Research Article

Evaluation of Anti-Inflammatory Effect of Green Synthesized Gold Nanoparticles on Imiquimod Induced Psoriasis in Mice as Model

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Abstract | Psoriasis is a systemic inflammatory autoimmune disease characterized by skin abrasions, with the plaque subtype accounting for around 85% of all cases. Green chemistry emerged as a realistic and simple alternative to more difficult chemical synthesis techniques for generating gold nanoparticles. This study evaluated the potential of *Syzygium aromaticum* gold nanoparticles (SaAuNPs) in treating psoriasis in mice. The study had two parts. The first part involved the extraction of clove oil and the synthesis of SaAuNPs using a green synthesis method. The second part involved the induction of psoriasis in mice using 5% imiquimod cream, followed by topical application of different concentrations of SaAuNPs, clove oil, and salicylic acid. The second part evaluated the effectiveness of SaAuNPs in reducing psoriasis symptoms in mice. The findings showed that SaAuNPs had a significant ($P \leq 0.05$) anti-inflammatory effect, with the groups treated with 0.003% and 0.005% SaAuNPs showing a significant reduction in psoriasis symptoms. The physicochemical properties of SaAuNPs were characterized using numerous techniques, including UV-visible spectroscopy, Zeta potential measurement, particle size analysis, X-ray diffraction (XRD), and scanning electron microscopy (SEM). The SaAuNPs showed a UV-visible spectrum at 562 nm, a zeta potential of 90mV, and a particle size of 48nm. The XRD revealed a crystalline nature of the particles, and the SEM showed regular spherical nanoparticles. The study also showed that clove oil and salicylic acid had similar results but to a lesser extent and with non-significant findings. The outcomes suggest that SaAuNPs could be an encouraging alternative approach for the treatment of psoriasis, which can be further explored in future studies.

Keywords | Clove oil, Imiquimod, Green synthesis, Psoriasis, Gold nanoparticles, Anti-inflammatory effect.

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INTRODUCTION

Global interest has grown in various elements of nanotechnology research, and a variety of new uses and advances for various types of nanoparticles have been found in the fields of energy, electronics, space, and medicine (Abdulsattar, 2015; Ali and Khudair, 2019; Shaker et al., 2022). Nanotechnology is widely employed in various sectors such as the agricultural sector, chemistry of the environment, veterinary medicine, drug detection (including cardiac medical treatment, oral health, diagnostic methods, skin therapy, and cancer therapy), as well as in the biological and medical fields (Ayad et al., 2019; Husain et al., 2019; Hussein et al., 2019).

Nanoparticles, characterized by their size, distribution, and shape, typically consist of atom clusters ranging from one to one hundred nm. These particles exhibit distinct and enhanced properties as compared to larger particles derived from the corresponding bulk materials (Saliem et al.,

2016; Hasan and Raheem, 2021).

Green chemistry has originated as a achievable and simple substitute to more complex chemical synthetic procedures for obtaining gold nanoparticle AuNPs (Al Shaabani et al., 2020).

Psoriasis can be defined as a systemic inflammatory autoimmune disease considered by skin abrasions, with the most public subtype presence being plaque kind. This kind establishes as sore, elevated lesions enclosed in dead dermatologic cells with a silvery presence. Mild types are treated topically and with a sanitary food regimen. This kind of therapy requires clinical adaptation because it is long-lasting. The silver-white scaly forms can be treated topically with various keratolytic or pickling ointments and creams, including salicylic acid. Vitamin D3 analogs are used to stop the growth of epidermal cells and modulate cell differentiation. Topical corticosteroids are also an effective way to manage localized psoriasis (Reynolds et al., 2019).

The selection of antipsoriatic drugs is typically based on the severity of the ailment and its impact on the patient, with additional factors like as cost, side effects, patient preference, and accessibility also being considered .Researchers have looked into the use of natural extracts and nanotechnology to enhance currently used therapies or uncover new classes of medications. It is well-recognized that plants with phenolic compounds in their roots, leaves, and flowers have excellent anti-inflammatory properties, and other active ingredients that stop free radicals from damaging cells are excellent antioxidants (Akbari et al., 2022).

Clove *(Syzygium aromaticum)* is belongs Myrtaceae intimate.The main oil compounds of clove are eugenol, eugenol acetate as well as β-caryophyllene constituents. The anti-inflammatory mechanism of Eugenol dimers involves the inhibition of cytokine expression in macrophages and modulation of the nuclear factor kappa B (NF-kB) gene expression, which regulates cell proliferation and survival. The chemo-preventive impact of clove oil primarily attributed to its eugenol components (Leem et al., 2011).

Gold nanoparticles have emerged as a highly investigated class of metal nanomaterials due to their distinctive characteristics. Gold nanoparticles have demonstrated significant potential in the field of medicine and other applications (Ko et al., 2022).

In the existing study, the goal was to evaluate the inflammatory inhibition action of gold nanoparticles prepared by green biosynthesis from clove oil on Imiquimoid-induced psoriasis in a mice model.

MATERIALS AND METHODS

Collection Of Plant

Clove buds (*Syzygium aromaticum*) were obtained from the local marketplace in Baghdad and subsequently subjected to disinfection using a 5% sodium hypochlorite (NaOCl) solution.The buds were then washed three times with filtered water and subsequently dehydrated.

