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RESEARCH ARTICLE

Titrimetric Assessment of Anti-urolithiatic Effects of Siver Nanoparticles Synthesized from Elaeagnus conferta Roxb Leaves

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Abstract: Medicinal plants play a great role in human health and diseases they are considered as backbone of all the traditional medicine. Elaeagnus conferta roxb is traditionally used for the management of ulcer, pulmonary complaint, heart pain, fever, sores, and other purposes. The present study focused on synthesizing, characterization, and evaluation of in vitro antiurolithatic activity of green synthesized silver nanoparticles from Elaeagnus conferta Roxb Leaves. To synthesized the silver nanoparticles were characterized by ultraviolet (UV)-visible, Fourier-transform infrared spectroscopy (FTIR), Scanning Electronic Microscopy (SEM) and Investigation of in vitro antiurolithiatic activity test by titrimetric method. The results shows that the synthesized nanoparticles from Elaeagnus conferta roxb leaves it were used to characterize using SEM analysis it was found to be mostly rod in shape and for FTIR spectrum shows absorption bands at 601.79,686.66, 1635.64 and 3325.28cm−1 indicating the presence of capping agent with the nanoparticles, the compounds corresponds to primary amines shows ranges of 3325.28 cm−1, 1635.64 cm−1 corresponds to carbonyl group,686.66 cm−1 and 601.79cm−1 corresponds to C–H bend alkyne respectively. Synthesized nanoparticles used its evaluation of antiurolithiatic activity using titrimetric method showed 4mM silver nanoparticles had the highest calcium oxalate dissolution as 91% compared with standard drug neeri is used with an effective of 80%. The green synthesized silver nanoparticles from Elaeagnus conferta Roxb leaves were successfully characterized and showed rod-like shapes with functional groups aiding stabilization. The nanoparticles exhibited strong antiurolithiatic activity, achieving 91% calcium oxalate dissolution, which was more effective than the standard drug Neeri (80%), highlighting their potential as a natural remedy for urolithiasis.

Keywords: Elaeagnus conferta Roxb; Silver Nanoparticles; SEM analysis; Antiurolithiatic activity.

INTRODUCTION

Nowadays we are often facing a problem on urolithiasis, Reoccurrence of stone formation it is a multifaced condition which leads to urinary tract infection, renal failure, cystine disorder and even genetic disorders. Nearly 2-3 times more common in males than in females that to they suffer from reoccurrence of formation of stones. Urolithiasis found in pediatric predisposing causes have been recognized in children more than 75%. Even though kidney dialysis, stone removal through endoscopic method were done frequently it were too expensive and reoccurrence of stone is also quite common (1). Hence there is many medications and remedies have been used during so many years to treat urinary stones. For the development of plant – based medicine as an alternative system to limit to enhance stone recurrence which serves as a cosmic source of new drug entities (2).

Medicinal plants play a great role in human health and diseases. Plant act as a valuable source in all medicinal purposes. Now a days traditional medicine is practiced in many countries like India, Japan, China, and Thailand (3). In our research we have focused on Elaeagnus conferta Roxb (family Elaeagnaceae) is a thorny shrub with elliptic, obovate leaves, besides the whole plant was edible it was used traditionally for the management of ulcers, pulmonary complaints, heart pain, fever, sores, and others. The plant is shown to exhibit biological activities such as blood alcohol clearance, anti-inflammatory, antioxidant, antibacterial, cytotoxic and antihelmintic activity (4).

Phytoconstituents are naturally occurring compounds it is present in plant materials which produce health benefits for humans. The most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. Pharmacists are more attractive in these compounds because of their therapeutic performance and low toxicity which could be used for the development of new drugs to which inhibit the bacterial and fungal infections (5).

