

In vitro anti-inflammatory activity of silver nanoparticle synthesized *Avicennia marina* (Forssk.) Vierh.: A green synthetic approach

R. Vijayaraj¹, G. Dinesh Kumar², N. Sri Kumaran¹

¹Department of Marine Biotechnology, Academy of Maritime Education and Training University, Chennai, Tamil Nadu, India, ²Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Thanjavur, Tamil Nadu, India

Abstract

Background: The *Avicennia marina* (Forssk.) Vierh., commonly known as gray mangrove, has been used as traditional medicine for decades with multifunctional biological activity. **Aim:** In the present study, the pharmacological significance was comparatively studied by crude extract and synthesized silver nanoparticles from *A. marina*. **Materials and Methods:** The identification of bioactive compounds was identified by gas chromatography–mass spectrometry (GC-MS). The synthesized silver nanoparticles were characterized using ultraviolet (UV)–spectrophotometry, scanning electron microscopy (SEM), and Fourier-transform infrared analysis (FTIR). The *in vitro* anti-inflammatory and antioxidant assays were followed by the standard methods. **Results:** In GC-MS analysis, the *A. marina* leaves revealed the existence of squalene (41.30), phytol (28.03), dodecanoic acid (18.55), and D-allose (18.33). The AgNPs *A. marina* was characterized by UV-Vis spectral analysis which shows a maximum absorption peak at 460.00 nm. The Fourier transform infrared spectroscopy analysis of the AgNPs Synthesized *A. marina* the presence of functional groups such as amides, alkynes (terminal), alkenes, aldehydes, nitriles, alkanes, aliphatic amines, carboxylic acids, and alkyl halides. The SEM analysis of AgNPs clearly showed the clustered and irregular shapes, mostly aggregated and having the size of 25–80 nm. The *in vitro* anti-inflammatory properties of crude and their synthesized AgNPs showed the inhibition of protein denaturation 68.92% and 72.1% and inhibition of antiproteinase activity 68.9% and 72.9%. The plant extract exhibited significant 2,2-diphenyl-1-picrylhydrazyl-hydrate radical scavenging activity value 62.7–98.5 µg/mL. **Conclusion:** This study exhibit that the *A. marina* contains various bioactive compounds and recommended as anti-inflammatory and pharmaceutical importance.

Key words: *Avicennia marina*, Fourier-transform infrared analysis, gas chromatography–mass spectrometry analysis, scanning electron microscopy, ultraviolet-spectroscopic analysis

INTRODUCTION

Medicinal plants are widely used by the traditional medicinal practitioners to cure different diseases due to their worldwide availability and fewer side effects. The herbal medicines occupy distinct position right from the primitive period to present day. Medicines that are used today are not definitely the same as those that were used in ancient times. India has a wealth of medicinal plants and most of which have been traditionally used in Ayurveda, Unani systems of medicine, and by tribal healers for generations. The medicinal value of this plant lies in the bioactive phytochemical constituents that

produce a definite physiological effect on the human body. These natural compounds signify the base of modern drugs as we use today. Phytocomponents are the natural bioactive compounds found in the plants. These phytocomponents work with nutrients and fibers to form an integrated part of

Address for correspondence:

Dr. N. Sri Kumaran, Department of Marine Biotechnology, Academy of Maritime Education and Training University, Kanathur, Chennai – 603 112, Tamil Nadu, India. Phone: +91-9952031362. E-mail: s.kumaran08@gmail.com

Received: 25-01-2018

Revised: 26-07-2018

Accepted: 08-08-2018

human defense system against various diseases and stress condition.

The systematic study of mangrove species has revealed that crude extracts of different plant parts in different solvents and compounds isolated from them exerted potential antibacterial, antifungal, antiviral, and antioxidant activities. The *Avicennia marina* is commonly known as gray mangrove tree classified in the plant family Acanthaceae and is commonly used for the treatment of ulcers, rheumatism, smallpox, and other ailments. Some studies were already reported for *A. marina* against parasites, fungi, and bacteria.^[1]

Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes.^[2] In medicines, silver and silver nanoparticles have an ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds.^[3] Silver nanoparticles are reported to have anti-inflammatory activity.^[4] The added advantage of using plants is phytoconstituents which act as capping agents, thereby conferring the silver nanoparticles with additional pharmacological properties. Hence, the present study was made to investigate identification of bioactive compounds and synthesis of silver nanoparticles from *A. marina* (Forssk.) Vierh. and its *in vitro* anti-inflammatory properties.