Preparation And Extraction Of Clove Oil (*Syzigyum Aromaticum*)

The process of hydro-distillation (water distillation) was employed to extract clove oil from dried buds. The process of extraction involved the use of the Clevenger equipment. 200g of dried buds were taken and add 500 ml boiled (100°C) of distilled water was in a round type bottom flask in the Clevenger apparatus until the oil distillation process finished after 4-5 hours. The amount of oil was detected, and the oil in the distillation product was dried out over anhydrous sodium sulfate Na_2SO_4 and kept in refrigerator at a temperature of 4°C (Kaur and Rani, 2019).

Identification Of Active Ingredients Of The Clove Oil

Gas Chromatography-Mass Spectrometry (GC-MS) analysis is a technique employed to identify the various constituents present in an oil sample and determine the relative abundance of each component as a percentage fraction. To identify the active components demonstrating antimycotic action, GC/MS was used to look at the phytochemicals of *S. aromaticum*. Matching the results of the analysis with the reference of the retention time allowed the chemical components of the clove bud extract to be identified (Yassin et al., 2020).

Preparation Of Gold Nanoparticles By *S. Aromaticum* Oil

Chloroauric acid ($AuCl_4$) bought by Sigma–Aldrich, was used deprived of any additional purification. For the production of gold nanoparticles using clove oil, 50 ml clove oil was added to about 50 mL of 0.004M $AuCl_4$ solution (aqueous) throughout rousing at 200 rpm. The reflection of weak brownish color in a few minutes specifies the development of nanoparticles of gold. After reduction, centrifugation of the solution was done and nanoparticles were composed and re-dispersed in water. The centrifugation procedure was repeated several times so that another impurities may become washed out (Singh et al., 2010; Azam et al., 2009). Then, to obtain a solid form of *Sa*AuNPs, the mixture components of solutions having *Sa*AuNPs were poured into ceramic-crucible plates and placed into the workroom heater adjusted at about 350°C for 2 hours to obtain a pale brown powder of *Sa*AuNPs (Debroy et al.,

2023).

To prepare a carbapol gel containing *Sa*AuNPs at concentrations of 0.001%w/w, 0.003%w/w, and 0.005%w/w, the amounts of *Sa*AuNPs needed were 1 mg , 3 mg, and 5 mg respectively which were added to 100 gram of carbapol gel to obtain the final desired concentrations.

Description Of *Sa*AuNPs

Uv-Visible Spectroscopy

The monitoring of the consistency and production of SaAuNPs was conducted using Ultra-violet Spectroscopy. The samples undergo analysis within the wavelength range of 250 nm to 800 nm. The solution's absorbance was measured at one-hour intervals over a period of 24 hours. The color change was observed gradually, with an increasing intensity of brown observed towards the end of the 24-hour period (Azam et al., 2009).

Zeta Potential

The determination of the stability and strength of each nanoparticle is reliant upon the zeta potential, which is contingent upon the surface charge. Additionally, it plays a crucial role in the first adsorption of particles onto the cellular sheath. The rate of endocytotic absorption subsequent to adsorption is affected by the size of the particles. (Rasmussen et al., 2020).

Particle Size

If the size collection is less than 100 nm, then the sample can also be called nanoparticles or nano-gold (Ghosh *et al*., 2008).

X-Ray Diffraction (Xrd) Analysis

The crystalline structure of SaAuNPs was determined through the utilization of X-ray diffraction (XRD) analysis, as demonstrated in previous studies (Ayad *et al*., 2019).

Scanning Electron Microscope (Sem)

In order to conduct scanning electron microscopy (SEM) investigation, it is necessary to prepare thin carbon-coated films on copper grids. The films were fabricated by employing a dipping approach to put a minimal amount of the sample onto the substrate, followed by the removal of any surplus solution using blotting paper. Following that, the films underwent further drying for an approximate duration of five minutes under a lamp. (Ankamwar, 2010).

Preparation Of Gel Formula

The gel formulation used Carbopol polymer, a non-sensitizing and inert substance used in pharmaceutical products, was prepared by dispersing 1 gram of Carbopol powder in 80 ml of deionized water. The solution was adjusted to 100 ml and triethanolamine (TEA) was added to neutralize the pH to about 7.

Experimental Animals

The study included male mice measuring between 20 and 25 grams, aged approximately 6 to 8 weeks. The animals were confined within plastic enclosures and situated within a designated housing facility affiliated with the Department of Physiology, Biochemistry, and Pharmacology within the College of Veterinary Medicine. The animals were provided with commercially viable laboratory animal feed and tap water, which was readily accessible without any limitations. The temperature within the apartment was consistently regulated between 20 to 25°C, while also ensuring continuous ventilation. Prior to commencing this investigation, ethical approval was obtained from the local committee of animal care and use at the College of Veterinary Medicine, Baghdad University (Number 1606).

Induction Of Psoriasis In Mice

Number of animals was 84 male mice at 7 weeks of age were used. The dominant part of the back of total animals (diameter of 3 cm) was clean-shaven. The mice underwent topical application of imiquimod (IMQ) treatment, specifically using 62.5 mg of commercially available IMQ cream (5%), administered daily for 10 consecutive days on the shaved back area (Van der Fits et al., 2009).

