RESEARCH PAPER

Preparation and characterization of silver nanoparticles using surian (*Toona sinensis*) leaf extract and the wound healing efficacy in mice

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ABSTRACT

Objective(s): Green synthesis is a method of forming silver nanoparticles (AgNP) that is widely developed because it uses natural reducing agents, which are safer and more environmentally friendly. This study investigates the formation of silver nanoparticles using surian (*Toona sinensis* (Juss.) M. Roem) leaf extract as a bioreductor and its wound healing effectivity in mice.

Materials and Methods: *T. sinensis* leaf was extracted by distilled water in 1:10 ratio at 90 °C for 15 min using magnetic stirrer. 0.5 mL of 0.1 M $AgNO_3$ was mixed with 0.25 mL of 10% *T. sinensis* leaf extract, diluted to 50 mL, and stirred for 4 hr. The UV-Vis spectrum was measured at 300-800 nm. Particle size, morphology, functional group, and crystal structure of silver nanoparticles were characterized. For wound healing properties, mice were divided into seven groups, and each group experienced wounds induced by HCl on their dorsal side, followed by various treatments. Wound healing was monitored over 18 days, and statistical analysis assessed the effect of silver nanoparticle concentration and treatment duration.

Results: The formation of colloidal silver nanoparticles was indicated by a change in the color from colorless to brown. The silver nanoparticles had the Surface Plasmon Resonance (SPR) band at 420 nm, spherical with an average size of 39 nm, and crystalline with a face-centered cubic structure. These silver nanoparticles could accelerate wound healing in mice compared to the negative control group, the group given silver sulfadiazine, $AgNO_3$, and *T. sinensis* leaf extract alone ($P<0.05$).

Conclusion: This shows that silver nanoparticles mediated by *T. sinensis* leaf extract have the potential to be developed into wound healing agents.

Keywords: *Colloidal silver, Plant extract, Reducing agents, Silver nanoparticles, Wound healing*

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INTRODUCTION

Nanotechnology has become a significant area of interest for scientists in recent times. Research on designing, synthesizing, and manipulating particles into nanoscale has been widely developed. Nanoparticles have distinctive properties with particle sizes around 1-100 nm [1]. These include optical, electronic, and superior bioactivity properties due to their smaller shape and size [2].

The use of nanoparticles for biomedical and pharmaceutical applications such as drug delivery, wound dressings, biosensors, and other medical purposes has been widely reported. Nanoparticles have been used for wound healing by initiating the healing process at various phases [3].

Wound management currently relies on developing new drugs and effective wound-dressing materials. In prolonged wounds, the inflammatory response can cause tissue damage. Pathogenic microbes in wounds also produce toxins and enzymes that can prolong the inflammatory response and delay wound healing [4]. This aspect represents a significant area of research and poses a considerable clinical challenge. In recent years, research and development of wound dressing materials have entered a new level [5].

Materials such as nanomaterials, nanofibers,

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and biomaterials are commonly used in wound care, especially materials with antibacterial, antimicrobial, and anti-inflammatory activities. Metal nanoparticles have been widely applied as diagnostic, drug delivery, and antimicrobial agents in health and cosmetic products. Silver nanoparticles have attracted the attention of researchers because of their simple synthesis process. They can be applied in various fields of science and technology, including the medical field [6].

Silver in the form of metallic silver, silver nitrate, and silver sulfadiazine, which has antibacterial properties, has been widely used to treat burns, dental care, catheters, and infection control [7]. Silver in the ionic form has greater toxicity than silver nanoparticles [8]. Therefore, nano preparations are more effective in killing resistant bacteria that infect wounds, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which accelerate wound healing [9,10].

The simplest method used to form silver nanoparticles is the chemical reduction method [11]. The disadvantage of this method is that it uses synthetic chemical compounds that are hazardous for medical applications and hazardous waste to the environment. To overcome this, a sustainable synthetic method known as the green synthesis approach has been developed [12]. The use of plants and microorganisms as reducing and stabilizing agents in the synthesis of silver nanoparticles was developed in this method to reduce the discharge of harmful substances into the environment. Plants that have secondary metabolites (alkaloids, flavonoids, phenolics, terpenoids, etc.) can be used as reducing agents [13].

Green synthesis method is very simple, requiring less time and energy in comparison to the physical and chemical methods. Using water as a solvent in extraction also reduces hazardous waste in the environment [14]. The extraction process is carried out by heating the plant extract. Ahmadi et al. (2018) and many others demonstrated that boiling the plant in water is an effective method for preparing extracts to synthesize silver nanoparticles [15–18]. Nguyen et al. (2023) also reported the increase in intensity of silver nanoparticle peak could be correlated with an enhancement in the extraction temperature [19]. This is attributed to the higher temperatures leading to a greater separation of the reducing agent, thereby explaining the heightened peak intensity.

Surian (*T. sinensis*) is a plant that contains a lot of secondary metabolites. *T. sinensis* is a species of the Meliaceae family widely distributed in Asia, especially in Indonesia. *T. sinensis* has been widely used traditionally to treat diseases such as infections, diarrhea, diabetes, and others. *T. sinensis* leaves have been reported to contain phenolic compounds (gallic acid, methyl gallic) and flavonoids (quercetin, quercetrin, kaempferol, catechin, rutine) [20]. The content of secondary metabolites that act as natural antioxidants can be used as medicine [21]. Secondary metabolites of *T. sinensis* leaf extract can play a role in reducing silver ions into silver nanoparticles. No previous studies have explored the use of silver nanoparticles mediated by *T. sinensis* leaf extract for wound healing. In this study, silver nanoparticles mediated by *T. sinensis* extract were characterized, and their efficacy in promoting wound healing was investigated.

