRI are some examples of nanoparticle application in the medical field [2–8]. Currently, different techniques are applied for the synthesis of nanoparticles including microwave-assisted synthesis, chemical and photochemical synthesis protocols, reduction in solution, and electrochemical synthesis route [9–12]. Green synthesis of nanoparticles through eco-friendly synthesis methods is gaining attention among the researcher community because it does not require high pressure, temperature, and toxic chemicals but bacteria, fungi, and plant extract are used for the synthesis of green nanoparticles [13–15]. These biological systems can synthesize nanoparticles in a safe, easy, and economical way [16]. The formation of green nanoparticles is due to the strong reducing ability of these biological systems, and this reducing ability is attributed to the enzymes and/or biomolecules in plant cells [17,18]. The development of environment-friendly plant extract-based nanoparticles through green synthetic route has vast application in modern science owing to their efficient drug delivery model and less toxicity [19,20]. Green synthesis of nanoparticles developed using algae, plants, fungi, and bacteria is renowned as a safe,

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efficient, and environmentally friendly approach in drug discovery [21,22].

Around the globe, saffron is most commonly used as a traditional food spice and medicine. *Crocus sativus* L. (saffron) is a perennial herb that belongs to the family Iridaceae and is also known as red gold. It is known to be the most expensive herb cultivated throughout the world [23]. Flowers of saffron are light purple having reddish thread-like stigmas characterized as spice possessing unique natural colorant (Figure 1). Approximately, 36,000 flowers of saffron yield 1 pound stigmas. To obtain a half kilogram of pure saffron, nearly 200,000 dried stigmas are required [24]. Clinical trials are being conducted to evaluate the effectiveness of saffron against mild depression [25]. Scientists have investigated the anti-inflammatory and antinociceptive properties of different parts of stigma flower including petals and stigmas [26]. It is considered a natural spice possessing a strong odor and intense natural yellow color other than its renowned therapeutic properties. Phytochemical characterization has demonstrated that the flavor, color, and bitterness of saffron are associated with safranal, crocin and crocetin, and glucoside picrocrocin, respectively [27].

The present study is designed to synthesize gold nanoparticles (Au-NPs) by reducing gold ions using alcoholic extract of the saffron flower. Biosynthesized Au-NPs were characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy

(EDX), and X-ray diffraction (XRD). Effects of varying concentrations of salt, pH, and temperature on the stability of synthesized gold nanoparticles were also investigated in this study. Furthermore, SS-AuNPs were screened for antifungal, antibacterial, enzyme inhibitory, antinociceptive, antiinflammatory, and sedative properties.

2 Materials and methods

2.1 Procurement of raw materials and chemicals

Pure dry saffron flower stigmas were obtained from Dubai. Analytical grade reagents and chemicals were procured and used in this study. Hydrogen tetrachloroaurate trihydrate [$\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$], deionized water, methanol, and sodium chloride were procured from Merck.

2.2 Extraction

Two hundred fifty grams of pure dry saffron flower stigmas was soaked in 250 mL of 50% alcohol for 1 week and then was filtered through Whatman no. 1 filter paper. A bright orange-red filtrate was obtained, which was treated as a source extract for further procedures.

Figure 1: Saffron (*Crocus sativus*) flower and stigmas.

2.3 Synthesis of saffron flower stigma-based gold nanoparticles

For the green synthesis of alcoholic extract of saffron flower stigma-based gold nanoparticles, different ratios (1:1, 10:1, 20:1, 30:1, 40:1, 50:1, and 60:1) of 1 mM (HAuCl_4) and extract were used. The obtained extract was added to 1 mM warm gold solution (HAuCl_4) already present in a titration flask and was uniformly stirred for a period of 4 h. The different ratios of the extract and the gold solution give different colors, i.e., blue, violet, pink, red, and cherry red as shown in Figure 2.

2.4 Characterization of SS-AuNPs

The synthesis of AuNPs was detected spectrophotometrically (UV-Vis spectrophotometer: Shimadzu UV-1800 Japan) at a wavelength varying from 200 to 900 nm by placing the sample in the optical path length (10 mm) quartz. For the characterization of the particle size, AuNPs were subjected to atomic force microscopy (AFM) and scanning electron microscopy (SEM) (SM-5910-JEOL, Japan). Energy-dispersive X-ray (EDX) (INCA-200, England) and X-ray diffraction (XRD) studies were carried out for elemental analysis and to determine the crystallinity of the biosynthesized saffron flower stigma-based gold nanoparticles (SS-AuNPs). The nanoparticle solution was subjected to centrifugation for 15 min at 10,000 rpm for the

removal of free proteins and other unwanted components. Afterward, the samples were dried under vacuum and ground in a mortar having finely divided potassium bromide (KBr) to form pellets. These KBr pellets were placed in a sample holder of FTIR for further measurement.