MATERIALS AND METHODS

Collection and Authentication of Plant

The leaves of *A. marina* were collected from the Muthupet mangrove forest which is located at the southern end of the Cauvery Delta, Tamil Nadu, India. It was taxonomically identified and authenticated by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Preparation of Extract

The coarse powder plant material was extracted with ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure to get the crude extract. Standard methods were used for preliminary phytochemical screening of the extract, which was performed to know the phytoconstituents in the extract.^[5]

Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS analysis of the sample was performed using a Shimadzu GCMS-QP2010 GC-MS interfaced with a Turbo Mass quadrupole mass spectrometer.

Identification of Compounds

Interpretation of mass spectrum of GC-MS was conducted using the mass spectral database of the National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Preparation of 1 mM Silver Nitrate Aqueous Solution

An accurately weighed 0.017 g of silver nitrate was dissolved in 100 mL of double-distilled water and stored in amber color bottle for further use.

Synthesis of Silver Nanoparticles from Ethanolic Extraction of *A. marina*

The synthesis of silver nanoparticle was performed by Sri Kumaran *et al.*,^[6] and 5 mL of the ethanolic leaf extract of *A. marina* was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this, 50 mL of 1 mM AgNO₃ solution was added dropwise with constant stirring of 120 rpm at 50–60°C. The color change of the solution was checked periodically. The color change of the medium from colorless to brown after 5 h was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the ethanolic extract of *A. marina* to generate extremely stable silver nanoparticles.

Characterization Techniques

Ultraviolet (UV)-visible spectroscopy

The silver nanoparticles were characterized in a Shimadzu-1800 UV-visible spectrophotometer. The optical properties (absorbance) of the sample were evaluated at the wavelength range of 300–600 nm. The double-distilled water was used as a blank reference.

Scanning electron microscope (SEM)

Thin films of the sample were prepared on a carbon-coated copper grid by dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper, and then, the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Fourier-transform infrared (FTIR) spectroscopy

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, after complete reduction, silver nanoparticles were concentrated by repeated centrifugation (3 times) of the reaction mixture at 15,000 rpm for 20 min. The supernatant was replaced by

distilled water each time. Thereafter, the purified suspension was freeze-dried to obtain a dried powder. Finally, the dried nanoparticles were analyzed by ALPHA FT-IR Spectrometer (from Bruker, Germany) for the detection of different functional groups by showing peaks from the region of 4000 cm^{-1} – 500 cm^{-1} .

Assessment of Anti-inflammatory Activity

Inhibition of protein denaturation

Inhibition of protein denaturation by the ethanolic extracts of crude and AgNPs synthesized *A. marina* was evaluated by the method of Mizushima *et al.*^[7] with slight modification. 500 μL of 1% bovine serum albumin was added to 100 μL of crude and AgNPs synthesized *A. marina*. This mixture was kept at room temperature for 10 min, followed by heating at 51°C for 20 min. The resulting solution was cooled down to room temperature, and absorbance was recorded at 660 nm. Acetylsalicylic acid was taken as a positive control. The experiment was carried out in triplicates, and the percentage inhibition for protein denaturation was calculated using the following equation:

$$\% \text{Inhibition of protein denaturation} = 100 - ((A1 - A2) / A0) * 100$$

Where A1 is the absorbance of the sample, A2 is the absorbance of the product control, and A0 is the absorbance of the positive control.

Protease inhibition assay

Inhibition of trypsin by the ethanolic extracts of crude and AgNPs synthesized *A. marina* was evaluated by the method of Sakat *et al.*^[8] 100 mL of bovine serum albumin was added to 100 μL of crude and AgNPs synthesized *A. marina*. This was incubated at room temperature for 5 min. Reaction was inhibited by the addition of 250 μL of trypsin followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Acetylsalicylic acid was used as a positive control. The experiment was carried out in triplicates, and the percentage inhibition of protease inhibition was calculated.

$$\% \text{Protease Inhibition} = 100 - (A1 - A2) / A0 * 100$$

Where A1 is the absorbance of the sample, A2 is the absorbance of the product control, and A0 is the absorbance of the positive control.

Determination of Antioxidant Efficacy

2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) radical scavenging activity of the ethanolic extract of *A. marina* was determined by the method of Binsan *et al.*^[9] The crude samples were prepared in concentrations of 50–200 $\mu\text{g/mL}$, and 0.1 mM of DPPH in 95% (v/v) methanol was added

to the sample. Ascorbic acid was taken as the reference standard, and in the blank, deionized water was used instead of the sample. The mixture was allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer.