MATERIALS AND METHODS

Material

The materials used in this study were surian leaves (*T. sinensis*), distilled water, $AgNO_3$ (Merck), HCl, aluminum foil, Whatman filter paper No.42, and mice.

Preparation of T. sinensis leaf extract

Fresh surian leaves (*T. sinensis*) were washed and airdried for two weeks. The dried leaves were ground into *T. sinensis* leaf powder. *T. sinensis* leaves were extracted with distilled water at a 1:10 w / v ratio and then stirred for 15 min using a magnetic stirrer at 90 °C. The crude extract was cooled and filtered. The extract was freeze-dried, stored in an airtight container, and placed at 4°C [21,22].

Formation of silver nanoparticles

0.5 mL of 0.1 M $AgNO_3$ solution was mixed with 0.25 mL of 10% *T. sinensis* leaf extract, and then the volume was added to 50 mL with distilled water. The silver nanoparticles were formed while stirring at room temperature using a magnetic stirrer at 150 rpm. After 1 hour of stirring, 2 mL of samples were taken, and the UV-Vis absorption spectrum was measured using spectrophotometer at the wavelength range of 300-800 nm.

Characterization of silver nanoparticles

Size and morphological characterization

The size of silver nanoparticles was determined using Particle Size Analyzer (PSA) (Horiba SZ-

100). The measuring range was 0.35-10,000 nm, the scattering angle of 90°, and the holder temperature was 25 °C.

The morphology of silver nanoparticles was determined using a Transmission Electron Microscope (TEM) (HT7700). A total of 5 µl of silver nanoparticle colloid samples were placed on a copper grid and then dried. Samples were observed at magnifications of 50,000 and 100,000 times. The voltage was set at 120kV. TEM micrograph showed the morphology of silver nanoparticles.

Crystal structure characterization

Silver nanoparticle crystal structure was analyzed using an X-Ray Diffractometer (XRD) (X'Pert Powder DY 3688). 10 mg of silver nanoparticles were placed on the sample holder (glass) and leveled. The measurement was carried out at room temperature under the following conditions: metal target Cu, Kα filter, voltage 40kV, current 30 mA. The analysis was conducted in the 2 theta 10°- 90° [23]. The peak intensity and theta value were obtained from the diffractogram pattern, which showed the crystal phase of the silver nanoparticles.

Functional group characterization

Functional group characterization was carried out using Fourier Transform Infrared (FTIR) to determine the functional groups of *T. sinensis* leaf extracts and silver nanoparticles that had been synthesized. A mixture of 2 mg sample and 200 mg KBr powder was made into pellets. The pellets were placed in the sample holder, and the infrared spectrum was recorded at 400-4000cm^{-1}. Various vibration modes were identified to determine the functional groups present in extracts and silver nanoparticles [23].

Wound healing properties of silver nanoparticles

Animal models of HCl-induced injury for wound healing efficacy were performed using male white mice (*Mus musculus*). The animals were utilized after receiving consent from The Committee of the Research Ethics of the Faculty of Medicine, Universitas Andalas (No.277/KEP/FK/2020).

All mice were acclimatized for ± 7 days with a 12-hour light/dark cycle and a pellet diet. Mice were divided into seven groups of 5 mice. The mouse's skin was shaved on the right dorsal side of the body. Mice were anesthetized with ether. The skin was then wounded using 0.1 mL of 20% HCl solution. Wounds in mice were regularly given treatment: group I would be used as the negative control with no treatment, group II would be used as the positive control (treated with 1% silver sulfadiazine), group III and group IV would be treated with $AgNO_3$ and *T. sinensis* leaf extract, group V, group VI, and group VII would be treated with silver nanoparticles at concentrations 0.5 mg/mL, 0.25 mg/mL, and 0.05 mg/mL.

Each treatment was applied to the wound area once a day for 18 days. The diameter of the wound was measured on days 1, 3, 6, 9, 12, 15, and 18. The wound area was calculated based on the wound diameter data. The percentage of wound healing was determined by calculating the reduction of the wound area using the following formula: [24, 25].

% WH = (WA1-WAn/WA1) x 100
Where:
WH = wound healing
WA1 = area of the wound on day 1
WAn = area of the wound on day n (n= 3, 6, 9, 12, 15 and 18)

Statistical analysis

Wound healing percentages were analyzed statistically using SPSS software. Two-way ANOVA and Tukey's post hoc test were used to analyze the data. Experiments were repeated 3 times for each data point, and the significance level was $P \leq 0.05$.

RESULTS

Formation of *silver nanoparticles using T. sinensis leaf extract as bioreductor*

Colloidal silver nanoparticles in this study showed a change in color from colorless to yellow to brown (Fig. 1).

The wavelength of maximum absorption of silver nanoparticles mediated by *T. sinensis* leaf extract was 420 nm. The shift of absorption maximum of $AgNO_3$ and surian leaf extract towards longer wavelength (from 302 and 271 nm to 420 nm) indicated that silver nanoparticles had

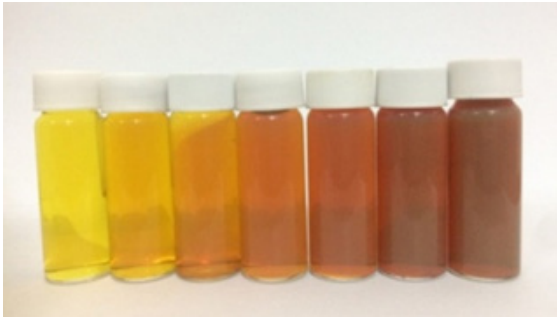

Fig. 1. Colloidal silver nanoparticles

been formed (Fig. 2).