2.5 Kinetic and stability studies of SS-AuNPs

To find the time-dependent synthesis of AuNPs, a kinetic study was performed. For this study, samples were drawn from the reaction mixture after a certain interval of time, and the UV data were recorded periodically. The stability of AuNPs was checked through varying the parameter conditions like pH, type of salt, salt concentration, and temperature. Drop-wise addition of either 1 M NaOH or HCl was done for adjustment of pH value of AuNPs solutions between 2 and 14. The effect of sodium chloride (NaCl) on the stability of AuNPs was determined by adding (1 mL) 0.1–1.5 M NaCl to the biosynthesized AuNPs (2 mL). Similarly, the effect of other salts with the same concentration (0.1 M) was also checked. The UV-Vis spectrum was obtained after each treatment. To assess the temperature stability of AuNPs, they were heated in a water bath at 30°C, 50°C, and 100°C for 30 min each.

2.6 Catalytic activity of SS-AuNPs

Reaction studies were performed to check the catalytic activity of saffron stigma-based nanoparticles for the degradation of Rhodamine B (RhB). RhB is one of the most commonly used dyes, which is widely used for industrial purposes, such as printing and dyeing in textile, paper, paints, and leathers. However, the organic dyes will cause serious environmental and biological problems, even capable to induce irritation to the skin, eyes. Thus, the removal of dye from water is a great challenge. The convention methods for removal of RhB include biochemical and physiochemical methods, such as liquid membrane, ozonation, and adsorption, which are expensive and not very effective. Heterogeneous nanocatalyst (metal nanoparticles) present promising application for the organic dye decomposition with superior activity [28].

In the aqueous medium, RhB shows maximum absorption at 552 nm. This reaction was monitored by

Figure 2: Different ratio of gold and saffron extract.

the UV–Visible spectroscopy in the wavelength between 200 and 700 nm at room temperature. The decrease of absorbance at the maximum wavelength (552 nm) with time was recorded and shown in Figures 17 and 18. The intense bright red color of the RhB solution faded and became colorless during the degradation process. The addition of biosynthesized nanoparticles improved the reduction process (dye degradation up to 95% within 13 min).

2.7 Antibacterial activity of SS-AuNPs

Antibacterial activity of the synthesized SS-AuNPs against *Acinetobacter*, *Providencia*, *Streptococcus*, and *Escherichia coli* was assessed by using the well diffusion method [28]. Standard antibiotics such as levofloxacin, ciprofloxacin, amoxicillin, and norfloxacin were used for comparison purposes. This assay was performed in triplicate.

2.8 Enzyme inhibition activity of SS-AuNPs

Biosynthesized gold nanoparticles and saffron stigma extract were analyzed for their enzyme inhibitory potential against three different types of enzymes, i.e., xanthine oxidase, urease, and carbonic anhydrase-II. Xanthine oxidase inhibition of all the experimented samples was evaluated by determining the hydroxylation rate of xanthine (substrate) followed by uric acid formation. This reaction produces a colorless end product, and absorption was noticed at 295 nm [29]. In this assay, allopurinol was used as a standard. Likewise, carbonic anhydrase-II inhibitory activities of the tested samples were analyzed using 4-nitrophenyl acetate (4-NPA) that is colorless. Hydrolysis of 4-NPA results in the formation of carbon dioxide (CO₂) and 4-nitrophenol (yellow). During this experiment, the formation of this yellow-colored compound was monitored. The reaction temperature was maintained between 25°C and 28°C, and acetazolamide was used as a standard inhibitor [30]. Samples were treated with urea for the determination of urease inhibitory activity of respective samples. Urease activity was investigated by measuring ammonia production through the indophenol method. In the urease inhibitory assay, thiourea was used as a standard inhibitor [31]. All the experimented assays were conducted in triplicate.

2.9 *In vivo* screening

2.9.1 Animals

BALB/c mice of either sex (18–22 g) procured from National Institute of Health (NIH), Islamabad, were used in this experimental work. Before the commencement of experimental procedures, all mice were examined for any physical or behavioral abnormalities. After initial screening, only the healthy animals were selected for the experimental procedure. The selected animals were acclimatized to standard (12 h light/dark cycle at 22 ± 2°C) animal house conditions with strict compliance to laboratory guidelines of NIH for animal care and use. All the experimental work was approved from the ethical committee number UOS/Pharm-234 of the University of Swabi, Pakistan. All mice were classified as the negative control group ($n = 6$), positive control group, and the tested group.

2.9.2 Sedative activity (open field test)

Apparatus used to assess the sedative activity of gold nanoparticles comprised an area of white wood (150 cm diameter) covered with stainless steel walls. The base of the apparatus was divided into 19 squares with black lines. This open-field apparatus was placed in a sound-attenuated room, and mice were acclimatized in the dim red light for 1 h before the initiation of the experiment. Mice were categorized into negative control group (distilled water; 10 mL/kg), positive control group (diazepam; 0.5 mg/kg), crude saffron stigma extract-treated groups (15, 25, 50, and 100 mg/kg), and saffron flower stigma-based gold nanoparticles-treated groups (2.5, 5, and 10 mg/kg). After 30 min administration of respective treatment, each mouse was placed in the center of the apparatus for 10 min, and the number of lines crossed by each mouse was counted and noted [31].

