

RESEARCH ARTICLE

Bioactivity Studies of *Datura metel*, *Aegle marmelos*, *Annona reticulata* and *Saraca indica* and their Green Synthesized Silver Nanoparticle

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Abstract

In this study, various plants like *Datura metel*, *Aegle marmelos*, *Annona reticulata* and *Saraca indica* were collected and subjected for extraction using various solvents, namely Water, Chloroform and Ethanol. The extracts were done with TLC bioautography for Antioxidant activity and Antibacterial activity, Minimum Inhibitory Concentration, Antibacterial activity, Antioxidant activity (DPPH and FRAP) and Phytochemical analysis. Plants used in this study showed antibacterial and antioxidant activity. These extracts were further utilized for Silver nanoparticle production and were characterized. Silver nanoparticles were utilized for *In vitro* antibacterial activity, where they did not show any antibacterial activity.

Keywords: Antioxidant activity; antibacterial activity; silver nanoparticles.

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INTRODUCTION

In the recent days, the awareness on the treasury that is the plant kingdom, which is flooded with such potential drugs has increased¹. Bacteria have developed multiple defense mechanisms against antimicrobial drugs where a few are becoming multidrug resistant as well². Hence novel methods must be simultaneously developed to eradicate such bacteria. Medicinal plants are known for their tremendous application in the medicinal world since long ago. These plants not only act as antimicrobial compounds but also suppress peroxidation by reacting with free radicals expressing anti-oxidant activity. The plants and their parts are known to contain certain bioactive compounds such as tannins, saponins, essential oils and more other metabolites³. These plants do also help in the production of nanoparticles⁴⁻⁶.

Nano biotechnology is the fast-rising sector in the present era. Metal nanoparticles are known for their structural flexibility and functionality⁷. Hence these nanoparticles find application in medicine, catalysts, contrasting agent etc⁸. Silver has been known for treating burns and wounds to prevent infections for centuries⁹. There are reports stating that silver nanoparticles are non-toxic to humankind but lethal to microorganisms¹⁰. There are a lot of methods exploited to synthesize silver nanoparticles viz., chemical, radiation, electro-chemical and photochemical methods¹¹. Although out of these methods, the chemical method produces a high yield for low cost, they have their own drawbacks such as contamination due to precursors, utilization of toxic solvents and generation of toxic byproducts. Hence biological approaches are adopted for the synthesis due to its abilities like cost effectiveness, non-toxic nature, ability to produce high yield, energy efficiency and environmental friendly¹². The biological methods of nanoparticle production involve the action of plants, enzymes and fungi. But out of these the plant based nanoparticles are found to be efficient and safe for human therapeutic usage¹³. Plant metabolites are responsible for the formation of silver ions by reducing silver nitrate⁴. With these evidences, the present investigation focuses on the understanding of the bioactivity of selected four medicinal plants viz., *Datura metel*, *Aegle*

marmelos, *Annona reticulata* and *Saraca indica*, the utilization of extracts of the same for silver nanoparticle production, the characterization of the nanoparticles produced and their antibacterial effect.

MATERIALS AND METHODS

Chemicals

The following chemicals were bought from SRL, India and all the reagents were analytical grade: Acetone, Benzene, Chloroform, Methanol, Petroleum ether, DPPH, Ferric chloride, Conc. Sulphuric acid, Conc. Hydrochloric acid, Dragendorff's reagent, Benedict's reagent, Potassium ferricyanide, Trichloroacetic acid, Ethanol, Sodium chloride, Ethyl acetate, Ammonium molybdate, Nitric acid, Copper acetate solution, Conc. Ammonium hydroxide, Sodium hydroxide, EDTA and Ferric chloride.

Sample collection

Datura metel, *Aegle marmelos*, *Annona reticulata* and *Saraca indica* leaves were chosen and procured from different localities of Chennai, Tamil Nadu. The healthy leaves were rinsed in water, shade dried, powdered and then stored for extraction.