Experimental Design

The mice (84 mice) were categorized into seven groups (12 mice/group) as the followings:
Group A: control Negative infection untreated.
Group B: control Positive induced psoriasis untreated.
Group C: Induced psoriasis and treated topically with 0.001% of SaAuNPs for 10 days.
Group D: Induced psoriasis and treated topically with 0.003% of SaAuNPs for 10 days.
Group E: Induced psoriasis and treated topically with 0.005 % of SaAuNPs for 10 days
Group F: induced psoriasis and treated topically with 0.5% of the clove oil extract alone for 10 days.
Group G: Induced psoriasis and treated topically with 5% salicylic acid for 10days.

Clinical signs

The animals undergo continuous monitoring to identify any changes in clinical manifestations, such as activity levels, behavioral patterns, and the presence of inflammatory indicators such as increased thickness, redness, and the appearance of silvery spots.

Gross Examination

The animals were subjected to skin visual inspection in order to monitor and assess any observable abnormalities or alterations in their skin, including but not limited to ery-

thema,irritation, scaling, itchiness, annoyance, thickness, and pattern of distribution of psoriatic lesions.

Measurement Of Serum Cytokines

Blood samples, measuring 1 ml, were obtained from the heart using the cardiac puncture technique following the administration of ketamine (60 mg/kg intraperitoneal .) and xylazine (16 mg/kg intraperitoneal) for anesthesia(Parasuraman *et al.*, 2010) .Blood samples were collected, centrifuged, and stored at -20 Co. Mouse IL-17 and TNF alpha ELISA was used to quantify IL-17, TNF, and VEGF levels in mouse serum, following manufacturer's recommendations (Na Takuathung et al., 2018).

Statistical Analysis

The study data were subjected to statistical analysis using Microsoft Programming and SAS (Statistical Analysis System, version 9.1). The analysis involved conducting a Two-Way Analysis of Variance (ANOVA) with a significance level set at $P<0.05$. Post-hoc analysis was performed using the least significant differences (LSD) method. (SAS, 2012).

RESULTS AND DISCUSSION

Extraction Yield

The hydro-distillation process resulted in the extraction of clove bud oil, which exhibited a vibrant yellow color and emitted the characteristic aroma associated with clove oil. The yield of the extracted oil was determined to be 50% based on the percentage of the powder obtained. This finding was reported by (Tambe E. and Gotmare S., 2020). The percentage yield of the extract can be calculated by dividing the weight of the extract (in grams) by the weight of the clove bud powder (in grams) and then multiplying the result by 100.

The oil yield percentage can be calculated by multiplying the weight of the extracted oil by 100 and dividing it by the weight of the clove bud powder.

The oil percentage of yield can be calculated using the formula: oil yield = (100 * 100) / 200, resulting in a yield of 50% w/w.

The findings of this study align closely with those of (Ishaq *et al.*, 2019), who reported a 48.84% yield of clove bud powder extract. Harvest season, extraction method, and place of origin can all affect yield differences (Ali and Ibrahim, 2023).

Overall, the Clevenger method is a very effective extraction method that yields high percentages of essential oil while preserving its chemical integrity and quality. The Clavenger method yielded 50% oil from clove buds, demonstrating its efficacy and reliability. It involves prolonged heating to extract most oil, ensuring high-quality, pure oils with minimal degradation of components (Nannaware et al., 2022).

Analysis Of Clove Oil By (Gc-Ms)

Active composites in clove buds extract with were listed in Table 1 and Figure 1. The plant buds screening showed the existence of twelve signal peaks of the individual constituents obtained involving 100% of the whole known compounds. Caryophylline was the main constituent that represented 28.9% of the total compounds, followed by humulene which comprised 21.06%, Eugenol 13.1%, cyclohexane 7.85%, alpha copaene 6.61%, 1.6.10-dodecatrien 3.73%, adamantane 3.28%, alloaromadendrene oxide 2.99%, naphthalene 2.96%, caryophylline oxide 1.53% and trancalamene 1.53%.

Table 1: Main components of Clove oil extraction

Compounds	Retention time	Area %
Caryophylline	13.9	28.9
Humulene	14.5	21.06
Eugenol	12.76	13.1
Cyclohexane	16.46	7.85
Alpha copaene	13.16	6.61
1.6.10-dodecatrien	15.99	3.73
Adamantane	17.27	3.28
Alloaromadendrene oxide	17.53	2.99
Naphthalene	15.41	2.96
Caryophylline oxide	17.73	1.53
Trancalamene	15.48	**1.53**