2.9.3 Acetic acid-induced writhing test

The acetic acid-induced writhing test was conducted to assess the antinociceptive potential of the saffron stigma extract and the biosynthesized gold nanoparticles. Two hours before the commencement of this assay, mice were withdrawn from food. Mice were divided into nine groups. Group-I (negative control) was administered to distilled

water (10 mg/kg). Group-II (positive control) was treated with diclofenac (10 mg/kg). Group-III, IV, V, and VI were subjected to the saffron stigma extract at a dose of 15, 25, 50, and 100 mg/kg, respectively. While group-VII, VIII, and IX received SS-AuNPs at corresponding doses of 2.5, 5, and 10 mg/kg. Acetic acid (1%) was injected (i.p.) to each mouse after 30 min administration of respective treatments. The number of abdominal constrictions (writhing) was counted for 10 min after 5 min administration of acetic acid injections [32]. The percent inhibition was calculated through the following equation:

$$\% \text{ analgesia} = 100 - N_{\text{wt}}/N_{\text{wc}} \times 100, \quad (1)$$

where N_{wt} is the number of writhing in tested animals and N_{wc} is the number of writhing in negative control animals.

2.9.4 Carrageenan-induced paw edema test

Carrageenan-induced paw edema test was performed to evaluate the antiinflammatory effect of all the tested treatments by following the standard protocols. Animals were categorized and treated as in acetic acid-induced writhing test. Inflammation was induced by administration of carrageenan (1%, 0.05 mL) treatment in the subplantar region of the right hind paw (edema). The volume of paw edema was recorded after 1, 2, 3, 4, and 5 h posttreatment of inflammatory drug. The induced inflammation was quantified using a plethysmometer. The percent inhibition in edema/inflammation was calculated through the following equation [31,32]:

$$\text{Percent inhibition} = \frac{A - B}{A} \times 100, \quad (2)$$

where A is the paw volume of the negative control and B is the paw volume of the other tested group.

2.9.5 Acute toxicity study

For acute toxicological profiling, the animals were treated with the saffron stigma extract (100, 250, 500, and 1,000 mg/kg) and AuNPs (10, 25, 50, and 100 mg/kg). After the aforementioned administration, animals were observed for the first 5 h for any gross changes, and then, mortality was recorded after 24 h by following the standard protocols [28].

2.10 Statistical analysis

Results of this study were stated as mean $\pm$ SEM. Significant differences ($p \leq 0.05$) among all the experimented groups were assessed using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

3 Results

3.1 Selection of SS-AuNPs and extract ratio

Figure 3 shows different peaks at the range of 530–580 nm, exhibiting different absorbance values, which indicated different sizes of gold nanoparticles. The sharpness of the peak showed the uniformity of gold nanoparticles. By considering the height of the peak from the baseline of the spectrum, it was observed that the peak for the saffron stigma-based gold nanoparticle (5:1) showed the highest absorbance at 545 nm wavelength. This indicated the presence of a maximum concentration of gold nanoparticles in the solution. However, other peaks showed lower peak height (measured from the baseline of the spectrum) and broadness, which indicates a greater number of nonuniform gold nanoparticles in the solution. Therefore, this ratio (5:1) was used for the preparation of bulk solutions for further investigation.

Figure 3: UV-Visible spectra of AuNPs of saffron.

3.2 Kinetic study of SS-AuNPs

The results for kinetic studies of the synthesized SS-AuNPs are shown in Figures 4–6. Outcomes of this study

Figure 4: UV-Visible data of kinetic study of AuNPs of saffron stigma (SS-AuNPs).

Figure 5: Kinetic study of AuNPs of saffron stigma (SS-AuNPs).

Figure 6: Color change of AuNPs of saffron stigma (SS-AuNPs) formation with time: (a) saffron extract, (b) gold solution, (c) initial mixing of gold solution and extract, (d) after 30 min, and (e) after 1 h.

reveal that the number and uniformity of nanoparticles increased with time. The regression analysis showed that $R^2 = 0.9895$, while the rate of reaction was 2.55×10^{-2} .

3.3 Stability of gold nanoparticles

3.3.1 Stability toward pH

To understand the effect of pH on the stability of AuNPs, the pH of AuNPs solution was adjusted between 1 and 14. The solution was kept for 24 h at room temperature, and the effect was obtained by recording UV-Visible spectra. The results showed that the AuNPs were more stable at pH between 3 and 12, while lower stability was observed toward more acidic and basic, i.e., 1–2 and 13–14 (Figure 7). The reason of the instability of AuNPs at lower and higher pH may be due to the removal of stabilizer (plant extract) from the gold surface to destabilize the nanoparticles. Moreover, very low pH caused the re-oxidation of neutral AuNPs [12]. However, maximum stability was noticed at alkaline pH, i.e., 8–10. At the pH range of 3–4, moderate stability of the AuNPs was observed as in this range gold nanoparticles revealed peak broadening [12].