RESULTS

Photochemical Analysis

The preliminary phytochemical screening results of *A. marina* showed [Table 1] various bioactive secondary metabolites constituents such as alkaloids (0.42%), flavonoids (0.62), saponins (4.5%), tannins (1.0%), terpenoids (0.20%), carbohydrates (0.16), and protein (2.5).

GC-MS Analysis of *A. marina*

The bioactive compounds present in the *A. marina* extract were identified by GC analysis. Totally 15 major compounds were identified *A. marina* extract. As shown in Figure 1, the GC-MS analyses of *A. marina* extract at the retention time (Rt) 14.87, 22.18, and 24.88 resulted in the identification of 15 major different compounds. The compound identified after comparison of the mass spectra with NIST library [Table 1] indicates the presence of various bioactive compounds.

Synthesis of Silver Nanoparticle from *A. marina*

The green synthesis of silver nanoparticles through plant extracts was carried out. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibration in silver nanoparticles. The appearances of the brown color in the reaction vessels suggest the formation of silver nanoparticles [Figure 2].

Characterization of Silver Nanoparticle

UV-visible spectrophotometer

The surface plasmon resonance (SPR) of AgNPs produced a peak at 420 nm [Figure 3], which suggests the dispersal of silver nanoparticles.

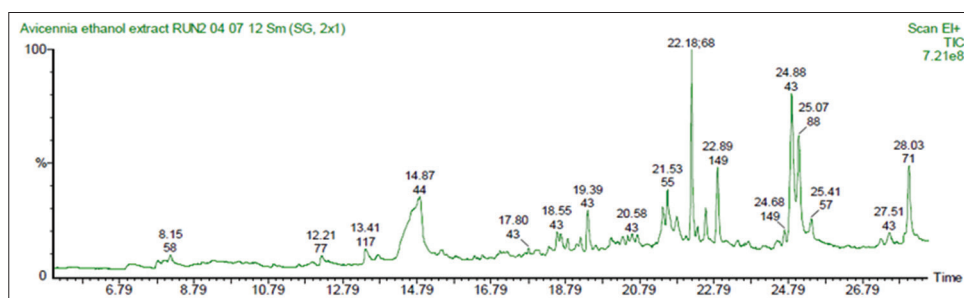
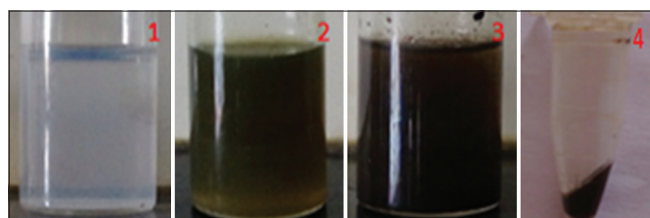
SEM analysis

The SEM analysis displays that the high-density AgNPs synthesized by the plant extract of *A. marina* confirm the presence of AgNPs of size ranging from 20 to 35 nm [Figure 4].

Table 1: GC-MS analysis of *Avicennia marina*

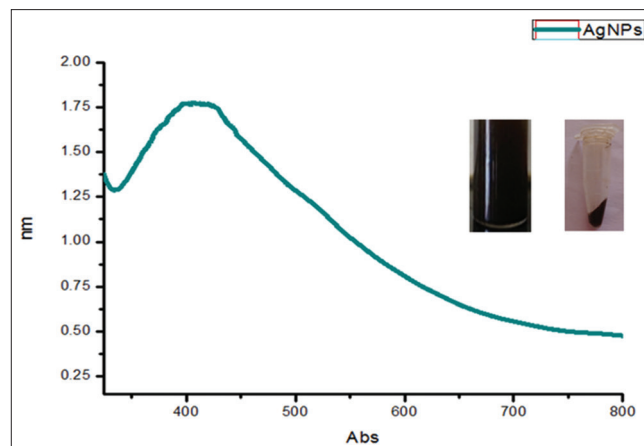
Peak name	Retention time	Molecular formula	Molecular weight (g/mol)
Triquinacene	11.59	C ₁₀ H ₁₀	130
Nonanoic acid	13.75	C ₉ H ₁₈ O ₂	158
D-Allose	18.33	C ₆ H ₁₂ O ₆	180
Dodecanoic acid	18.55	C ₁₂ H ₂₄ O ₂	200
Nonanoic acid, 3-methylbutylester	18.85	C ₁₄ H ₂₈ O ₂	228
Heptanoic acid, 3,5,5-triethyl-	19.2	C ₁₃ H ₂₆ O ₂	214
Benzonitrile, 4-ethenyl-	20.47	C ₉ HN	129
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	20.71	C ₁₁ H ₁₄ O ₃	194
Benzoic acid, 3,4,5-trimethoxy-	21.78	C ₁₀ H ₁₂ O ₅	212
n-Hexadecanoic acid	24.88	C ₁₆ H ₃₂ O ₂	256
Phytol	28.03	C ₂₀ H ₄₀ O	296
(E)-9-Octadecenoic acid ethylester	29.04	C ₂₀ H ₃₈ O ₂	310
Octadecanoic acid, 2-methyl-, methylester	29.5	C ₂₀ H ₄₀ O ₂	312
Cis-9-Hexadecenal	32.96	C ₁₆ H ₃₂ O	238
Squalene	41.3	C ₃₀ H ₅₀	410