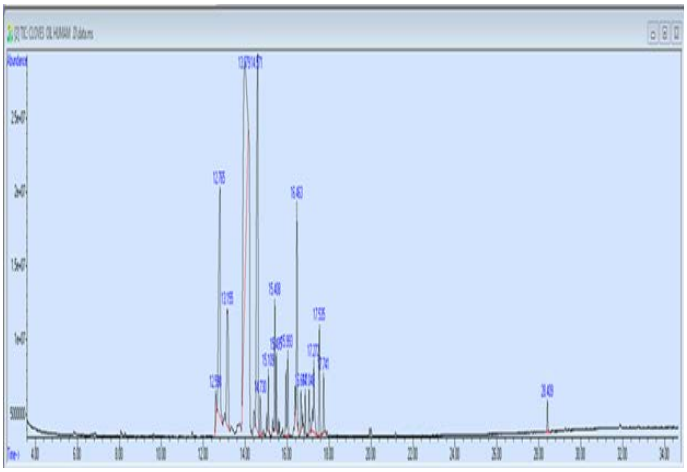


Figure 1: Phytochemicals analysis of *S. aromaticum* essential oil by GC-MS

The active compounds of the essential oil observed revealed that caryophylline was the most dominant active component (28.9%),after that humulene (21.06%) and eugenol (13.1%). The present data revealed the effect of the grind-

ing (crushing) technique of clove buds on the essential oil composition depending, especially, at the time of hydro-distillation.Whole clove oil contains higher eugenol components, while crushed clove bud oil has higher caryophyllene content. Crushing clove buds increases caryophyllene content but reduces eugenol levels (Safrudin et al., 2015).

Formulation Of Gold Nanoparticles

The extract of the clove was applied for the purpose of reduction of chloroaurate ions. The disappearance of the faint yellow solution of gold and the observation of brownish color within a few minutes indicates the formation of *Sa*AuNPs as shown in Figure 2A. Then after the use of a ceramic crucible and laboratory furnace, a pale brown powder of *Sa*AuNPs was obtained as shown in Figure 2B, which corresponds to (Singh et al., 2010).

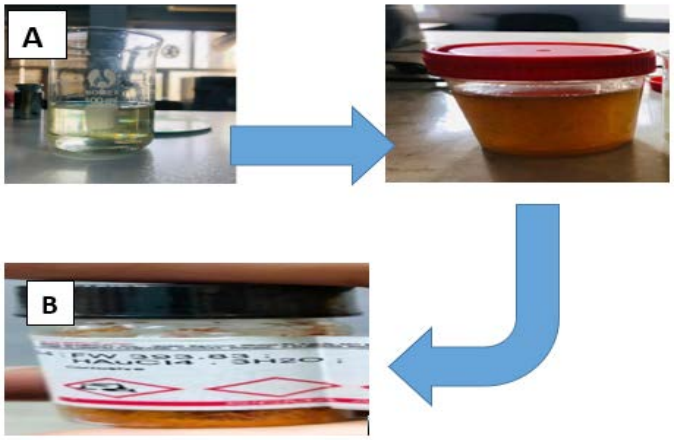


Figure 2: (A)Formation of gold nanoparticles solution, (B) Formation of gold nanoparticles powder

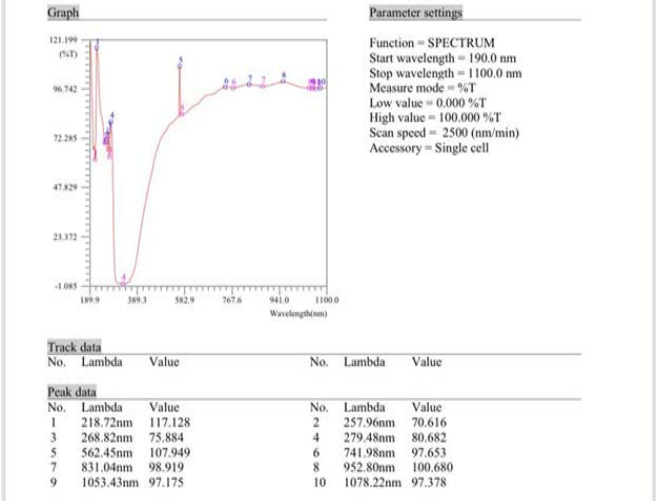
Clove oil reduces gold ions to gold nanoparticles through electron transfer from the eugenol molecule, acting as a reducing mediator. The gold nanoparticles stabilize through the capping effect of eugenol molecules (Panda and Mandal, 2022).

Characterization Of *Sa*AuNPs: Uv Spectroscopy

The UV-Visible spectra of synthesized gold nanoparticles (AuNPs) showed a wide absorption peak at 562 nm, indicating that these nanoparticles strongly absorb light at this wavelength, reducing light intensity.The absorption band was observed at a wavelength of 562 nm, indicating the presence of gold particles. The emission maxima observed at a wavelength of 562 nm are attributed to inter-molecular charge transfer phenomena. The occurrence of an emission band at the elongated wavelength is attributed to the head-to-tail arrangement of the particles, also known as J-aggregation (Nee Kamaldeeb et al., 2014).

Zeta Potential

The mean of the Zeta Potential obtained was 90.26 mV as shown in Figure 4, which indicates that the *Sa*AuNPs were stable and Unaggregated. A high zeta potential value of 90.26 mV for SaAuNPs indicated a significant electrostatic repulsion between the particles. The high zeta potential indicates strong repulsion between nanoparticles, preventing aggregation and settling out of solution, crucial for nanoparticle stability in biomedical applications, as it quantifies electrostatic charge (Carone et al., 2023).