Characterization of silver nanoparticles

Morphological and size characterization of silver nanoparticles

TEM results showed the morphology of the silver nanoparticles was spherical and dispersed without any aggregation between particles (Fig. 3).

The particle size distribution spectrum using PSA showed two peaks. The first peak showed the size distribution of particles with a mean diameter of 2 nm. The second peak showed the particle size distribution with a mean diameter of 40 nm. The average diameter of bioreduction silver nanoparticles using *T. sinensis* leaf extract was 39 nm (Fig. 4).

Crystal structure characterization of silver nanoparticle

XRD of silver nanoparticles showed a sharp peak in the 2θ region at 38.16, 44.27, 64.52, 77.46, and 81.50. The peaks were indexed in planes (111), (200), (220), (311), and (222), respectively, which are specific diffraction peaks of silver with a face-centered cubic structure (Fig. 5). The degree of crystallinity of the silver nanoparticle sample was 85%, with a crystal size of 12 nm.

Functional groups characterization of silver nanoparticle

FTIR spectrum of colloidal silver nanoparticles and *T. sinensis* leaf extract showed wide absorption

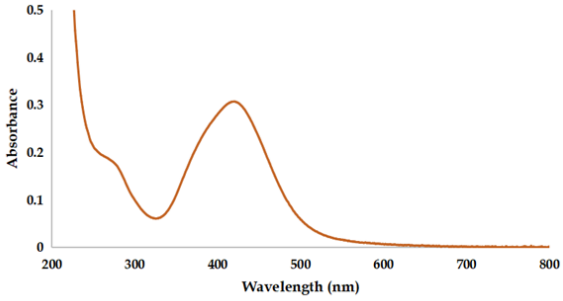

Fig. 2. UV-Vis spectrum of silver nanoparticles mediated by *T. sinensis* leaf extract

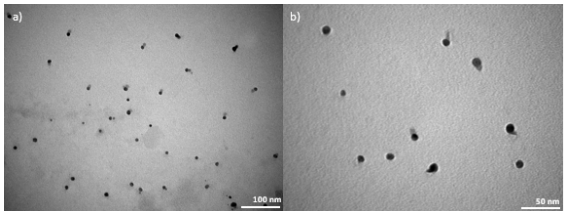

Fig. 3. TEM micrographs of silver nanoparticles at magnifications of (a) 50,000 and (b) 100,000 times

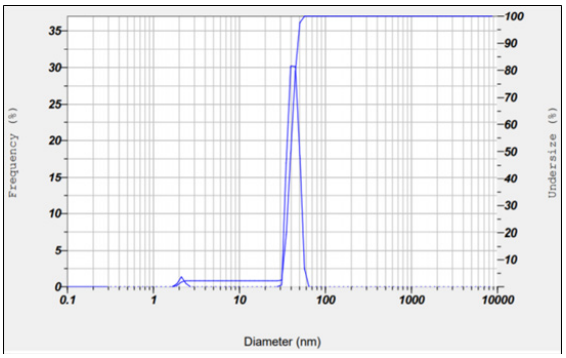

Fig. 4. Particle size distribution of silver nanoparticles using *T. sinensis* leaf extracts as bioreductor

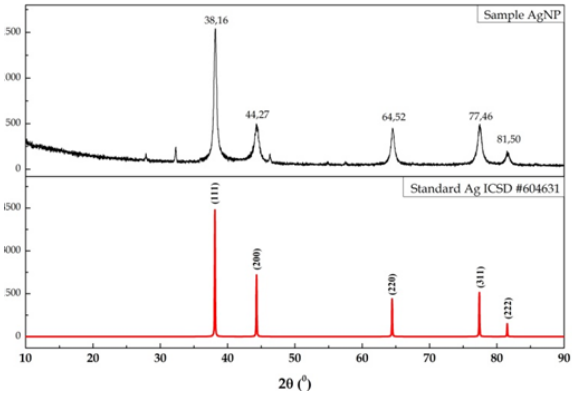

Fig. 5. X-ray diffractogram of silver nanoparticles using *T. sinensis* leaf extracts as bioreductor

in the 3332 cm^{-1} and 3333 cm^{-1} wavenumber regions which were phenolic –OH stretching, 1637 cm^{-1} and 1636 cm^{-1} which were C=O stretching, and absorption at the wavelength region of 678 cm^{-1} and 710 cm^{-1} which were the C–H vibration (Fig. 6).

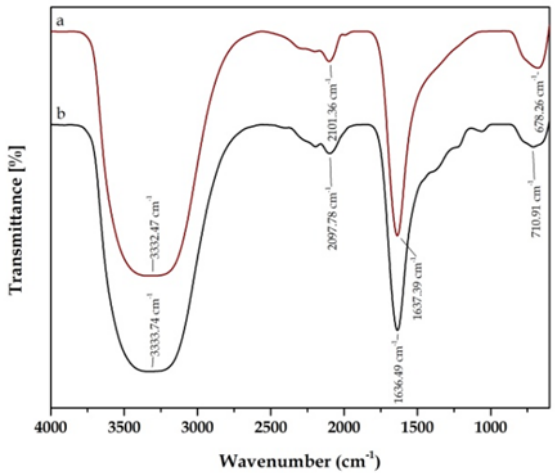

Fig. 6. FTIR spectrum of (a) silver nanoparticles using *T. sinensis* leaf extract as bioreductor, and (b) *T. sinensis* leaf extract