GC-MS: Gas chromatography–mass spectrometry

**Figure 1:** Gas chromatography–mass spectrometry chromatogram of ethanolic extract of *Avicennia marina***Figure 2:** Synthesis of silver nanoparticles from the ethanolic extraction of *Avicennia marina*. (1) mM silver nitrate solution, (2) 1 mM silver nitrate solution and ethanolic leaf extraction of *A. marina*, (3) Silver nanoparticle synthesized *A. marina* after 5 h of inhibition (brown color), (4) Synthesized silver nanoparticles

FTIR analysis

FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the plant extracts. Figure 5 shows the FTIR spectrum of *A. marina* mediated synthesized AgNPs, the silver nitrate salt, and dried *A. marina* extract, and in AgNO₃ peaks were observed at 3697 cm⁻¹, 1761 cm⁻¹, 1390 cm⁻¹, and 831 cm⁻¹ which are associated OH stretching, C=C stretching, CH stretching, and CH stretching, respectively.

**Figure 3:** Ultraviolet-visible spectra of AgNPs in *Avicennia marina*

Assessment of Anti-inflammatory Activity

Inhibition of protein denaturation activity

The *A. marina* extracts were effective in inhibiting heat-induced albumin denaturation. *A. marina* was observed

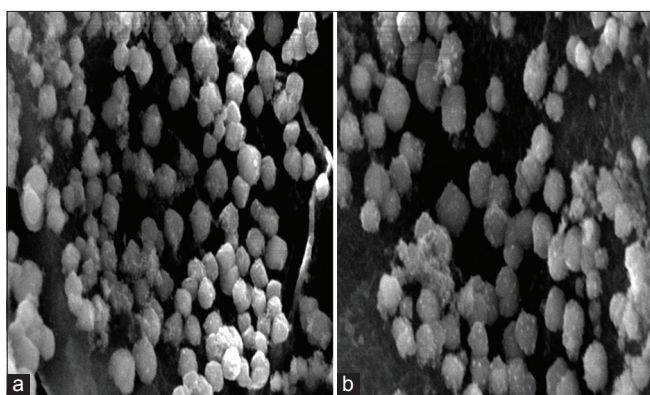


Figure 4: (a-b) Scanning electron microscope analysis of AgNPs in *Avicennia marina*

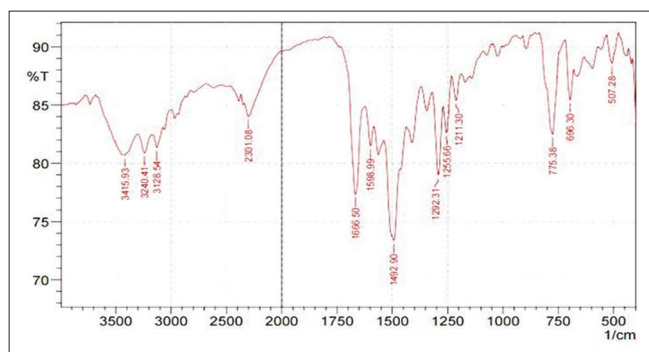


Figure 5: Fourier-transform infrared analysis of *Avicennia marina*

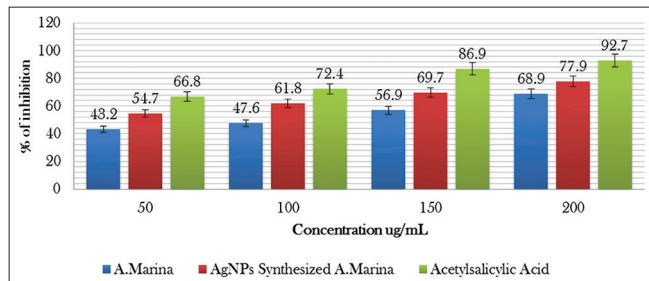


Figure 6: Inhibition of protein denaturation activity

as cured extraction was 68.92% and AgNPs synthesized *A. marina* 72.1%, respectively. Aspirin was used as a standard anti-inflammation drug as shown in Figure 6.