Nowadays nanoparticles play an important role in modern medicine with applications ranging from contrast agents in medical imaging to carriers for gene delivery into individual cells. Nanoparticles have a few properties that distinguish them from total materials simply by virtue of their size, such as chemical reactivity, energy absorption, and biological mobility (6,7). Chemical synthesis of silver nanoparticles can lead to hazardous and environmentally harmful products. Plants have been identified as a safe alternative platform for synthesizing nanoparticles. Plant-derived nanoparticles are gaining attention for their potential health advantages for humans and animals (8).

AgNPs are recognized for their broad antibacterial range and excellent antimicrobial activity. Additionally, several investigations have demonstrated the nematicidal and antihelmintic action of silver nanoparticles (9). AgNPs have being studied for potential applications in cancer diagnosis and treatment. The biological function of AgNPs is determined by surface chemistry, distribution, size and shape. The physicochemical features of nanoparticles impact their behavior, safety, and effectiveness (10). Therefore, we have put forward our research on using synthesized nanoparticles in the study of urolithiasis property. Hence, our research focuses on Silver nanoparticles synthesized from Elaeagnus conferta roxb leaves for its evaluation of anti-urolithiatic activity using titrimetric method.

MATERIALS AND METHODS

2.1. Collection of Plant material

The Elaeagnus conferta Roxb leaves were collected from Nilgiris Biosphere Nature Park, Annaikatti, Coimbatore District, Tamil Nadu, India. Authenticated from Botanical Survey of India, TNAU. No BSI/SRC/5/23/3018/2785/Tech.

2.2 Preparation of plant extract

The collected plant leaves were shade dried until it was free from moisture and powdered. The plant extract was prepared by Soxhlet extraction method. 25g of powdered plant material was extracted with 250ml of water. The process of extraction was carried out until the solvent in siphon tube of an extractor became colourless. The extract was filtered and allowed to dry. Further, the dried extract was maintained in a refrigerator at 4 °C for further anti-urolithiatic activity (11).

2.3 Synthesis of Silver Nanoparticles

Synthesis of AgNPs was performed based on the method, 100 ml of 2mM and 4 mM silver nitrate solution was prepared in conical flask. Then 25 ml of Elaeagnus conferta Roxb leaves extract was added to 100 ml silver nitrate solution following by heating for 15 to 20 min at 70 °C–80 °C. After the color changed from colourless to dark brown, the samples were kept at room temperature in dark for complete reduction of silver ions. The overnight samples were centrifuged repeatedly and wash with distilled water, the AgNPs were freeze-dried for getting a black colored silver nano powder and stored at −80 °C for further characterization (12).

2.4 Characterization of Silver Nanoparticles

2.4.1 UV–visible spectroscopy

The formation of the reduced silver nanoparticles in colloidal solution was monitored by using a UV–vis spectrophotometer. The absorption spectra of the supernatants were taken between 300 and 700 nm, using a UV–vis spectrophotometer. The deionized water was used as the blank (13).

2.4.2 Fourier transform infrared (FTIR) spectroscopy

Fourier Transform InfraRed (FTIR) Spectrum was used to determine the functional groups responsible for the synthesis of silver nanoparticles and record the FTIR spectrum. These functional groups may aid in the capping, reduction, and stabilization of silver nanoparticles. FTIR was performed in the spectral array from 400–4000 cm-1(14).

2.4.3 SEM Analysis

To determine the structure of the obtained nanoparticles, SEM was used. The dried samples were placed on a double conductive tape fixed on a sample holder at a normal temperature. A platinum-gold coating was applied to the samples to increase conductivity. After this, the samples were visualized at 80 kV voltage (14).

2.5 Preparation of Semipermeable membrane from eggs

The eggs were thoroughly washed with distilled water before being placed in a beaker containing 500ml of 10% acetic acid. It was left overnight, resulting in complete decalcification of the semipermeable membrane.

The next day, semipermeable membranes were carefully removed from decalcified egg using syringe to remove the entire content of the egg, washed thoroughly with distilled water, placed in ammonia solution to neutralize acid traces, and then rinsed with distilled water. It was kept in the refrigerator for further use.