The wound healing activity of silver nanoparticles mediated by T. sinensis leaf extract

Types of treatment (silver nanoparticles with different concentrations, negative control, positive control, $AgNO_3$, and *T. sinensis* leaf extract), duration of administration, and their interaction statistically gave a very significant effect ($P<0.005$) on the percentage of wound healing in mice (Table 1). The group of mice given silver nanoparticles with a concentration of 0.5 mg/mL had the highest percentage of wound shrinkage compared to the other groups, which was 60.93%. Then followed by a group given silver nanoparticles with a concentration of 0.25 mg/mL, silver sulfadiazine, silver nanoparticles with a concentration of 0.05 mg/mL, $AgNO_3$, *T. sinensis* leaf extract, and negative control with the percentage of wound healing was 57.48%; 52.73%; 50.35%; 46.25%; 43.93%; and 40.03% respectively. The percentage of wound healing in all mice increased with more prolonged treatment. The average percentage of wound healing in mice on days 3, 6, 9, 12, and 15 were 2.99%, 19.77%, 51.89%, 81.44%, 95.60%, and 100%.

In negative control mice, the percentage of wound healing on day 3 was almost the same as the group given $AgNO_3$. The following day, the percentage of wound healing in negative control mice was slower than in all groups given the drug. Meanwhile, mice given $AgNO_3$ on the 3rd day initially had a smaller percentage of wound healing than those given *T. sinensis* leaf extract. However, on the 6th day, the percentage of wound healing was almost the same and even slightly higher on the 9th, 12th, and 15th days (Figure 7).

The group given silver nanoparticles with a concentration of 0.05 mg/mL from day 1 to day 6 showed almost the same percentage of wound healing as the group given $AgNO_3$ and *T. sinensis* leaf extract. On the 9th day, there was a significant

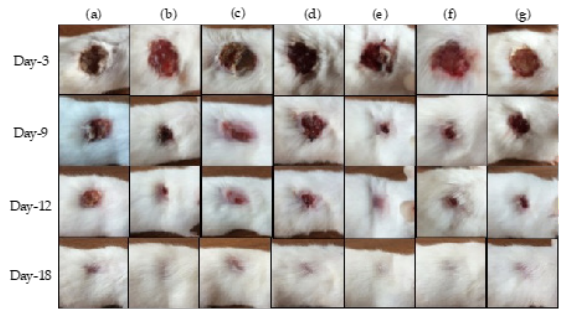

Fig. 7. Wound healing efficacy in mice with the treatment of (a) no treatment, as negative control, (b) marketed silver sulfadiazine as positive control, (c) silver nitrate, (d) *T. sinensis* leaf extract, and silver nanoparticles mediated by *T. sinensis* leaf extract with the concentration (e) 0.5 mg/mL, (f) 0.25 mg/mL, and (g) 0.05 mg/mL

increase in the percentage of wound healing of groups given silver nanoparticles. This percentage was greater than the group given $AgNO_3$ and *T. sinensis* leaf extract. The increase in the percentage of wound healing also exceeds the positive control group given silver sulfadiazine. However, on days 9 to 15, the percentage of wound healing in the group given 0.05 mg/mL silver nanoparticles was slightly smaller than the group given silver sulfadiazine (Fig. 8).

The percentage of wound healing in the group given 0.5 mg/mL silver nanoparticles until the 6th day was almost the same as the group given 0.25

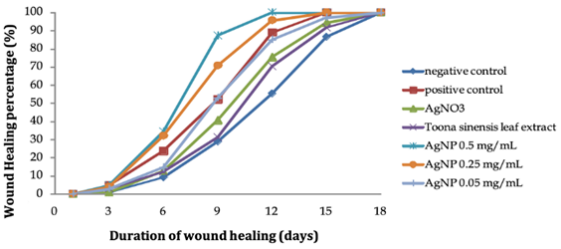

Fig. 8. Wound healing percentage in mice

Table 1. Wound healing percentage in mice

Duration (days)	Wound healing percentage (%) ± SD							Average
	Negative control	Positive control	$AgNO_3$	*T. sinensis* leaf extract	AgNP 0.5 mg/mL	AgNP 0.25 mg/mL	AgNP 0.05 mg/mL	
1	0,00 ±0,000	0,00 ±0,000	0,00 ±0,000	0,00 ±0,000	0,00 ±0,000	0,00 ±0,000	0,00 ±0,000	0,00 ±0.000
3	0,94 ±2,104	4,80 ±3,450	0,92 ±2,056	2,80 ±4,164	4,84 ±4,876	3,86 ±4,068	2,81 ±2,566	2,99 ±1.633^{p}
6	9,09 ±5,032	23,56 ±6,390	12,67 ±3,839	12,03 ±2,703	34,47 ±5,867	32,09 ±10,146	14,47 ±4,309	19,77 ±10.287^{q}
9	28,71 ±4,804	51,95 ±3,195	40,53 ±3,152	31,02 ±8,475	87,17 ±8,764	70,82 ±6,833	53,01 ±9,189	51,89 ±21.236^{r}
12	55,17 ±6,896	88,83 ±3,043	75,28 ±5,610	70,16 ±6,200	100,00 ±0,000	95,57 ±2,759	85,07 ±6,611	81,44 ±15.636^{s}
15	86,32 ±1,689	100,00 ±0,000	94,32 ±4,998	91,46 ±2,474	100,00 ±0,000	100,00 ±0,000	97,13 ±2,522	95,60 ±5.250^{t}
18	100,00 ±0,000	100,00 ±0,000	100,00 ±0,000	100,00 ±0,000	100,00 ±0,000	100,00 ±0,000	100,00 ±0,000	100,00 ±0.000
Average	40,03 ±41.199^{a}	52,73 ±44.162^{c}	46,25 ±43.583^{b}	43,93 ±42.616^{b}	60,93 ±46.235^{e}	57,48 ±44.849^{d}	50,35 ±44.626^{c}	