Inhibition of antiproteinase activity

The *A. marina* exhibited significant antiproteinase activity. The percentage of inhibition was observed in *A. marina* extract. The standard aspirin 92.87% drug showed maximum proteinase inhibitory action. The *A. marina* was observed as crude extraction 68.9% $\mu\text{g/mL}$ and AgNPs synthesized *A. marina* 72.9% $\mu\text{g/mL}$, respectively. Results are shown in Figure 7.

Determination of Antioxidant Efficacy

In the present study, the mangrove extracts have high DPPH scavenging capacity, which increased with increasing concentration [Figure 8].

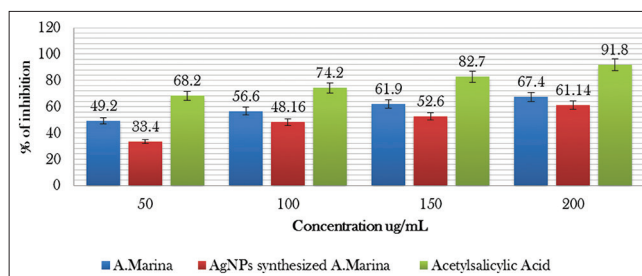


Figure 7: Inhibition of proteinase activity

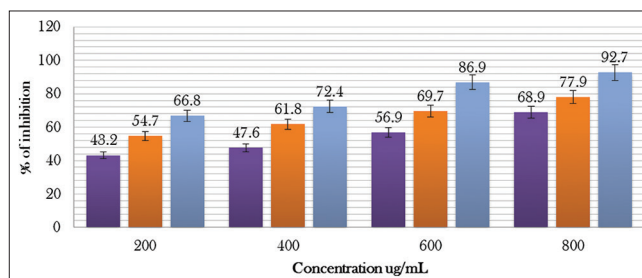


Figure 8: 2,2-diphenyl-1-picrylhydrazyl-hydrate assay of *Avicennia marina*

The DPPH assay was carried out at different concentrations of mangrove samples, namely 200 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$, 600 $\mu\text{g/mL}$, and 800 $\mu\text{g/mL}$. DPPH assay did not show any significant difference at 200 $\mu\text{g/mL}$ and 400 $\mu\text{g/mL}$ concentrations in *A. marina*; however, it was significant for 600 $\mu\text{g/mL}$ and 800 $\mu\text{g/mL}$ for the extracts. DPPH is a relatively stable free radical.

DISCUSSION

Medicinal plants are the major source of therapeutic agents to cure human diseases. Recent researches in medicinal and aromatic plants made the health-care enhancement for the purpose of humankind. The vast floral resources of mangrove forest are best known for their medicinal properties. Vast studies have been made on mangrove forest plants and their bioactive compounds during these days due to the medical importance. The mangrove herbal extracts have been practiced as a common method for the treatment of health disorders for many centuries. The bioactive compounds of mangrove plants are unique in their actions. Since they possess competence in many bioactive principles against disease producing microbial organisms,^[10] secondary metabolites such as alkaloids, steroids, phenols, and terpenoids have been chemically characterized from mangroves which have toxicological, pharmacological, and ecological importance.^[11]

The GC-MS analysis supports the presence of important bioactive compounds. The relative concentrations of various compounds were calculated by the use of gas chromatogram which gives many peaks. The height of the peak corresponds to the relative concentration of compound. The compounds which are eluted at different timings through

gas chromatogram are picked up by the mass analyzer and produce particular fragmentation pattern. This fragmentation pattern is compared to the compounds present in the reference library (NIST) in which the structure of compounds is determined. This provides the unique chemical fingerprint that shows the importance of plant under study.

GC-MS chromatogram of an ethanolic extract of *A. marina* is presented in Figure 1. The relative *Rt* and mass spectra of the extract components were compared with those of authentic samples and with mass spectra from a data library. As shown in Table 1, GC-MS analysis of *A. marina* extract at the *Rt* 14.87, 22.18, and 24.88 resulted in the identification of 15 major different compounds. The compound identified after comparison of the mass spectra with NIST library [Table 2], indicating the presence of various bioactive compounds.