2.6 Investigation of in vitro anti-urolithiatic activity test by titrimetry method

- Group I : 1mg of calcium oxalate (Blank)
- Group II : 1mg of calcium oxalate + 10 mg of neeri (Positive control)
- Group III : 1mg of calcium oxalate + 10 mg of 2nm synthesized Nanoparticles of Elaeagnus
    - conferta Roxb leaves.
- Group IV : 1mg of calcium oxalate + 10 mg of 4nm synthesized Nanoparticles of Elaeagnus conferta Roxb leaves.

Each group was packed in an egg semipermeable membrane tied with thread at one end and suspended in a beaker containing 100 ml of 0.1 M Tris buffer. For 2 hours, all groups were kept in an incubator preheated to 37°C. Each groups entire content was removed from the sutured semipermeable membrane and transferred into a separate test tube. Add 2ml of 1N sulphuric acid to all the 4 test tubes and titrated with 1N KMNO4 till a light pink colour end point obtained. To determine the total amount of dissolved calcium oxalate by various solvent extracts, the amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment (15).

RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles

The aqueous extract of Elaeagnus conferta Roxb leaves change their colors when warmed. Elaeagnus conferta Roxb leaves extract changes colour from colourless to dark brown while adding AgNO3 solution. Color changes are possible because some of the Ag ions begin to be reduced due to the effects of heat and produces Ag+ complex. This color change indicates the formation of Ag nanoparticles (16).

3.2 Characterization of silver Nanoparticles

3.2.1. UV–visible spectroscopy

After the addition of 2 mM and 4 mM silver nitrate solution, the colour of Elaeagnus conferta Roxb leaf extract changed from colourless to reddish brown after heating at 70 °C–80 °C for 15-20 min (Fig 1), indicating the reduction of Ag ions by Elaeagnus conferta Roxb leaf extracts. AgNPs showed maximum absorption at 410 nm and 420 nm by recording the surface plasmon resonance using visible spectroscopy, which is consistent with previous studies (12).

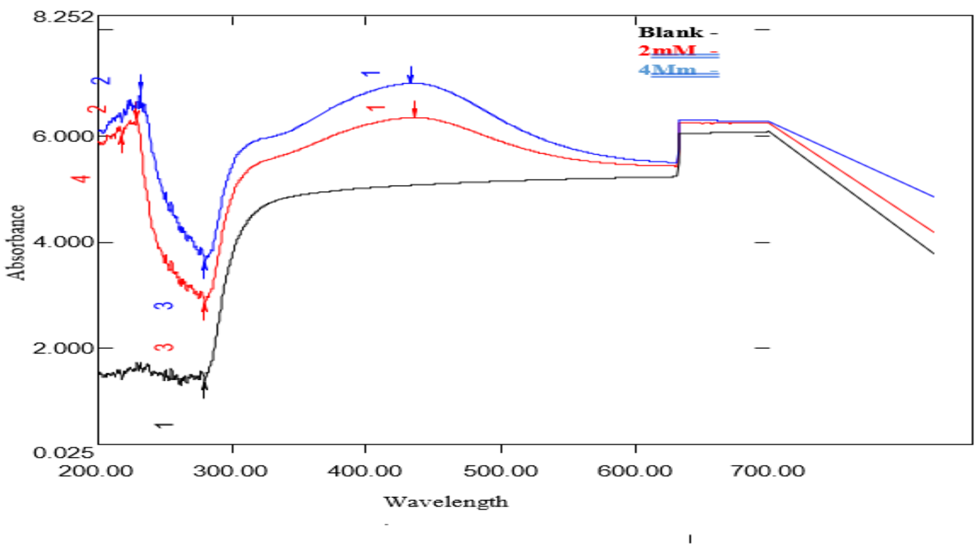

Fig1: UV analysis for synthesis of nanoparticles from Elaeagnus conferta Roxb leaf extract