No.	Lambda	Value	No.	Lambda	Value
1	218.72nm	117.128	2	257.96nm	70.616
3	268.82nm	75.884	4	279.48nm	80.682
5	562.45nm	107.949	6	741.98nm	97.653
7	831.04nm	98.919	8	952.80nm	100.680
9	1053.43nm	97.175	10	1078.22nm	97.378

Figure 3: UV-Visible spectra for *Sa*AuNPs

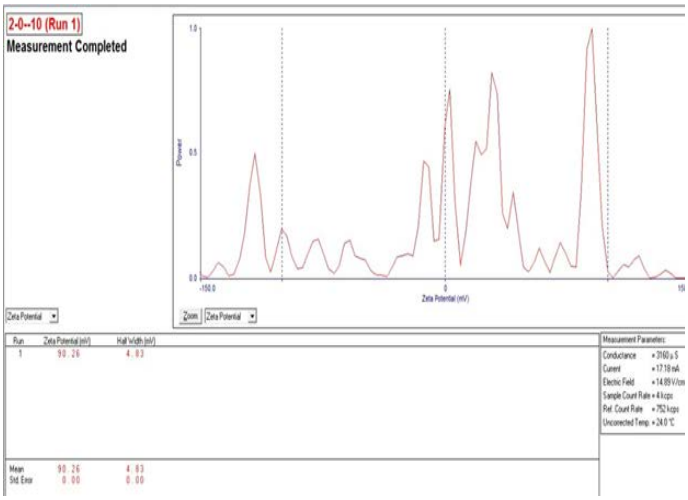


Figure 4: Zeta potential for *Sa*AuNPs

Particle Size

The *SaAuNP's* effective particle size was brought to an average diameter of 48 nm as shown in figure 5. The specific size of the particles found to be 48 nm and it was still within the range of what is considered to be "nanoscale" particles, which typically have dimensions between 1 and 100 nanometers. The optical properties of SaAuNPs are significantly influenced by their particle size, with larger particles exhibiting a wider absorption spectrum. The surface area of gold nanoparticles is directly correlated with

their dimensions, impacting their reactivity, catalytic activity, and engagement with other molecules. Accurate determination of nanoparticle dimensions is crucial for their efficacy, leading to increased attention towards environmentally friendly nanogold production (Bayda et al., 2019).

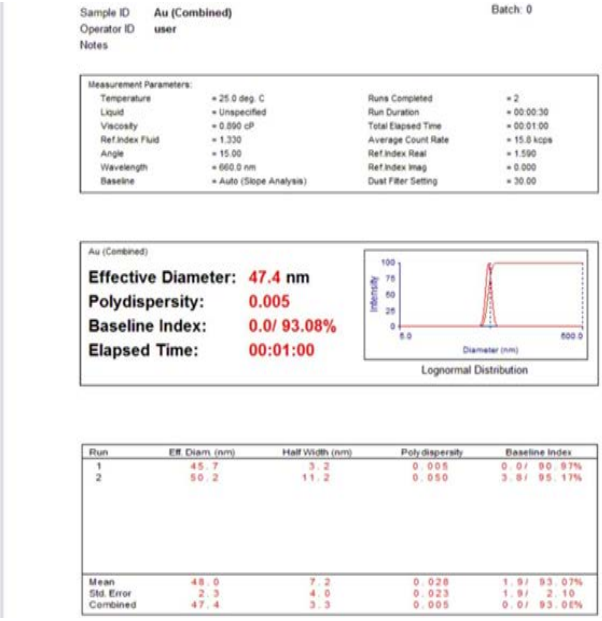


Figure 5: Particle size of SaAuNPs

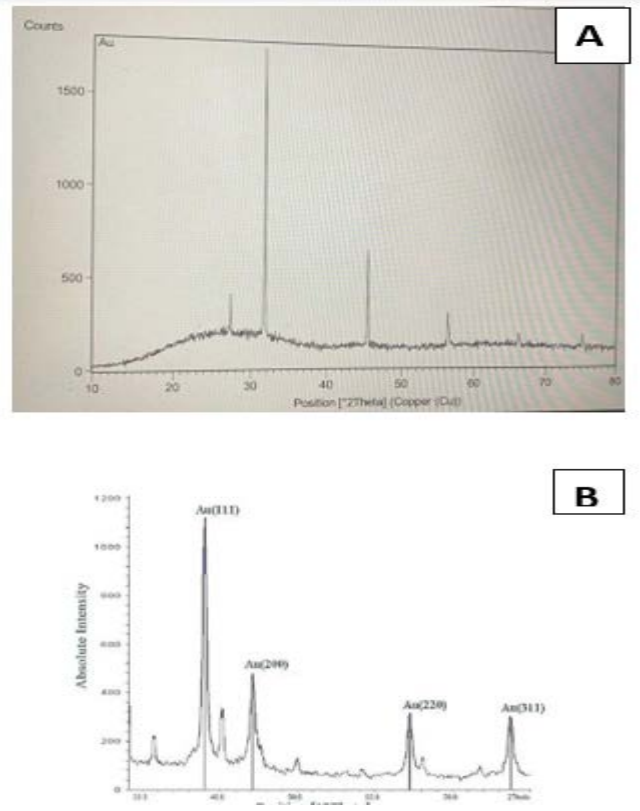


Figure 6: XRD pattern of AuNPs from *S.aromaticum* A) XRD analysis of Gold NPs B) standard Bragg reflections (Vilas *et al.*, 2016)
SEM photograph of *Sa*AuNPs

X-Ray Diffraction Examination Of *Sa*AuNPs

The X-ray diffraction (XRD) analysis revealed the presence of crystalline nanoparticles, which were characterized by four distinct peaks that corresponded to the conventional Bragg reflections. This is illustrated in Figure 6. X-ray diffraction (XRD) is a widely employed technique in the field of materials science for the purpose of investigating the crystal structure and phase composition of various materials (Vilas et al., 2016).