Biological methods of nanoparticles synthesis using plant or plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods. Biological synthesis process provides a wide range of environmentally acceptable methodology, low-cost production, and minimum time required. At the same time, the biologically synthesized silver nanoparticles have many applications in the field of medicine and agriculture.^[28]

The biosynthesis of silver nanoparticles through plant extracts was carried out. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to the excitation of surface Plasmon vibration in silver nanoparticles. The appearances of brown color in the reaction vessels suggest the formation of silver nanoparticles [Figure 2].

The UV absorption peak of silver nanoparticles ranges from 400 to 450 nm according to Ramteke *et al.*^[29] Figure 3 shows the UV absorption peaks of *A. marina*. UV-visible spectra show the peaks approximately at 460.00 nm, clearly indicating the formation of spherical AgNPs in the plant extracts. The occurrence of the peak at 460 nm is due to the phenomenon of SPR, which occurs due to the excitation of the surface Plasmon present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field.^[30]

The silver nanoparticles are cubical, rectangular, triangular, and spherical in shape with uniform distribution. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilizes the nanoparticles.^[31] The measured sizes of the agglomerated nanoparticles were in the range 287.5–293.2 nm. However, the average size of an individual particle is estimated to be 70 nm. In the present study, SEM analysis provides the morphology and size details of the

Table 2: Pharmacological properties of *A. marina*

Peak name	Pharmacological properties	Reference
Triquinacene	Cytotoxic, anti-inflammation. Phenylbutenoid, as antioxidant and anticancer dimmer proved to have cytotoxic activity	[12,13]
Nonanoic acid	Cytotoxicity and antimicrobial activity, antifungal,	[35]
D-Allose	Antioxidant activity	[35]
Dodecanoic acid	Hyperglycemic and hypoglycemic, anti-inflammatory, hepatoprotective activity, antimicrobial, anti-inflammatory	[20-27]
Nonanoic acid, 3-methylbutyl ester	Hypocholesterolemic, antiandrogenic, flavor, hemolytic 5-alpha-reductase inhibitor, anti-inflammatory, antiviral, anti-inflammatory	[16]
Heptanoicacid, 3,5,5-triethyl-	Cytotoxic activity, antimutagenic, and anticarcinogenic activities.	[36]
Benzonitrile, 4-ethenyl-	Anti-microbial, hypoglycemic, antioxidants	[37]
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	Antioxidants, hypochlosterolenic, and hemolytic	[37]
Benzoicacid, 3,4,5-trimethoxy-	Antimicrobial and anti-inflammatory	[19]
n-Hexadecanoic acid	Hypocholesterolemic	[17,18]
Phytol	Antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory	[14]
(E)-9-Octadecenoicacidethylester	Antimicrobial, cytotoxicity activity	[36]
Octadecanoic acid, 2-methyl-, methyl ester	Antitumor, immunostimulant, chemopreventive, lipoxygenase inhibitor	[15]
cis-9-Hexadecenal	Anti-inflammatory	[19]
Squalene	Antitumor, immunostimulant, chemopreventive, lipoxygenase inhibitor	[36]

A. marina: *Avicennia marina*

nanoparticles. The high-density AgNPs synthesized by the plant extract of *A. marina* confirm the presence of AgNPs of size ranging from 20 to 35 nm [Figure 4]. Particle size, size distribution, and shape of silver nanoparticles are the important parameters that govern the properties, and hence, it has wide applications in medicinal fields.

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules.^[32] When AgNPs are produced by chemical synthesis, three main components are needed: a silver salt, a reducing agent to reduce ions to 0-charged molecules and a stabilizer or capping agent to control the growth of the NPs and prevent them from aggregation. In this context the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the plant extracts. Figure 5 shows the FTIR spectrum of *A. marina* mediated synthesized AgNPs, the silver nitrate salt, and dried *A. marina* extract, and in AgNO₃, peaks were observed at 3697 cm⁻¹, 1761 cm⁻¹, 1390 cm⁻¹, and 831 cm⁻¹ which are associated OH stretching, C=C stretching, CH stretching, and CH stretching, respectively.