Preparation of the extract

Plant powder was extracted with three solvents, namely Water, Ethanol and Chloroform. 10g plant powder was taken in 100mL of respective solvent (water, ethanol and chloroform). Plants leaves were macerated, filtered and concentrated using oven at 50°C.

Phytochemical analysis

Phytochemicals were screened for all the four plants¹⁴.

Antibacterial activity

Briefly, *Pseudomonas aeruginosa* was spread over nutrient agar under aseptic conditions and wells were filled with different concentrations (2µg, 4µg, 8µg and 16µg) of the crude extracts and incubated at 37°C for 24h. Inhibition zone was noted. Ciproflaxacin was exploited as positive control and their respective solvents as negative control¹⁵.

TLC and bioautography analysis

Each solvent extract was subjected to TLC bioautography (Merck, F245). The TLC plates were cut into required sizes and were solvent

vapor-saturated in TLC glass chamber¹⁶. Sample was loaded onto TLC plate using capillary tube and run against the mobile phase i.e. Chloroform: Methanol (8:2). The Retention factor (Rf) was noted after exposing them to iodine. Antioxidant and antibacterial activity were detected on TLC plate as mentioned earlier^{17, 18}. The Rf values of the samples were calculated.

Antioxidant assays

Various antioxidant assays were performed for the metabolites namely FRAP assay¹⁹, DPPH assay²⁰.

Synthesis of silver nanoparticles

The four various plant leaf extracts were subjected for silver nanoparticles as described by earlier studies,²¹ except for chloroform extracts of the plants since they are immiscible in water.

Characterization of silver nanoparticles

Silver nanoparticles were characterized by UV-Vis analysis^{22,23}, SEM (Scanning Electron Microscopy)²⁴.

Antibacterial activity

The antibacterial activity of silver nanoparticles was evaluated against the gram-negative bacterial species *Pseudomonas aeruginosa* by following Saravanakumar et al.²⁵.

RESULT AND DISCUSSIONS

Phytochemical Analysis

Aqueous, ethanol and chloroform extracts of *Datura metel*, *Annona reticulata*, *Aegle*

marmelos, *Saraca indica* leaves were quantitatively analyzed (Table 1). Screening confirmed Alkaloids, Saponins and Tannins presence in all extracts. Carbohydrates were noticed to be present in all three solvents of *Saraca indica* and aqueous extract of *Datura metel*. Phytosterols were absent in almost all extracts except aqueous extract of *Datura metel* and *Saraca indica* whereas phenols were found in chloroform extracts of *Aegle marmelos* and *Saraca indica*. *Datura metel* was reported to possess saponins and other phytochemicals²⁶. Biologically active compounds can play a vital role in the pharmaceutical industries possessing pharmacological activities. Each of the phytochemicals have different bioactivities like antibiotic, analgesic etc²⁷.

Antibacterial Activity

Datura metel's ethanolic extract was having antibacterial activity (Table 2), which is on par with the earlier report of Akharaiyi²⁶. Chloroform extract of *Datura metel* was found to be non-effective against *P.aeruginosa* used in this study, which is contradictory to the result of Vadlapudi and Kaladhar²⁸, where they found chloroform extract of *Datura metel* to have antibacterial activity against *Erwinia caratovara* and *Pseudomonas syringae*. Aqueous extract of *Annona reticulata* shows maximum zone of inhibition followed by ethanol extract of *Aegle marmelos* against the bacteria *Pseudomonas aeruginosa*.

Table 1. Phytochemical analysis of medicinal plants chosen for this study

Phytochemical	<i>D. metel</i>			<i>A. marmelos</i>			<i>S. indica</i>			<i>A. reticulata</i>		
	H	E	C	H	E	C	H	E	C	H	E	C
Alkaloids	++	+++	+	+++	++	+	+++	-	+	++	-	++
Carbohydrates	++	-	-	+	-	-	++	+++	++	+	+	-
Glycosides	-	-	-	-	+	-	-	-	-	-	-	-
Saponins	++	++	++	++	++	++	++	++	++	+++	+	++
Phytosterols	+	-	-	-	-	-	+++	-	-	-	-	-
Phenols	-	-	-	-	+	+	-	-	++	+	+	-
Tannins	-	+++	++	+++	+++	+++	+++	+++	-	-	-	+++
Flavonoids	-	-	+	-	-	-	-	-	-	-	-	+
Protein and aminoacids	-	-	-	-	-	-	++	-	-	-	-	-
Diterpenes	-	-	-	-	-	-	-	-	-	-	-	-