3.2.2. FTIR analysis

Fourier Transform-infrared spectroscopy. The synthesis of nanoparticles from Elaeagnus conferta Roxb leaf extract were analyzed by FT-IR spectroscopy using SHIMADZU in the range of 600-3600cm-1 and the spectral range ,4000-400cm-1 to confirm the functional groups. The FTIR analysis was carried out to identify major functional groups present in the Elaeagnus conferta Roxb leaf extract, which are responsible for the synthesis of Ag NPs. These functional groups might be responsible for the reduction of silver ions to silver nanoparticles (17). For synthesis of 2mM silver nanoparticles FTIR spectrum shows absorption bands at 601.79,686.66, 1635.64 and 3325.28cm−1 indicating the presence of capping agent with the nanoparticles. 3325.28 cm−1 which corresponds to primary amines (18), 1635.64 cm−1 corresponds to carbonyl group (19), 686.66 cm−1 and 601.79cm−1 corresponds to C–H bend alkynes (20) stretch as shown in (fig 2). These results obtained correlate the findings of previous scientific literatures. For synthesis of 4mM silver nanoparticles FTIR spectrum shows absorption bands at 501.49, 555.50, 601.79, 678.94, 1635.64 and 3332.99cm−1. 3332.99 cm−1 which corresponds to primary amines, 1635.64 cm−1 corresponds to carbonyl group, 678.94 cm−1 and 601.79cm−1 corresponds to C–H bend alkynes (21) and 555.50 and 501.49 cm-1 corresponds to alkyl group shown in (fig 3). These results obtained correlate the findings of previous scientific literatures (19)

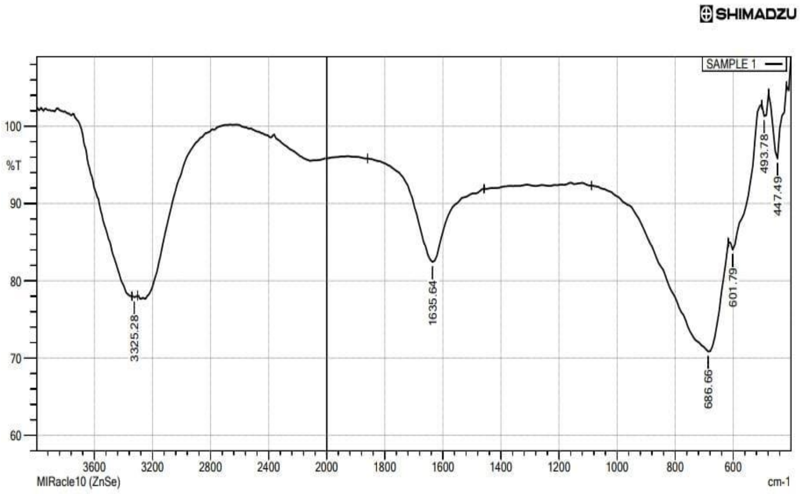

Fig 2: FTIR analysis for synthesis of 2mM silver nanoparticle

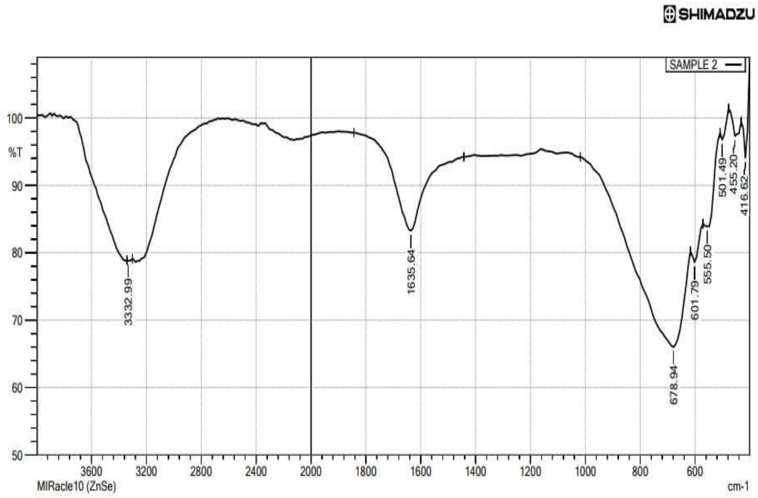

Fig3: FTIR analysis for synthesis of 4mM silver nanoparticles.