The Scanning electron microscope (SEM) technique was utilized to realize the size and figure of *Sa*AuNPs. The result typically showed a field of closely packed, individual nanoparticles with a spherical shape and a relatively uniform size distribution as shown in Figure 7.

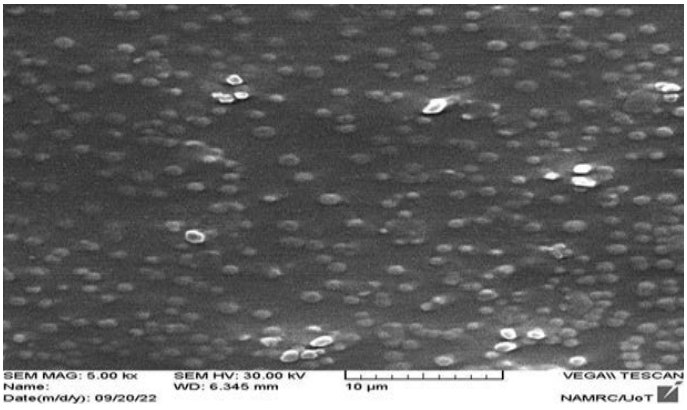


Figure 7: SEM image of *Sa*AuNPs

The finding of SEM revealed a sphere-shaped results. These results revealed the strong relationship between the plasmonic properties and morphology of the SaAuNPs which make these nanoparticles ideal for use in medical applications such as the delivery of drugs, tumor therapeutics, diagnosis and treatment of cancer, and many other conditions (Do et al., 2020). SEM results show a strong correlation between gold nanoparticles' plasmonic properties and morphology, making them suitable for medical applications like drug delivery, tumor therapeutics, cancer diagnosis, and treatment (Husain and Ibrahim, 2019).

Induction Of Psoriasis In Mice

Clinical Symptoms

In terms of behavior, since the negative control mice did not experience the inflammation that the imiquimod-treated mice did, they behaved normally or at baseline. Whereas, the positive control group during the total 10 days of imiquimod application showed an increased grooming behavior, a symptom of discomfort or irritability, due to the induction of inflammation on the back skin beginning from 3-5 days of imiquimod application and increased until the 10 day of completing the application. They displayed less activity, which suggested less acceptable behavior and more

anxiety. While the groups that were treated with 0.001%, 0.003%, and 0.005% of *Sa*AuNPs returned gradually normal behavior and activity with less or no irritable effects between the animals. In addition, clove extract and salicylic acid reduce these symptoms but by less degree than *Sa*AuNPs.

Gross Observation

Negative Control Group

Normal control mice displayed smooth, intact skin with no visible lesions or abnormalities. There were no symptoms of irritation, infection, or inflammation on the skin. The negative control group showed intact skin with no breaks, scrapes, scars, cuts, or abnormalities. In addition, there were no skin lesions (Such as papules, nodules, or ulcers), no redness or erythema, and no pruritis or itching as shown in Figure (8 A).

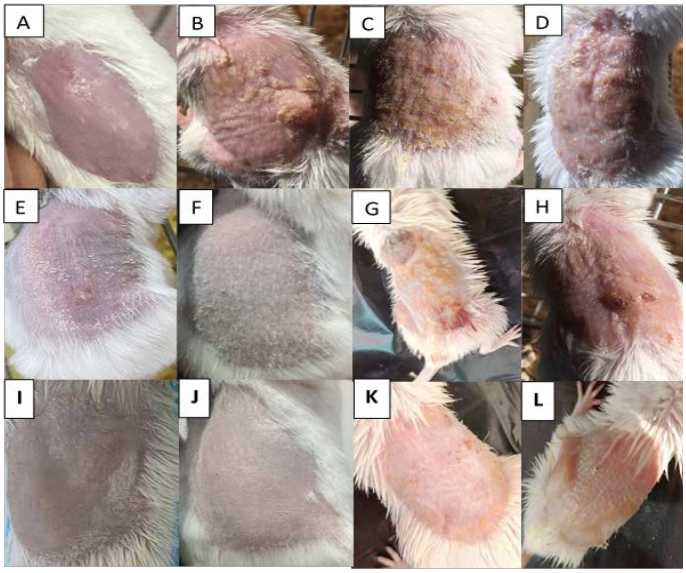


Figure 8: Evaluation of dorsal skin (A) negtive group(B) positive group after 5 days of induction (C) positive group after 10 days of induction (D) after 10 days of treatment by 0.001% *Sa*AuNPs (E) after 5 days of treatment by 0.003% *Sa*AuNP (F) after 5 days of treatment by 0.005% *Sa*AuNPs (G) after 5 days of treatment by 0.5% clove (H) after 5 days of treatment by Salicylic acid (I) after 10 days of treatment by 0.003% *Sa*AuNPs (J)after 10 days of treatment by 0.005% *Sa*AuNPs (K)after 10 days treatment by 0.5% clove (L) after 10 days of treatment by Salicylic acid .