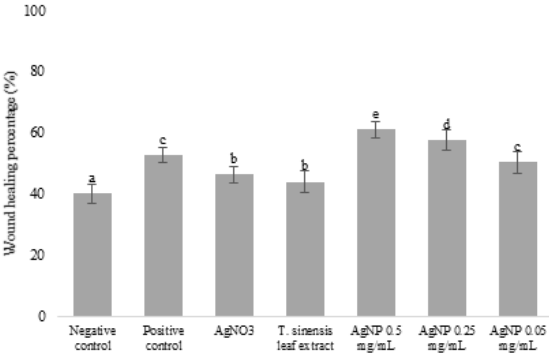

Fig. 9. Effect of treatment in wound healing percentage. Different alphabets on each bar indicate significant differences in mean values (P<0.05)

mg/mL silver nanoparticles. However, on the 9th day, there was an acceleration of wound healing in the group given 0.5 mg/mL silver nanoparticles compared to the group given 0.25 mg/mL silver nanoparticles. By day 12, the wounds in mice treated with 0.5 mg/mL silver nanoparticles had healed and were the fastest compared to all the treated and negative control groups (Figure 8).

It can be concluded that all treated groups had a higher percentage of wound healing compared to negative controls. Based on Tukey's post hoc test, all groups gave a significantly different percentage of wound healing (P<0.05) except between the group given silver sulfadiazine and the group given silver nanoparticles with a concentration of 0.05 mg/mL (P>0.05), and between the group given $AgNO_3$ and the group given *T. sinensis* leaf extract (P> 0.05) (Fig. 9).

DISCUSSION

T. sinensis leaf extract used in this study contained compounds from the phenolic, flavonoids, triterpenoids, and saponins groups [26]. This group of compounds has the potential to be used as bioreductor in the formation of silver nanoparticles. The combination of biomolecules present in plant extracts, such as alkaloids, phenolics, flavonoids, tannins, terpenoids, proteins, enzymes, and polysaccharides, can reduce and stabilize silver ions in the formation of silver nanoparticles [27]. The groups of biomolecular compounds that play a role in the bioreduction of silver ions are hydroxyl and carboxylate groups [1]. Previous studies on the formation of silver nanoparticles by bioreduction using plant extracts have been reported, including *Carica papaya*, *Calliandra haematocephala*, *Azadirachta indica*, and *Aloe vera* extracts [28–31].

The formation of silver nanoparticles in this study used 1 mM $AgNO_3$ and 0.05% surian leaf extract. The reduction of silver ions resulted in color change of the solution from colorless to yellow and to brown. This change occurs due to the excitation of Surface Plasmon vibrations from silver nanoparticles. The color of silver nanoparticles could be affected by the concentration of $AgNO_3$ and the extract added. Arokiyaraj et al. (2017) also reported silver nanoparticles synthesized using 2 mmol $AgNO_3$ and 5 mL of *Rheum palmatum* extract produced colloids with brown color [32].

Silver nanoparticles were also confirmed by the shift in the maximum absorption wavelength of silver nitrate and *T. sinensis* leaf extract, which initially was 302 nm and 271 nm to a wavelength range of 420 nm. This alignment with previous research indicates that colloidal silver nanoparticles absorb light at approximately 400-450 nm [33]. Similar results were also found in research on the green synthesis of silver nanoparticles using plant extracts of *Calliandra haematocephala*, *Punica granatum*, and *Amomum villosum* [31, 34, 35].

The silver nanoparticles were characterized using several instruments, including PSA to obtain information about the particle size distribution, TEM to analyze particle morphology, XRD to determine the crystal structure of particles, and FTIR to identify the functional groups of the silver nanoparticles.

From the particle size analysis, silver nanoparticles mediated by *T. sinensis* leaf extract had an average particle size of 39 nm. It was supported by the analysis of particle shape and size using TEM. It can be concluded that silver nanoparticles were spherical and dispersed with sizes below 100 nm. According to the National Nanotechnology Initiative, nanoparticles have a particle size between 1-100 nm [33]. Previous studies have reported bioreduction of silver nanoparticles by several plant extracts, such as *Azadirachta indica*, *Euphorbia milii,* and *Punica granatum,* had particle sizes of 34 nm, 20-50 nm, 48 nm respectively, and were spherical [28, 34, 35].

Based on the crystal structure characterization using XRD, silver nanoparticles mediated by *T. sinensis* extract gave sharp peaks similar to the X-Ray diffraction pattern of standard ICSD #604631. The sharp peaks on the diffractogram indicated the degree of crystallinity of silver nanoparticles was relatively high at 85%. Based on the Scherrer equation, the crystalline size of the silver nanoparticles was 12 nm. These

peaks were indexed in fields (111), (200), (220), (211), and (222). The peak accordingly showed a specific diffraction peak of silver with a face-centered cubic structure [36]. Esmaile et al. (2020) reported a similar crystal structure of silver nanoparticles with the crystal size of 23.5 nm from the bioreduction of 4 mM $AgNO_3$ by 5 mL of *Ziziphora clinopodioides* extract. The difference in crystal size may occur due to differences in extract content and reactant concentration during the formation of silver nanoparticles [37].