3.2.3. Scanning electron microscope

Among various electron microscopy techniques, SEM is a surface imaging method, fully capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales. Using SEM, we can probe the morphology of particles and derive a histogram from the images by either by measuring and counting the particles manually, or by using specific software. The combination of SEM with energy-dispersive X-ray spectroscopy (EDX) can be used to examine silver powder morphology and conduct chemical composition analysis. The limitation of SEM is that it is not able to resolve the internal structure, but it can provide valuable information regarding the purity and the degree of particle aggregation (22). The silver nanoparticles synthesized by Elaeagnus conferta Roxb were mostly rod in shape (fig 4). In addition, elemental analysis was also performed to confirm the presence of silver nanoparticles in the solution. The EDS analysis showing an intense signal indicates the presence of elemental silver (23).

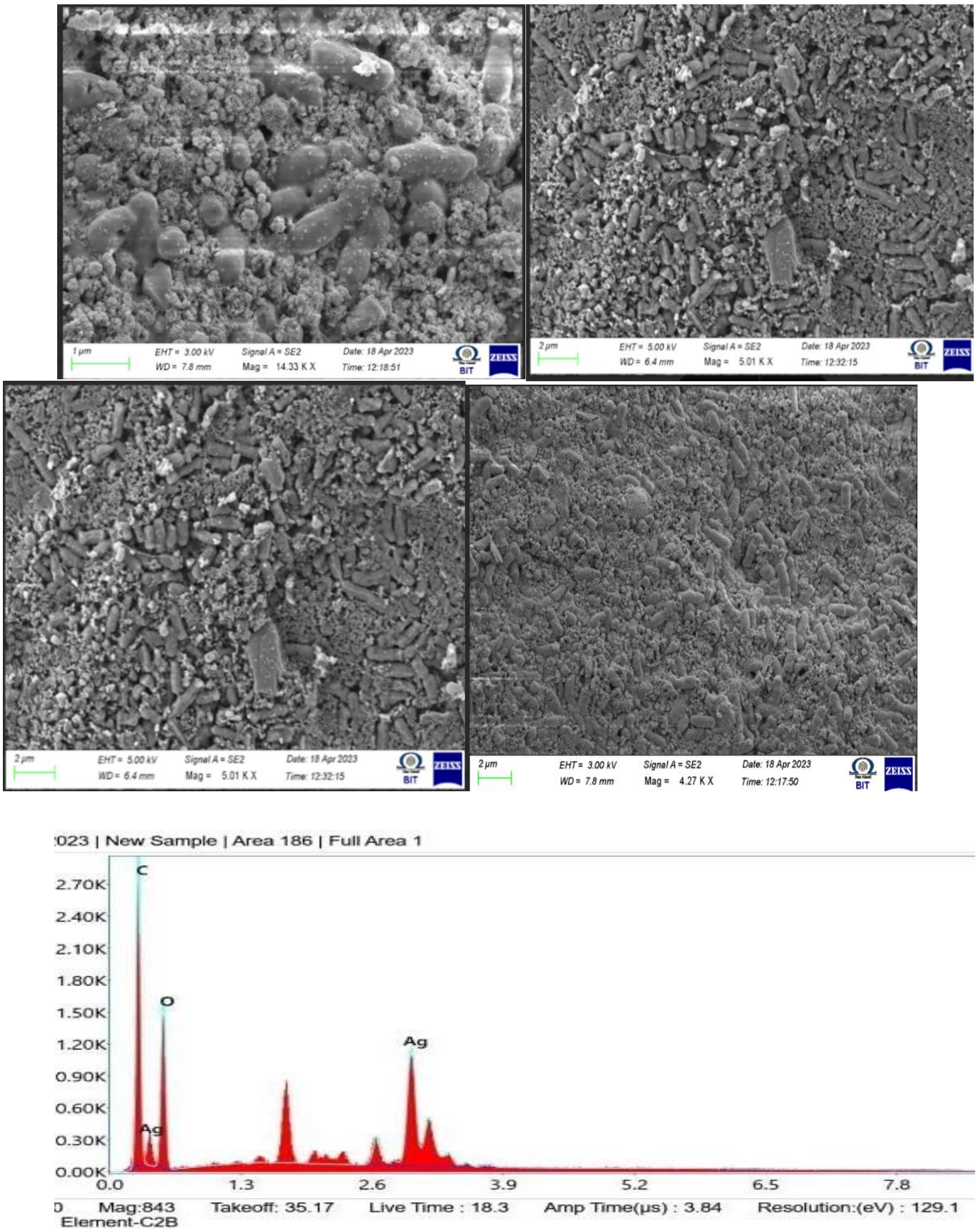

Fig4: SEM micrograph of silver nanoparticles synthesis

3.3 Preparation of semipermeable membrane from eggs

The semipermeable membrane of egg was prepared by using 500ml of 10% acetic acid being placed in a beaker with eggs. The next day eggs become decalcified shown in (fig 5) and eggs were punctured by syringe to remove entire content of egg it were used for antiurolithiatic activity (Niharika et al.,2018).

Fig 5: Decalcified eggs

3.4 Investigation of in vitro antiurolithiatic activity test by titrimetric method

Each group was placed in a beaker filled with 100 ml of 0.1 M Tris buffer and suspended in an egg semipermeable membrane that was secured with thread at one end. All groups were housed in an incubator that was heated to 37°C for two hours. The full substance of each group was taken out of the sutured semipermeable membrane and put into a different test tube (Sanjuna et al.,2019). Substance from each test tube was titrated separately till a light pink color end point obtained it was shown in table 1.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by synthesized 2nm and 4nm silver nanoparticles from Elaeagnus conferta Roxb leaves.