3.3.2 Stability toward different salts

To find the stability of AuNPs of saffron in the solution of different salts, 0.1 molar concentration of different salts (NaCl, CaCl₂, CuCl₂, NiCl₂, Hg₂Cl₂, PbCl₂, ZnCl₂, and CdCl₂) was prepared. Equal volumes of AuNPs were taken in a separate test tube, and 2 mL of each salt solution was added to these test tubes. After 24 h of mixing of the salt solution with AuNPs, UV data were recorded, which show the disruption of these nanoparticles due to salt. This disruption was obvious from their color change and precipitation (Figure 8).

Figure 7: AuNPs of saffron stigma (SS-AuNPs) at different pH values.

Figure 9: UV-Visible data of effect of NaCl on saffron stigma AuNPs (SS-AuNPs).

Figure 8: UV-Visible data of effect of different salts on saffron stigma AuNPs (SS-AuNPs).

3.3.3 Stability toward NaCl

The effects of salt (NaCl) on gold nanoparticles of saffron were determined by varying the salt concentration on the synthesized gold nanoparticles. To find the salt effect, 2 mL of the gold nanoparticles were mixed with 1 mL of NaCl solution having a concentration in the range of 0.1–1 M. The intermixed salt and gold nanoparticles solution were kept for 24 h, and then, UV data were recorded. It was observed that by increasing the salt concentration, the gold nanoparticles start precipitating, and the solution become colorless at the high salt concentration (Figures 9 and 10).

Figure 10: Effect of NaCl on saffron stigma AuNPs (SS-AuNPs) (after 24 h).

3.3.4 Stability toward heat

To determine the stability of gold nanoparticles toward heat, the synthesized gold nanoparticles of saffron were heated at different temperature (25°C, 40°C, 60°C, and 80°C) for 30 min. UV data obtained at different temperatures clearly show that the gold nanoparticles destabilized at high temperature, i.e., at 80°C (Figure 11).

3.4 Characterization of gold nanoparticles (SS-AuNPs)

Comparison of FTIR spectra of saffron extract and its biosynthesized gold nanoparticles (SS-AuNPs) showed

Figure 11: Effect of heat on saffron stigma AuNPs (SS-AuNPs).

broadening of certain peaks in the spectrum of nanoparticles. In the FTIR spectra of SS-AuNPs, sharp and broader peak with higher amplitude was noticed at $3,427\text{ cm}^{-1}$ compared to that in the simple saffron extract ($3,462\text{ cm}^{-1}$), indicating that NH or OH groups present in the saffron extract reduced Au^+ ion to Au^0 metal and therefore indicating the formation of saffron stigma-based gold nanoparticles SS-AuNPs. Another indication from the figure was the shifting of the peak of the carboxylic ketonic group from $1,776$ to $1,664\text{ cm}^{-1}$ and $1,473\text{ cm}^{-1}$. Thus, the carboxylate group was involved in the stabilization of gold nanoparticles (Figure 12).

Saffron-loaded AuNPs were mostly in the size range of 25–35 nm. Most of the nanoparticles were in spherical shapes; however, a small amount of irregular nanoparticles (nanorods and nanotriangles) was also found (Figure 13). The EDX analysis authenticates the occurrence of the metallic gold (Au) in the synthesized saffron stigma-based AuNPs (SS-AuNPs). In SS-AuNPs, strong signals were observed at 0.5 and 2.3 keV, whereas a weak signal was observed at 9.8 keV. EDX spectra reveal the Si signal due to the use of silicon lattice; however, N signal demonstrated the occurrence of nitrogen-containing organic components in the experimented sample. Furthermore, strong signals for O and C may be owing to the presence of various biomolecules responsible for the capping of AuNPs. Likewise, the presence of Cl in the HAuCl_4 molecule was responsible for the chlorine signal in the obtained EDX spectra. In the EDX analysis, the presence of metallic Au supported the reduction of Au^{3+} (metal cations) to Au^0 (elemental form) (Figure 14).