FTIR analysis was carried out to determine the possible functional groups involved in the reduction of silver ions (Ag+) to silver nanoparticles (Ago). The infrared spectrum of *T. sinensis* leaf extract showed a strong absorption at a wavenumber of 3333 cm^{-1} which could be attributed to the stretching vibration of the hydroxyl group (O-H) of phenolic compounds or N-H stretching of amine groups from amino acids/proteins contained in the extract. Meanwhile, the absorption peak at 1636 cm^{-1} showed a stretching vibration of C=O, indicating amides in the extract. The weak signal at 710 cm^{-1} was the vibration of C-H aromatic ring bending of phenolic compounds in the extract.

In the infrared spectrum of silver nanoparticles, there was a shift in the absorption peak at 3340 cm^{-1} which confirmed the interaction of the hydroxyl groups and silver nanoparticles, resulting in the partial destruction of the hydrogen bonds between phenolic molecules in *T. sinensis* leaf extract. The carbonyl group has a strong ability to bond with metals by forming a layer that covers silver nanoparticles and acts as a stabilizing agent that prevents agglomeration. This interaction was confirmed by a shift in the absorption peak of the carbonyl group at 1637 cm^{-1}. The presence of hydroxyl (-OH) and amine (N-H) groups in the extract was the main factor involved in the reduction and stabilization of silver nanoparticles [38].

Similar results were observed in the formation of silver nanoparticles using *Ziziphora clinopodioides* extract. The hydroxyl (-OH), carboxylate (-COOH), and amine (N-H) groups contained in the extract play role in the synthesis of silver nanoparticles (Esmaile et al., 2020). The reduction of silver ions to silver nanoparticles is caused by the presence of phenolic compounds, flavonoids, amino acids, vitamins, tannins, and others. [31, 39].

The use of silver nanoparticles as a wound healing agent was investigated in this work using mice as experimental animals. The effectiveness of silver nanoparticles was determined by the shrinkage of the wound area. Silver nanoparticles could heal wounds in a shorter time compared to the positive control, $AgNO_3$, *T. sinensis* leaf extract, and negative control group.

Wound healing by *T. sinensis* leaf extract can be attributed to its anti-inflammatory and antibacterial activity. Kuo et al. (2020) reported that surian leaf extract has antioxidant properties and can inhibit the release of cytokines to prevent damage to cells [40]. Surian leaf extract also has antibacterial effects on *E.coli*, *Salmonella*, and *Staphylococcus* with Minimum Inhibitory Concentration (MIC) of 0.25, 0.125, 0.25, and 0.25 g/mL, respectively [41].

Silver sulfadiazine was used as a comparison in this study because it is the "gold standard" in the topical treatment of burns. Silver sulfadiazine and silver nitrate are broad-spectrum antibacterial agents. Giving these antibacterial agents to wounds can prevent and treat wound infection, thereby accelerating wound healing [42]. The silver ion in silver nitrate dissociates rapidly in solution, resulting in a rapid release of silver. The silver ion interacts with the chloride in the wound fluid, reducing its antibacterial effectiveness. Silver sulfadiazine has a slower release of silver ions than silver nitrate, so it has a longer-lasting effect. Silver nanoparticles act as reservoirs of silver atoms. The effect of silver nanoparticles can last longer than silver nitrate and silver sulfadiazine, even at low concentrations [43].

Silver nanoparticles have a high surface-to-volume ratio, making them more reactive and allowing the particles to remain effective at low concentrations. This also reduces the possibility of side effects [42]. Previous studies have reported the broad-spectrum antibacterial properties of silver nanoparticles. The antibacterial mechanism of silver nanoparticles involves disruption of the cell wall, blocking DNA replication, and destabilizing bacterial ribosomes [9, 12, 27].

Al-Shmgani et al. (2017) reported the effectiveness of wound healing of biosynthesized silver nanoparticles with *Catharanthus roseus* leaf extract. Based on this study, mice given silver nanoparticles showed 98% wound healing on day 12, while the negative control group was 85% [44]. Another study found that after 14 days, rabbits given silver nanoparticles synthesized using *Bryonia laciniosa* extract had wound shrinking of 92.6%. Meanwhile, rabbits given silver sulfadiazine

and negative control had wound shrinkage of 78.1% and 47.1%, respectively. There was a faster shrinkage and closure of the wound area in rabbits given silver nanoparticles compared to silver sulfadiazine and the negative control group [4].

Based on the study of Tian et al. (2007), silver nanoparticles also accelerate wound healing in diabetic mice and mice with burns. In diabetic mice with an excision wound model, the group was given silver nanoparticles healed within 16 days after injury, while the control group took 18 days. In mice with burns, the group given silver nanoparticles experienced faster healing with better epidermal histology and hair follicle growth than those given silver sulfadiazine and negative control [45]. Heydarnejad et al. (2014) also reported that silver nanoparticles have an anti-inflammatory effect in accelerating wound healing and reducing scarring by decreasing serum levels of anti-inflammatory factors (of TGF- β, C3, RF, CRP) [46]. The wound healing effect of silver nanoparticles is related to their antimicrobial properties, reduction of wound inflammation, and modulation of fibrogenic cytokines. Through cytokine modulation, silver nanoparticles also reduce scar formation and inflammation [4].

Silver nanoparticles have been successfully synthesized from bioreduction of silver nitrate with surian leaf extract (*T. sinensis*). Characterization of silver nanoparticles using PSA, TEM, XRD, and FTIR showed that the nanoparticles formed were spherical and crystalline, with a particle size of less than 50 nm. The silver nanoparticles were then tested on mice to see their effect on wound healing. Wounds in mice healed faster than the marketed wound medication (silver sulfadiazine). The role of bioreduced silver nanoparticles using *T. sinensis* leaf extract as antibacterial, anti-inflammatory, and antioxidant agents remains to be explored in future research.