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study, the protein denaturation bioassay was selected for *in vitro* assessment of the anti-inflammatory property of silver nanoparticles synthesized *A. marina*. The Denaturation of proteins is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain arthritic disease is due to denaturation of protein. The mechanism of denaturation involves an alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding.^[33]

In the presence study, denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the extract to inhibit protein denaturation was studied. Selected extracts were effective in inhibiting heat-induced albumin denaturation. *A. marina* was observed as cured extraction was 68.92% and AgNPs synthesized *A. marina* 72.1%, respectively. Aspirin was used as a standard anti-inflammation drug as shown in Figure 6.

The *A. marina* exhibited significant antiproteinase activity. The percentage of inhibition was observed in *A. marina* extract. The standard aspirin 92.87% drug showed maximum proteinase inhibitory action. The *A. marina* was observed as crude extraction 68.9% µg/mL and AgNPs synthesized *A. marina* 72.9% µg/mL, respectively. Results are shown in Figure 7.

Free radicals are chemical species containing one or more unpaired electrons that make them highly unstable and cause

damage to other molecules by extracting electrons from them to attain stability. The free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system, injury, gastritis, cancer, and AIDS. In recent years, much attention has been devoted to natural antioxidant and their association with health benefits.^[34]

There are several methods available to assess the antioxidant activity of compounds. DPPH free radical scavenging assay is an easy, rapid, and sensitive method for the antioxidant screening of plant extracts. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.^[35]

In the present study, the mangrove extracts have high DPPH scavenging capacity, which increased with increasing concentration [Figure 8] The DPPH assay was carried out at different concentrations of mangrove samples, namely 200 µg/ml, 400 µg/ml, 600 µg/ml, and 800 µg/ml. DPPH assay did not show any significant difference at 200 µg/ml and 400 µg/ml concentrations in *A. marina*; however, it was significant for 600 µg/ml and 800 µg/ml for the extracts. DPPH is a relatively stable free radical. DPPH radical react with suitable reducing agents, the electrons become paired off, and the solution losses color stoichiometrically depending on the number of electrons taken up. Hence, this assay provided information on the reactivity of test samples with a stable free radical. The decrease in the absorbance of the DPPH radical caused by test samples was due to the scavenging of radical by electron donation.

CONCLUSION

In the present study, 15 phytochemical constituents have been identified from ethanol leaf extract of *A. marina* by GCMS analysis. The phytoconstituent analysis revealed presence of alkaloids, flavonoids, saponin, and phytosterols. which complements the biochemical and pharmacological background of therapeutic applications. The biosynthesis of silver nanoparticles with leaf of the ethanolic extract of *A. marina* provides potential source for the anti-inflammatory properties. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize and prevent denaturation. Hence, this study gives an idea that the compound of *A. marina* can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation.

REFERENCES

1. Kumar GD, Rajakumar R. Gas chromatography mass spectrometry analysis of bioactive components from the ethanol extract of *Avicennia marina* leaves. *Innov J Sci* 2016;4:9-12.
2. Jiang HM, Wong AC, Denes FS. Plasma enhanced