The nature of saffron stigma-based gold nanoparticles (SS-AuNPs) was also evaluated through the XRD

analysis. The XRD profile of the synthesized nanoparticles revealed that peak positions are consistent with the metallic gold. In the case of gold-containing sample, the typical diffraction peaks of the FCC metallic gold phase were observed at 38.21° , 44.39° , 64.62° , and 77.59° . Like other crystalline phases, no absorption peaks were noticed, highlighting the purity of the products. Among all the peaks of SS-AuNPs, the diffraction peak observed at 38° was relatively intense. The Debye Scherrer equation ($D = k\lambda/\beta^{1/2} \cdot \cos \theta$) was used for derivation of mean particle diameters of SS-AuNPs. In this equation, θ , k , $\beta^{1/2}$, and λ are known as peak width at an angle, shape factor, the width of XRD peak at half height, and wavelength, respectively. The average particle size of SS-AuNPs was calculated to be nearly 25 nm (Figure 15). The AFM images showed that the AuNPs of saffron were spherical and oval with a size of about 25 nm (Figure 16). The kinetic study of AuNPs of saffron is shown in Figure 17. The number and the consistency of silver nanoparticles amplified over time. The regression analysis showed $R^2 = 0.9891$, while the rate of reaction was 2.26×10^{-2} (Figure 18).

3.5 Antibacterial activities

Saffron extract and synthesized SS-AuNPs were examined against *Acinetobacter*, *Providencia*, *Streptococcus*, and *E. coli*, respectively. The antibacterial effect of saffron extract and AuNPs of saffron (zone of inhibition in mm) is presented in Table 1. The maximum effect was observed by extract against *E. coli* (22 mm). The results indicate the mild broad-spectrum antibacterial potential of saffron extract and SS-AuNPs.

3.6 Enzyme inhibition activities

Saffron stabilized the gold nanoparticles, and their extract was screened against enzyme to investigate their enzyme inhibitory property (Tables 2–4). The tested samples (extract and SS-AuNPs) inhibited urease after applying at the same concentration ($0.2\text{ }\mu\text{g}$). The extract demonstrated significant (89.12%) urease inhibitory action compared to AuNPs (54.98%). The IC_{50} values of the tested samples are presented in Table 2. The CA-II inhibitory potential of the extract was comparatively weak than AuNPs in terms of the percent inhibitory effect of 64.29% and 38.74%, respectively, as presented in Table 3. The nonsignificant α -chymotrypsin inhibitory effect was shown

Figure 12: FTIR spectra of saffron extract (upper) and its SS-AuNPs (lower).

in terms of the experimented samples, i.e., saffron extract and SS-AuNPs that are presented in Table 4.

SS-AuNPs were more potent than simple extract of saffron.

3.7 Analgesic effect

The tested samples (saffron extract and SS-AuNPs) inhibited the acetic acid-induced writhing at different experimented doses (Table 5). The maximum analgesic effect was noticed at a higher dose in the case of both saffron extract (100 mg/kg) and SS-AuNPs (10 mg/kg). The percent antihypergesic effect at these higher doses was 66.87% and 84.98%, respectively. Results revealed that

3.8 Sedative effect

The sedative potential of saffron extract and SS-AuNPs at different doses is presented in Table 6. The saffron extract demonstrated a significant ($P < 0.001$) effect at lowest dose (15 mg/kg) compared to examined higher doses (100 mg/kg). The SS-AuNPs also significantly ($P < 0.01$) hindered the movement of animals at all tested doses as presented in Table 6.

Figure 13: Scanning electron microscopic (SEM) image of saffron-loaded AuNPs (SS-AuNPs).

3.9 Antiinflammatory effect

Both tested samples, i.e., saffron extract and SS-AuNPs, showed the significant antiinflammatory effect. The saffron extract demonstrated 71.22% antiinflammatory effect at the dose of 100 mg/kg, and similarly, the SS-AuNPs showed a high antiinflammatory effect (88.98%) at the dose of 10 mg/kg. The result of antiinflammatory effects at higher doses is depicted in Figure 19.

3.10 Acute toxicity

Results of the acute toxicity study have revealed that both the experimented samples, i.e., saffron extract and SS-AuNPs, proved to be free of mortality and any other unwanted effect as presented in Table 7.

4 Discussion

Medicinal plant-based remedies have a key role in the treatment of various pathological and nonpathological disorders. Synthesis, characterization, and applications of plant-based nanoparticles are novel and trending concepts in sciences and technology [33]. The utilization of plant materials for the biosynthesis of nanoparticles possesses more advantages as it requires less elaborative processes [34]. Gold nanoparticles are being employed in the field of biomedicine, packaging, cosmetics, and electronics efficiently [35]. Extracts from various parts of plants like seed, fruit, and leaf are being significantly used for the synthesis of gold-/silver-based nanoparticles [36]. Keeping in view the importance of plant-based gold nanoparticles, this study was designed to examine the effectiveness of alcoholic saffron stigma extracts and its gold nanoparticles (SS-AuNPs) in different *in vitro* and *in vivo* biological assays. The synthesis of gold nanoparticles due to the reduction of gold ions during the reaction with saffron stigma extract was determined by a

Figure 14: EDX spectra of saffron loaded gold nanoparticles (SS-AuNPs).

Figure 15: XRD spectra of AuNPs of saffron (SS-AuNPs).

Figure 18: Kinetic study of catalytic degradation of Rhodamine B.

Figure 16: AFM image of AuNPs of saffron (SS-AuNPs).