CONCLUSION

Surian (*T. sinensis*) leaf extract has the potential as a bioreductor in the formation of silver nanoparticles. The silver nanoparticles bioreduced using *T. sinensis* leaf extract were spherical with the particle size around 39 nm and had a face-centered cubic crystal structure. Silver nanoparticles could accelerate wound healing induced in mice. The silver nanoparticles effectiveness in wound healing was better than the group given silver sulfadiazine, $AgNO_3$, *T. sinensis* leaf extract, and negative control (P<0.005). These findings suggest that silver nanoparticles mediated by *T. sinensis* leaf extract can potentially serve as promising candidates for drug therapy in wound management.

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CONFLICTS OF INTEREST STATEMENT

The authors declared no conflict of interest.

REFERENCES

1. Hassan Korbekandi, Iravani S. The Delivery of Nanoparticles. Silver Nanoparticles. 2012. 3–23.
2. Ivanova N, Gugleva V, Dobreva M, Pehlivanov I, Dobreva M, Pehlivanov I, et al. Silver Nanoparticles as Multi-Functional Drug Delivery Systems. Nanomedicines. 2018;(November).
3. Ovais M, Ahmad I, Khalil AT, Mukherjee S, Javed R, Ayaz M, et al. Wound healing applications of biogenic colloidal silver and gold nanoparticles: recent trends and future prospects. Appl Microbiol Biotechnol. 2018;102(10):4305–4318.
4. Dhapte V, Kadam S, Moghe A, Pokharkar V. Probing the wound healing potential of biogenic silver nanoparticles. J Wound Care. 2014;23(9):431–441.
5. Rajendran NK, Kumar SSD, Houreld NN, Abrahamse H. A review on nanoparticle based treatment for wound healing. J Drug Deliv Sci Technol. 2018;44:421–430.
6. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: Synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci. 2016;17(9):1534.
7. Chopra I. The increasing use of silver-based products as antimicrobial agents: A useful development or a cause for concern? Journal of Antimicrobial Chemotherapy. 2007;59(4):587–590.
8. Basu A. Metals in Medicine: An Overview. Sci Revs Chem Commun. 2015;5(2):77–87.
9. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, et al. Silver nanoparticles as potential antibacterial agents. Molecules. 2015;20(5):8856–8874.
10. Gunasekaran T, Nigusse T, Dhanaraju MD. Silver nanoparticles as real topical bullets for wound healing. J Am Coll Clin Wound Spec. 2011;3(4):82–96.
11. Iravani S, Korbekandi H, Mirmohammadi S v., Zolfaghari B. Synthesis of silver nanoparticles: Chemical, physical and biological methods. Res Pharm Sci. 2014;9(6):385–406.
12. Siddiqi KS, Husen A, Rao RAK. A review on biosynthesis of silver nanoparticles and their biocidal properties. J Nanobiotechnology. 2018;16(1):14.
13. Firdhouse MJ, Lalitha P. Biosynthesis of silver nanoparticles and its applications. J Nanotechnol. 2015; 2015(September 2014).
14. Chouhan N. Silver Nanoparticles: Synthesis, Characterization and Applications. In: Silver Nanoparticles