Positive Control Group Without Treatment

Gross examination of the imiquimod-induced group typically revealed scaly, and well-defined plaques that were similar in appearance to plaque psoriasis. The lesions were more localized to the area of imiquimod application. The plaques were dry and appeared gradually with increasing intensity from day 5 (Figure 8 B) to day 10 (figure 8 C) of induction. These clinical symptoms were similar to a study on the psoriasis-induced skin of mice (Noor et al., 2021).

On day 5 of induction, the shaved skin began to be mildly dried with a slight keratinized area and silvery-colored area. At day 10 of the complete induction, highly keratinized silvery-red erythematous patches on the skin appeared, which indicated the development of psoriatic lesions. Cutaneous inflammation in mice generated by the administration of imiquimod resulted in a range of clinical symptoms. These symptoms included redness, scaling or flaking of the skin, increase dermatological thickness (Acanthosis) or hyperkeratosis, and creation of papules or plaques. These symptoms were typically observed in the areas of the skin where imiquimod was applied or where the skin was in close contact with the imiquimod-treated skin.

Imiquimod activates toll-like receptors in the skin, leading to the production of cytokines like TNF alpha and IL 17, triggering a pro-inflammatory immune response, resulting in the characteristic scaly erythema and silvery-red plaques of psoriasis in mice (Ding et al., 2022).

Induced Group Treated With 0.001%, 0.003% And 0.005% *Sa*AuNPs

Groups treated with 0.001%, 0.003%, and 0.005% concentrations of *Sa*AuNPs were used to evaluate the effects on the psoriatic skin, and the results showed that 0.003% and 0.005% exhibited a pronounced reduction in skin symptoms such as scaling, plaques, and thickening (Figures 8 D, E, I, F, J).

However, on treatment with *Sa*AuNPs at concentrations 0.003% and 0.005%, the results revealed a marked recovery from psoriasis symptoms as there was a gradual decrease in the thickness, redness, and scaling of the skin across day 5 (Figure 8 E, F) into the day 10 of treatment (Figure 8 I, J), while the concentration of 0.001% showed no obvious changes (Figure 8 D). On the other hand, Clove oil and salicylic acid showed a little gradual extent of symptomatic reduction in the skin features in comparison with *Sa*AuNPs (Figures 8 G, K, H, L).

Physical examination of the skin of an induced mouse after complete treatment by 0.003% *Sa*AuNPs (Figure 8 I) showed a decrease in the keratinization and redness with the disappearance of scares, while after complete application of 0.005% of *Sa*AuNPs (Figure 8 J) showed a disappearance of the keratinized and red patches with loss of scars. Overall, these findings suggested that *Sa*AuNPs have potential as a therapeutic agent for reducing skin symptoms in psoriasis in mice.

Induced Group Treated With Clove Extract

Clove oil Extract of 0.5% (Figure 8 G, K) topical application reduced the symptoms of psoriasis in the skin of mice. However, clove oil extract, especially when used on inflamed skin, can cause skin irritation (Redness, itching, and burning sensation), mainly due to eugenol content in the oil, and also can cause photosensitivity that requires the use of sunscreens if clove oil used topically (Sahlan et al., 2021). Therefore, the use of *Sa*AuNPs can be used as an alternative agent to clove oil in treating symptoms of psoriasis in mice.

Induced Group Treated With Salicylic Acid

Physical examination of the skin of an induced mouse after complete application of 5% Salicylic acid (Figure 8 L) showed a slight reduction of the keratinization and red patches with a lower incidence of scar formation. Salicylic acid is a common topical treatment for psoriasis that works by promoting the shedding of dead skin cells and reducing the thickness of psoriatic plaques (Jacobi et al., 2015). However, Salicylic acid can cause skin irritation, burning sensation, and dryness of the skin that can exacerbate psoriasis symptoms, therefore, regular moisturizing of the skin is required when salicylic acid is used to prevent skin dryness (Chularojanamontri et al., 2014). For this reason, *Sa*AuNPS can be used as an alternative to Salicylic acid in the treatment of psoriasis symptoms.

Measurement Of Serum Cytokines

Estimation Of Tumor Necrosis Factor

According to the data presented in Figure 9, there were no statistically significant differences (P≤0.05) observed across all groups prior to induction. The group treated with SaAuNPs at concentrations of 0.003% and 0.005% exhibited a statistically significant drop (P≤0.05) in TNF alpha concentration after 5 and 10 days, in comparison to the group treated with clove and salicylic acid, as well as the untreated group. The group of animals treated with 0.001% SaAuNPs, clove, and salicylic acid exhibited a progressive decline in the TNF value over a period of 5 and 10 days. However, it is essential to note that the TNF value remained elevated compared to the negative control group.

The serum levels of TNF-alpha were found to be significantly higher in the induced mice (Positive control) than in healthy not induced mice (negative control), suggesting that TNF-alpha had a vital part in the pathogenesis of psoriasis in mice

Imiquimod increases TNF alpha production by binding and activating TLR and induces transcriptional upregulation of the TNF alpha gene. However, A study has reported elevated serum levels of TNF in mice with induced mice compared to negative control mice. For example, one study reported that TNF levels were significantly increased in the serum of mice with imiquimod induced mice compared to control mice (Vander Fits et al., 2009).