Figure 17: UV-Visible data of catalytic degradation of Rhodamine B.

color change and UV-Vis spectra. UV-Vis spectroscopy is considered one of the most significant analytical tools to determine the stability of the synthesized nanoparticles in the aqueous solution [37]. UV-Vis spectrum revealed that the absorption peak sharpness depends on the volume ratio of the extract as maximum sharpness of peak was noticed for 5:1 ratio. This reaction mixture ratio was further used for the preparation of the bulk solution due to its effectiveness in the formation of gold nanoparticles. The particle size of biosynthesized nanoparticles was in the range of 25–35 nm and 25 nm as shown by SEM and AFM images, respectively. Similar to our study, Sadeghi *et al.* [38] revealed uniform distribution of gold nanoparticles prepared using the stevia leaf extract, showing the spherical shape of NPs having a particle size between 21 and 45 nm. UV-Vis spectra of all the other experimented ratios (1:1, 10:1, 20:1, 30:1, 40:1, 50:1, and 60:1) of saffron-based gold nanoparticles exhibited broad peaks and lower intensities at the wavelength of 540 nm (plasmon resonance of gold nanoparticles). Aggregation of nanoparticles and/or production of large anisotropic particles may be the reason behind this. Surface plasmon resonance absorbance is quite sensitive to the shape, nature, size, and interparticle distance among formed particles [39]. Particle size and gold nanoparticles content may be ascertained via the UV-Vis spectroscopy [31]. The results of the XRD analysis conducted in this study are in agreement with the results of red algae (*Gelidium amansii*)-based AuNanocrystals synthesized in the study by Kumar *et al.* [40]. Similar results of the XRD profile for the synthesized stevia leaf-based nanoparticles were documented by Sadeghi *et al.* [38]. In this study, energy-dispersive X-ray spectroscopy was conducted to evaluate the elemental composition of the prepared gold nanoparticles. The results of the current study were in

Table 1: Antibacterial effect of saffron extract and AuNPs of saffron (SS-AuNPs) (zone of inhibition in mm)

Samples	<i>Acinetobacter</i>	<i>Providencia</i>	<i>Streptococcus</i>	<i>Escherichia coli</i>
Saffron extract	10	05	12	22
AuNPs of Saffron (SS-AuNPs)	08	10	—	05
Levofloxacin	30	25	35	22
Norofloxacin	38	27	37	19
Ciprofloxacin	50	35	40	19
Amoxicillin	25	16	20	30

Table 2: Urease inhibition effect of saffron extract and SS-AuNPs

Samples/standard	Concentration (mg/mL)	% Inhibition	IC ₅₀ (µg/mL)
Saffron extract	0.2	89.12	38.16 ± 0.92
AuNPs of saffron (SS-AuNPs)	0.2	54.98	177.73 ± 5.20
Standard	0.2	98.21	21.24 ± 0.011

Table 3: Carbonic anhydrase-II inhibition effect of saffron extract and SS-AuNPs

Samples/standard	Concentration (mM)	% Inhibition	IC ₅₀ (µg/mL)
Saffron extract	0.2	38.74	—
AuNPs of saffron (SS-AuNPs)	0.2	64.29	170.66 ± 1.39
Standard	0.2	89.01	0.12 ± 0.03 µM

Table 4: α-Chymotrypsin inhibition effect of saffron extract and SS-AuNPs

Samples/standard	Concentration	% Inhibition	IC ₅₀ (µg/mL)
Saffron extract	0.2	22.43	—
AuNPs of saffron (SS-AuNPs)	0.2	26.09	—
Standard	0.2	98.87	5.72 ± 0.1

accordance with the earlier findings of Abu-Tahon et al. [20]. They also reported a strong peak at nearly 2.2 keV that is typically designated as absorption of metallic AuNPs.

Table 5: Analgesic activity of saffron extract and SS-AuNPs

Treatment	Dose (mg/kg; i.p)	% Inhibition of writhing
Saline	10 mL/kg	—
Diclofenac sodium	10	83.60 ± 0.64***
Saffron extract	15	32.98 ± 1.00
	25	39.70 ± 2.01
	50	51.98 ± 2.45**
	100	66.98 ± 2.99**
SS-AuNPs	2.5	64.87 ± 3.01**
	5	76.23 ± 3.29***
	10	84.98 ± 3.80***

** $p < 0.01$; *** $p < 0.001$.

4.1 EDX analysis

The presence of metallic gold Au in saffron-loaded AuNPs was confirmed from the EDX studies. Strong signals were observed from Au atoms in AuNPs at approximately 0.5 and 2.3 keV, while a weak Au signal was observed at 9.8 keV. The appearance of the Si signal corresponded to the use of a silicon lattice in the EDX study, while the signal for N shows the presence of nitrogen-containing

organic compounds in the biopolymer. Moreover, the other strong signals for C and O were due to the presence of different organic molecules capping the AuNPs. The appearance of the Cl signal in the EDX spectra of gold nanoparticles was due to the presence of chlorine in the tetra chloro auric acid trihydrate (HAuCl₄) molecule. The appearance of the elemental Au in the EDX analysis supports the reduction of metal cations (Au³⁺) to elemental form (Au⁰).