- Fabrication, Characterization and Applications. InTech; 2018.
15. Ahmadi O, Jafarizadeh-Malmiri H, Jodeiri N. Eco-friendly microwave-enhanced green synthesis of silver nanoparticles using Aloe vera leaf extract and their physico-chemical and antibacterial studies. Green Processing and Synthesis. 2018 Jun 27;7(3):231–240.
16. Balavijayalakshmi J, Ramalakshmi V. Carica papaya peel mediated synthesis of silver nanoparticles and its antibacterial activity against human pathogens. J Appl Res Technol. 2017 Oct 1;15(5):413–422.
17. Asif M, Yasmin R, Asif R, Ambreen A, Mustafa M, Umbreen S. Green Synthesis of Silver Nanoparticles (AgNPs), Structural Characterization, and their Antibacterial Potential. Dose-Response. 2022;20(2):1–11.
18. Sarli S, Kalani MR, Moradi A. A Potent and Safer Anticancer and Antibacterial Taxus-Based Green Synthesized Silver Nanoparticle. Int J Nanomedicine. 2020;15:3791–3801.
19. Nguyen LAT, Van Mai B, Van Nguyen D, Nguyen NQT, Van Pham V, Pham TLM, et al. Green synthesis of silver nanoparticles using Callisia fragrans leaf extract and its anticancer activity against MCF-7, HepG2, KB, LU-1, and MKN-7 cell lines. Green Processing and Synthesis. 2023;12(1): 20230024.
20. Yang H, Gu Q, Gao T, Wang X, Chue P, Wu Q, et al. Flavonols and derivatives of gallic acid from young leaves of Toona sinensis (A. Juss.) Roemer and evaluation of their anti-oxidant capacity by chemical methods. Pharmacogn Mag. 2014;10(38):185–190.
21. Hseu YC, Chang WH, Chen CS, Liao JW, Huang CJ, Lu FJ, et al. Antioxidant activities of Toona Sinensis leaves extracts using different antioxidant models. Food and Chemical Toxicology. 2008;46(1):105–114.
22. Chang HL, Hsu HK, Su JH, Wang PH, Chung YF, Chia YC, et al. The fractionated Toona sinensis leaf extract induces apoptosis of human ovarian cancer cells and inhibits tumor growth in a murine xenograft model. Gynecol Oncol. 2006;102(2):309–314.
23. Dipankar C, Murugan S. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. Colloids Surf B Biointerfaces. 2012;98:112–119.
24. Adibhesami M, Ahmadi M, Farshid AA, Sarrafzadeh-Rezaei F, Dalir-Naghadeh B. Effects of silver nanoparticles on Staphylococcus aureus contaminated open wounds healing in mice: An experimental study. Vet Res Forum. 2017;8(1):23–28.
25. Arya G, Kumari RM, Sharma N, Gupta N, Kumar A, Chatterjee S, et al. Catalytic, antibacterial and antibiofilm efficacy of biosynthesised silver nanoparticles using Prosopis juliflora leaf extract along with their wound healing potential. J Photochem Photobiol B. 2019;190:50–58.
26. Hanifa D, Sarina G, Djamaan A. Toona sinensis Mediated Green Synthesis of Silver Nanoparticles. IOSR J Pharm Biol Sci. 2020;15(2):8–15.
27. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. J Adv Res. 2016;7(1):17–28.
28. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract . J Radiat Res Appl Sci. 2016;9(1):1–7.
29. Jackson TC, Agboke AA, Udofa EJ, Ucheokoro AS, Udo BE, Ifekpolugo NL. Characterization and Release Kinetics of Metronidazole Loaded Silver Nanoparticles Prepared from Carica papaya Leaf Extract. Adv Nanopart. 2019;08(03):47–54.
30. Logaranjan K, Raiza AJ, Gopinath SCB, Chen Y, Pandian K. Shape- and Size-Controlled Synthesis of Silver Nanoparticles Using Aloe vera Plant Extract and Their Antimicrobial Activity. Nanoscale Res Lett. 2016;11(1): 520.
31. Raja S, Ramesh V, Thivaharan V. Green biosynthesis of silver nanoparticles using Calliandra haematocephala leaf extract , their antibacterial activity and hydrogen peroxide sensing capability. Arabian Journal of Chemistry. 2015;10(2):253–261.
32. Arokiyaraj S, Vincent S, Saravanan M, Lee Y, Oh YK, Kim KH. Green synthesis of silver nanoparticles using Rheum palmatum root extract and their antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. Artif Cells Nanomed Biotechnol. 2017;45(2):372–379.
33. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, et al. Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev. 2011;63(6):470–491.
34. Pradyutha AC, Rao VU, Rao YT. Biosynthesis, Characterization and Antibacterial Activity of Silver Nanoparticles of Euphorbia Milii Des Moul. Leaf Extract. International Research Journal Of Pharmacy. 2018;9(6):154–157.
35. Saratale RG, Shin HS, Kumar G, Benelli G, Kim DS, Saratale GD. Exploiting antidiabetic activity of silver nanoparticles synthesized using punica granatum leaves and anticancer potential against human liver cancer cells (HepG2). Artif Cells Nanomed Biotechnol. 2018;46(1):211–222.
36. Alaqad K, Saleh TA. Gold and Silver Nanoparticles: Synthesis Methods, Characterization Routes and Applications towards Drugs. J Environ Anal Toxicol. 2016;6(4):1000384.
37. Esmaile F, Koohestani H, Abdollah-Pour H. Characterization and antibacterial activity of silver nanoparticles green synthesized using ziziphora clinopodioides extract. Environ Nanotechnol Monit Manag. 2020;(February):100303.
38. Sadeghi B, Gholamhoseinpoor F. A study on the stability and green synthesis of silver nanoparticles using Ziziphora tenuior (Zt) extract at room temperature. Spectrochim Acta A Mol Biomol Spectrosc. 2015;134:310–315.
39. Kuppusamy P, Yusoff MM, Maniam GP, Govindan N. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. Saudi Pharmaceutical Journal. 2016;24(4):473–484.
40. Kuo CEA, Wu SY, Lee CH, Lai YR, Lu CH, Chen PC, et al. Toona sinensis modulates autophagy and cytokines in lipopolysaccharide-induced RAW 264.7 macrophages. Biomedicine and Pharmacotherapy. 2020;129:110386.
41. Peng W, Liu Y, Hu M, Zhang M, Yang J, Liang F, et al. Toona sinensis: a comprehensive review on its traditional usages, phytochemisty, pharmacology and toxicology. Brazilian Journal of Pharmacognosy. 2019;29(1):111–124.
42. Adhya A, Bain J, Dutta G, Hazra A, Majumdar B, Ray O, et al. Healing of burn wounds by topical treatment: A randomized controlled comparison between silver sulfadiazine and nano-crystalline silver. J Basic Clin Pharm. 2015;6(1):29.
43. Hayes TR, Su B. Electrospinning for Tissue Regeneration. Electrospinning for Tissue Regeneration. 2011;317–339.
44. Al-Shmgani HSA, Mohammed WH, Sulaiman GM, Saadoon AH. Biosynthesis of silver nanoparticles from Catharanthus roseus leaf extract and assessing their antioxidant, antimicrobial, and wound-healing activities. Artif Cells Nanomed Biotechnol. 2017;45(6):1234–1240.
45. Tian J, Wong KKY, Ho CM, Lok CN, Yu WY, Che CM, et al. Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem. 2007;2(1):129–136.
46. Saeed Heydarnejad M, Rahnama S, Mobini-Dehkordi M, Yarmohammadi P, Aslnai H, Heydarnejad MS. Sliver nanoparticles accelerate skin wound healing in mice (Mus musculus) through suppression of innate immune system. Nanomed J. 2014;1(2):79–87.