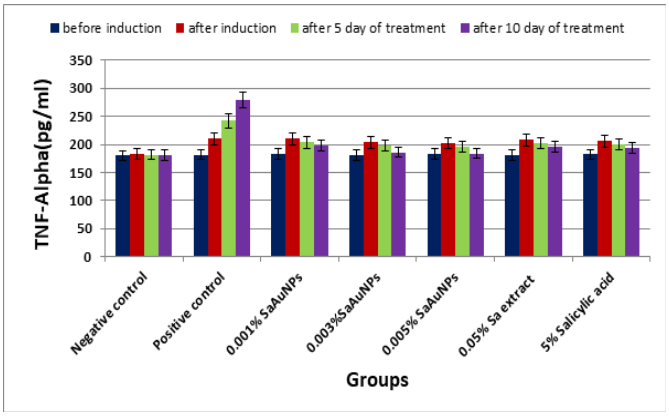


Figure 9: Serum TNF alpha of the negative and positive controls and different treatment concentrations of *Sa*AuNPs, clove and salicylic acid

Among the three concentrations of the *Sa*AuNPs, 0.003% and 0.005% were found to exhibit a substantial decrease in TNF in comparison with clove and salicylic acid. They can inhibit TNF-alpha production in psoriasis by modulating the action of transcription causes Such as activating-protein -1 (AP-1), which are involved in the regulation of TNF-alpha expression and they can inhibit the activation of these transcription factors in psoriatic cells, thereby reducing the expression of TNF-alpha (Sooklert et al., 2020). In addition, clove extract decreases the level of TNF- alpha to a lesser extent than *Sa*AuNPs, which indicates that SaAuNPs had a higher anti-TNF alpha activity than clove oil alone.

Salicylic acid (SA), moreover, reduced the level of TNF alpha in a significant manner, that indicates the influence of salicylic acid on induced-psoriasis in mice .

Estimation Of Interleukin 17 (IL-17)

As shown in Figure 10, there are no obvious significant differences (P≤0.05) among the entire groups before. The treated group with 0.003% and 0.005% *Sa*AuNPs showed a decrease significantly (P≤0.05) in the concentration of IL-17 compared with the group treated with clove and salicylic acid and induced untreated group, while non significantly with the negative control group. Animals treated with 0.001% *Sa*AuNPs, clove, and salicylic acid showed a gradual decrease in IL-17 but still higher than the negative control value.

The IL 17 measured levels were found to be meaningfully greater in the induced mice (Positive control) than in healthy not induced mice (negative control), suggesting that it had a vigorous part in the pathogenesis characters

of psoriasis in mice. However, several studies had stated an increased serum IL 17 in induced mice compared to negative control mice. For example, one study reported that IL-17 levels were significantly increased in the serum of mice with imiquimod-induced psoriasis-like skin inflammation compared to control mice (Van Der Fits et al., 2009).

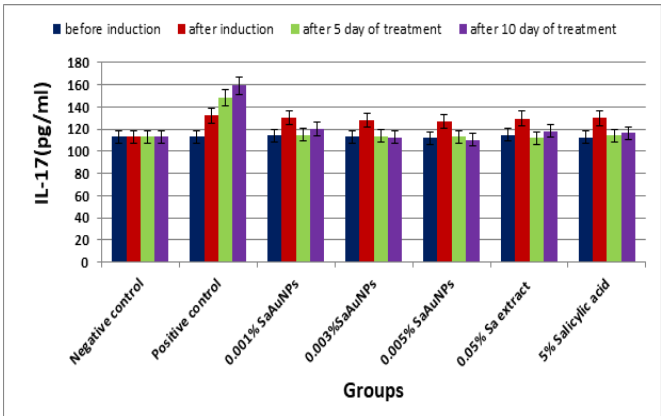


Figure 10: Serum IL-17 of the negative and positive controls and different treatment concentrations of *Sa*AuNPs, clove and salicylic acid

Among the three concentrations of the *Sa*AuNPs, 0.003% and 0.005% were found to exhibit a significant drop in serum IL-17. One mechanism by which *Sa*AuNPs can inhibit IL-17 production in psoriasis is by controlling the activity of transcription causes like signal transducer / activator of the transcription (STAT), which is involved in the regulation of IL-17 expression. Studies have shown that SaAuNPs can inhibit the activation of STAT in psoriatic cells, thereby reducing the expression of IL-17 (Shen et al., 2022).

CONCLUSIONS AND RECOMMENDATIONS

1: Green synthesis process of the nanoparticles of gold offers benefits like extent of size and shape uniformity, reduced toxicity, and crystalline stability, making them attractive for various applications.
2: Formulating gold nanoparticles with carbopol can result in sustained release and a prolonged therapeutic effect.
3: The use of imiquimod can lead to psoriasis-like skin injuries, and gold nanoparticles synthesized by clove oil have potential as a treatment alternative for psoriasis dealing with their studied anti-inflammatory properties.

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NOVELTY STATEMENT

The novelty of the study is its focus on pharmacologically active gold nanoparticles formulated by green synthesis that can be employed as novel anti-psoriatic pharmaceuticals due to the challenges and problems associated with psoriasis disease and the lack of readily accessible, secure, and efficient medicines for psoriasis that is resistant to many synthetic treatments, it is imperative to seek into alternate sources of anti-psoriasis medications.

CONFLICT OF INTEREST

No conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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