- deposition of silver nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics. *J Appl Polym Sci* 2004;93:1411-22.
3. Durán N, Marcato PD, Alves OL, Souza GI, Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several fusarium oxysporum strains. *J Nanobiotechnology* 2005;3:8.
4. Vijayaraj R, Kumar NK, Mani P, Senthil J, Kumar GD, Jayaseelan T. Green synthesis of silver nanoparticles from ethanolic seed extract of *Acranythes aspera* (linn.) and its anti-inflammatory activities. *Int J Pharm Ther* 2016;7:42-8.
5. Wong KK, Cheung SO, Huang L, Niu J, Tao C, Ho CM, *et al.* Further evidence of the anti-inflammatory effects of silver nanoparticles. *ChemMedChem* 2009;4:1129-35.
6. Jeyaraj M, Sathishkumar G, Sivanandhan G, MubarakAli D, Rajesh M, Arun R, *et al.* Biogenic silver nanoparticles for cancer treatment: An experimental report. *Colloids Surf B Biointerfaces* 2013;106:86-92.
7. Rao TA. Records of the Botanical Survey of India; 1961. P. 2.
8. Harborne JB. *Phytochemical Methods*. 3rd ed. New York: Chapman and Hall Int; 1998.
9. Kumaran NS, Vijayaraj R. biosynthesis of silver nanoparticles using *Abutilon indicum* (Link): An investigation of anti-inflammatory and antioxidant potential against carrageen induced paw edema in rats. *Asian J Pharm* 2017;11:92.
10. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol* 1968;20:169-73.
11. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *Int J Pharm Pharm Sci* 2010;2:146-56.
12. Kikuzaki H, Nakatani N. Antioxidant effect of some ginger constituents. *J Food Sci* 1993;58:1407-10.
13. Piyusha G, Shelar S, Reddy VK, Shelar GS, Reddy V. Medicinal value of mangroves and its antimicrobial properties - A review. *Cont J Fish Aquat Sci* 2012;6:26-37.
14. Philip K, Sinniah SK, Muniandy S. Antimicrobial peptides in aqueous and ethanolic extracts from microbial, plant and fermented sources. *Biotechnology* 2009;8:248-53.
15. Han AR, Kim MS, Jeong YH, Lee SK, Seo EK. Cyclooxygenase-2 inhibitory phenylbutenoids from the rhizomes of *Zingiber cassumunar*. *Chem Pharm Bull (Tokyo)* 2005;53:1466-8.
16. Murakami A, Takahashi D, Kinoshita T, Koshimizu K, Kim HW, Yoshihiro A, *et al.* Zerumbone, a southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: The alpha, beta-unsaturated carbonyl group is a prerequisite. *Carcinogenesis* 2002;23:795-802.
17. Inoue M, Yoshimi I, Sobue T, Tsugane S, JPHC Study Group. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: A prospective study in japan. *J Natl Cancer Inst* 2005;97:293-300.
18. Gallina G, Ferretti G, Merlanti R, Civitareale C, Capolongo F, Draisci R, *et al.* Boldenone, boldione, and milk replacers in the diet of veal calves: The effects of phytosterol content on the urinary excretion of boldenone metabolites. *J Agric Food Chem* 2007;55:8275-83.
19. Sahin N, Erdogan IK. Investigation of antimicrobial activities of nonanoic acid derivatives. *Fresenius Environ Bull* 2006;15:141-3.
20. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer Res* 2002;22:2587-90.
21. Graikou K, Kapeta S, Aligiannis N, Sotiroidis G, Chondrogianni N, Gonos E, *et al.* Chemical analysis of Greek pollen-antioxidant, antimicrobial and proteasome activation properties. *Chem Cent J* 2011;5:33.
22. Praveenkumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *Afr J Biochem Res* 2010;4:191-5.
23. Ciccia G, Coussio J, Mongelli E. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J Ethnopharmacol* 2000;72:185-9.
24. Nakatsuji T, Kao MC, Fang JY, Zouboulis CC, Zhang L, Gallo RL, *et al.* Antimicrobial property of lauric acid against *Propionibacterium acnes*: Its therapeutic potential for inflammatory acne vulgaris. *J Invest Dermatol* 2009;129:2480-8.
25. Yang D, Pornpattananangkul D, Nakatsuji T, Chan M, Carson D, Huang CM, *et al.* The antimicrobial activity of liposomal lauric acids against *Propionibacterium acnes*. *Biomaterials* 2009;30:6035-40.
26. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. *Am J Clin Nutr* 2003;77:1146-55.
27. Thijssen MA, Mensink RP. Fatty acids and atherosclerotic risk. In: Eckardstein AV, editor. *Atherosclerosis: Diet and Drugs*. Germany: Springer; 2005. p. 171-2.
28. Ahmad A, Sastry MA. Biological synthesis of triangular gold nanoparticles. *Nat Mater* 2004;3:482-8.
29. Ramteke C, Chakrabarti T, Sarangi BK, Pandey RM. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. *J Chem* 2013;2013: Article ID: 278925.
30. Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. *J Exp Med* 1962;115:597-608.
31. Nethradevi C, Sivakumar P, Renganathan S. Green synthesis of silver nanoparticles using *Datura metel* flower extract and evaluation of their antimicrobial activity. *Int J Nanomater Biostruct* 2012;2:16-21.

32. Janakiraman N, Sathish SS, Johnson M. UV-Vis and FTIR spectroscopic studies on *Peristrophe bicalyculata* (RETZ.) Nees. Asian J Pharm Clin Res 2011;4:125-9.
33. Matkowski A, Tasarz P, Szypuła E. Antioxidant activity of herb extracts from five medicinal plants from *Lamiaceae*, subfamily *Lamioideae*. J Med Plants Res 2008;2:321-30.
34. Sudhanshu NR, Rao N, Mittal S, Menghani E. *In vitro* antioxidant activity and phytochemical screening of the methanolic extract of *Cichorium intybus*. Int J Chem Pharm Sci 2012;2:13-6.
35. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, *et al.* Indian medicinal herbs as sources of antioxidants. Food Res Int 2008;41:1-15.

Source of Support: Nil. **Conflict of Interest:** None declared.