Table 6: Sedative activity of saffron extract and SS-AuNPs

Treatment	Dose (mg/kg; i.p)	Number of lines crossed in 10 min
Saline	10 mL/kg	145 ± 3.44
Diclofenac sodium	0.5	8.60 ± 0.64
Saffron extract	15	5.45 ± 0.03***
	25	115.55 ± 3.21*
	50	110.76 ± 3.88*
	100	104.42 ± 3.66*
SS-AuNPs	2.5	80.62 ± 3.01**
	5	65.67 ± 3.14***
	10	50.98 ± 3.99***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 19: Antiinflammatory activity of extract and AuNPs of *Crocus sativus* (extract 100 mg/kg; AuNPs 10 mg/kg) on carrageenan paw edema on mice. All values were represented as $\pm$ SEM for groups of six animals. The data were analyzed by ANOVA followed by Dunnett's test.

The FTIR analysis was conducted to study the possible biomolecules present in saffron stigma extracts, resulting in the capping and effective stabilization of saffron stigma-based gold nanoparticles. Absorption bands observed at 3,462, 2,969, 1,615, and 1,281 cm^{-1} correspond to $-\text{OH}/\text{NH}_2$, $-\text{CH}_2$, $\text{C}=\text{C}$, and $\text{C}-\text{O}$ stretching, respectively. The FT-IR

spectrum of saffron stigma-based nanoparticles showed a shift in $-\text{OH}$ stretching in band from 3,462 to 3,427 cm^{-1} , $-\text{CH}_2$ band from 2,969 to 2,974 cm^{-1} , and from 1,615 to 1,621 cm^{-1} ($\text{C}=\text{O}$). Another indication from the figure was the shifting of the peak of the carboxylic ketonic group from 1,776 to 1,664 cm^{-1} and 1,473 cm^{-1} . Thus, the carboxylate group was involved in the stabilization of gold nanoparticles. Results indicated that the synthesized gold nanoparticles using saffron stigma extract are surrounded by metabolites having functional groups of alcohols, carboxylic acid, amines, aldehydes, and ketones. Polyphenols present in the saffron extract are responsible for the reduction and stabilization of the biosynthesized gold nanoparticles [41,42]. Hydroxyl and NH -containing proteins and/or carbohydrates are vital for the synthesis and the capping of the prepared nanoparticles. Furthermore, the band observed in the region 2,969 cm^{-1} corresponds to CH stretching of aromatic compounds [20].

Stability studies of plant-based nanoparticles at different conditions like varied NaCl (sodium chloride) concentrations, pH, and temperature range are vital for assessing the environmental implications and potent human health risks [43]. The stability of produced nanoparticles could be evaluated by UV-Vis spectroscopy as precipitation, decomposition, and aggregation results characteristic changes in absorption of nanoparticles in the UV-Vis region (UV-Vis spectra). Synthesized nanoparticles were examined at different pH values ranging from 1 to 14. It was noticed that the synthesized nanoparticles were most stable at pH 7–8 followed by pH 5–6, pH 3–4, and pH 11–12. The least stability was observed in a medium having pH 1–2 and pH 13–14. Furthermore, at 0.1 M NaCl concentration, maximum absorbance was revealed, and the least was observed in the case of 1 M NaCl . Extreme peak broadening was noticed in the case of 0.7, 0.8, and 1 M NaCl , indicating the aggregation of SS-AuNPs. Exposure to varied pH values and salt

Table 7: Toxicological study of saffron extract and AuNPs

Treatment	Dose	Number of died animals/5	% Mortality	Gross behaviors changes
Normal saline	10 mL/kg	0/5	—	—
Saffron extract	100	0/5	—	—
	250	0/5	—	—
	500	0/5	—	—
	1,000	0/5	—	—
	10	0/5	—	—
SS-AuNPs	25	0/5	—	—
	50	0/5	—	—
	100	0/5	—	—

concentrations results in surface and structural changes of nanoparticles to aid drug delivery at the targeted site [44]. Hence, compositional features of gold nanoparticles play a vital role in the stability of synthesized nanoparticles. Likewise, the temperature is another main environmental factor influencing the activity, chemical characteristics, and stability of nanoparticles [31]. The impact of different temperatures (25°C, 40°C, 60°C, and 80°C) on the synthesized SS-AuNPs was examined by heating nanoparticle solutions for 30 min. A decrease in absorption revealed that elevation in the temperature resulted in the aggregation of SS-AuNPs. In any ecosystem, the toxicity, bioavailability, and mobility were determined by the overall stability of nanoparticles [45]. Therefore, the synthesis of stable gold nanoparticles is important as it decreases ion dissolution and helps in retaining its physiochemical properties. The results of this study will be helpful in optimizing the conditions for effective use of saffron stigma-based nanoparticles in different therapies.