roups	Percentage of dissolution Calcium oxalate	Synthesized silver nanoparticles from Elaeagnus Conferta Roxb leaves
roup1	Blank	0
roup2	Positive control	80
roup3	2nm synthesized nanoparticles	85
roup4	4nm synthesized Nanoparticles	91

The synthesized 2nm and 4nm silver nanoparticles from Elaeagnus conferta Roxb leaves are examined in this study for their antiurolithiatic properties. The synthesized 4nm silver nanoparticles from Elaeagnus conferta Roxb leaves had the highest percentage of calcium oxalate (CaOx) dissolution,91%, followed by the synthesized 2nm silver nanoparticles from aqueous extract of Elaeagnus conferta Roxb leaves, which had a percentage of calcium oxalate dissolution of 85%. The standard drug Neeri was found to be less effective at dissolving calcium oxalate. This study revealed that synthesized 4nm silver nanoparticles from aqueous extract of Elaeagnus conferta Roxb leaves had the highest calcium oxalate dissolution.

CONCLUSION

The present study highlights the potential of silver nanoparticles synthesized from Elaeagnus conferta Roxb leaves in treating urolithiasis. When combined with the herbal formulation Neeri, these 4 mm nanoparticles showed improved solubility and enhanced activity compared to the medication alone. These findings suggest that Elaeagnus conferta-based silver nanoparticles could serve as an effective component in antiurolithiatic therapies. The results lay the groundwork for future investigations and hold promise for development in pharmaceutical applications.

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COMPETING INTEREST

There are no competing interests among the authors.

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