Nowadays, scientists are keen and focused in developing safe, efficient, and environment-friendly techniques for the formulation of plant-based therapeutic products [46]. Hence, silver and gold nanoparticles are most commonly being used to biosynthesize stable plant extract-based nanoparticles having enhanced medicinal properties [47]. In this study, saffron stigma-based gold nanoparticles were assessed for their antibacterial and enzyme inhibitory properties. Saffron extract and synthesized SS-AuNPs were examined against *Acinetobacter*, *Providencia*, *Streptococcus*, and *E. coli*. The highest antibacterial effect was observed by the saffron extract against *E. coli* (22 mm). The results indicate the mild broad-spectrum antibacterial potential of saffron extract and SS-AuNPs. Saffron extract demonstrated the significant (89.12%) urease inhibitory action compared to SS-AuNPs (54.98%). However, the CA-II inhibitory potential of the saffron extract was comparatively weak than SS-AuNPs in terms of the percent inhibitory effect, i.e., 38.74% and 64.29%, respectively. Nonsignificant α -chymotrypsin inhibitory effect was shown in terms of experimented samples, i.e., saffron extract and SS-AuNPs. The gold nanoparticles surrounded by a number of drug moieties now act as a single group against the microbial organisms, thereby increasing the microbial activity [48].

The literature review has highlighted the role of the saffron extract against different diseased experimental subjects owing to its rich polyphenolic content. The acetic acid-induced writhing test was used to assess the antinociceptive properties of any medicinal agents. In this study, saffron extract and synthesized SS-AuNPs inhibited the acetic acid-induced writhing at different experimented doses. The maximum analgesic effect was noticed at a higher dose

in the case of both saffron extract (100 mg/kg) and SS-AuNPs (10 mg/kg). The percent antihypergesic effect at these higher doses was 66.87% and 84.98%, respectively. The results revealed that SS-AuNPs were more potent than simple extract of saffron. Similarly, saffron extract demonstrated a significant ($P < 0.001$) sedative effect at the lowest dose (15 mg/kg) compared to examined higher doses (100 mg/kg). The SS-AuNPs also significantly ($P < 0.01$) hindered the movement of animals at all tested doses. Both of the tested samples, i.e., saffron extract and SS-AuNPs, resulted in the significant anti-inflammatory effect. The saffron extract demonstrated 71.22% antiinflammatory at the dose of 100 mg/kg, and similarly, the SS-AuNPs also showed a high antiinflammatory effect (88.98%) at the doses of 10 mg/kg, respectively. The results of the acute toxicity study have revealed that both the experimented samples, i.e., saffron extract and SS-AuNPs, proved to be free of mortality and any other unwanted effect. The painkiller and antiinflammatory properties of saffron are well known, and safranal is one of the chemical constituents responsible for its analgesic effect [26]. This constituent has been reported with a significant analgesic effect [49]. It is further recommended to test the nanoparticles of safranal for the said pharmacological actions. According to the published reports, the saffron is a prostaglandin (PG) blocker, which is a pain, inflammatory, and pyrexia mediator. In addition to the PG blocker, the saffron is considered to be opioids receptor blocker [49]. This finding supports the use of saffron in the treatment of various inflammatory painful conditions. The nanoparticles of saffron with analgesic and antiinflammatory effect are reported first time here. These AuNPs are recommended to further process for mechanistic studies and dosage form development. Saffron is the most commonly used for neurological disorders such as antidepressant, anxiolytic, muscle relaxant, and anticonvulsant [50]. However, we find the significant sedative effect of the saffron extract and SS-AuNPs. This sedative effect is adjuvant for the said effects especially anxiolytic and anticonvulsant. The sedative effect of our tested samples might be attributed to the release in various neurotransmitters. The interactions of extract and AuNPs with GABA receptors resulted in the anxiolytic, muscle relaxant, sedative, and anticonvulsant effects, while the inhibition of serotonin and other biamines causes antidepressant effects. The application of nanotechnology modifies the chemical and behavioral properties of a substance by enhancing the biological activities of plant extract, promoting the release of bioactive metabolite, and decreasing the side effects [51]. As compared to conventional drug delivery systems, nanotechnology helps in promoting the synthesis of more stable,

bioavailable, efficient, and nontoxic plant-based medicinal drugs [52].

5 Conclusion

The biosynthesis of plant-based nanoparticles is gaining importance in the field of nanotechnology as they are considered safe, effective, and ecofriendly. In this study, saffron stigma-based gold nanoparticles were synthesized and evaluated for their biological effectiveness through various *in vitro* and *in vivo* assays. These biosynthesized gold nanoparticles were found to have maximum antibacterial activity against *E. coli*. Furthermore, the results of this study conclude that the prepared saffron stigma-based nanoparticles possessed analgesic, anti-inflammatory, and sedative properties.

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