+++ indicates immediate change, ++ indicates change which occurred within 5 min, + indicates change that occurs after 5 min; - indicates no such change

Thin Layer Chromatography

Thin Layer Chromatography Exposed to Iodine

Separation of compounds was done using thin layer chromatography and the TLC plates were exposed to iodine in iodine chamber¹⁸. Ethanol and chloroform extracts of *Datura metel* has shown the maximum of 3 bands (Fig. 1). The separated compounds were seen under ultra violet ray for the appearance of any extra bands, but no extra bands were found under ultra violet ray (Fig. 2), which is commonly used for observing other metabolites²⁹.

Most of the extracts showed antioxidant activity, 3 bands of Chloroform extract of *Datura metel* has shown anti-oxidant activity, even chloroform extract of *Aegle marmelos* showed antioxidant activity (Fig. 3). TLC bioautography for antioxidant activity was identified in *Aegle marmelos*³⁰.

TLC bioautography for antibacterial activity helps in identifying the compound which shows antibacterial activity in TLC plate itself. Antibacterial activity of *Punica granatum*, *Acacia senegal* and *Mangifera indica*^{18,31, 32} has been reported by TLC bioautography. Ethanolic and chloroform extracts exhibited antibacterial activity in TLC plate itself (Fig. 4).

Assay for Antioxidant activity

All the plants showed antioxidant activity

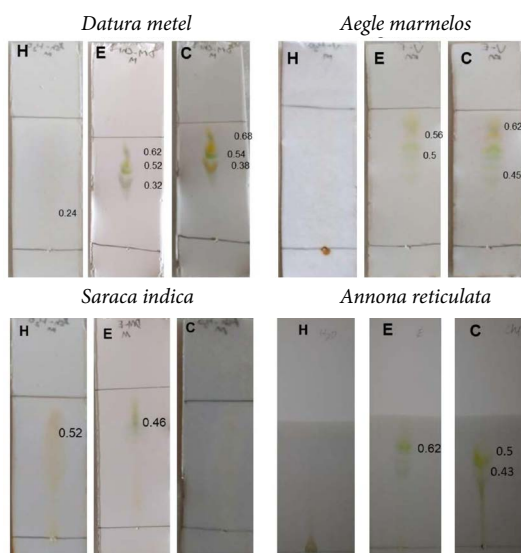


Fig. 1. Iodine exposed Thin layer chromatography of medicinal plants chosen for this study. H – aqueous extract, E – ethanol extract, C- Chloroform extract

(Fig. 5 and 6). In a study, it is reported by FRAP assay that leaf of *Datura metel* has more antioxidant activity than bark²⁶. Chloroform extract of *Annona reticulata* possessed more scavenging activity (Fig. 6). The antioxidant activity was even comparable with ascorbic acid^{33,34}. Reducing powers and scavenging activity of various medicinal plants were reported earlier^{35,36}.

Characterization of silver nanoparticles

UV-Visible Spectroscopy

Phytochemical compounds do reduce the Ag^+ to Ag^0 , but it also imparts stability of nanoparticles³⁷. Silver nanoparticles produced using ethanol extracts showed peaks near 420nm (Fig. 7) but water extract mediated silver nanoparticles were showing peak nearing 200-250nm. For the Ethanol extract of *Saraca indica*, *Annona reticulata* and *Aegle marmelos*, the peaks range at 600-630nm. Efforts were made to produce silver nanoparticles using *Annona muricata* and the particles were found to show absorbance at 420nm³⁸. In this study, silver nanoparticle produced using ethanol extract of *Datura metel* was found to show absorbance near 420nm to 440nm, where it is on par with the absorbance of silver nanoparticles produced^{39,40}.

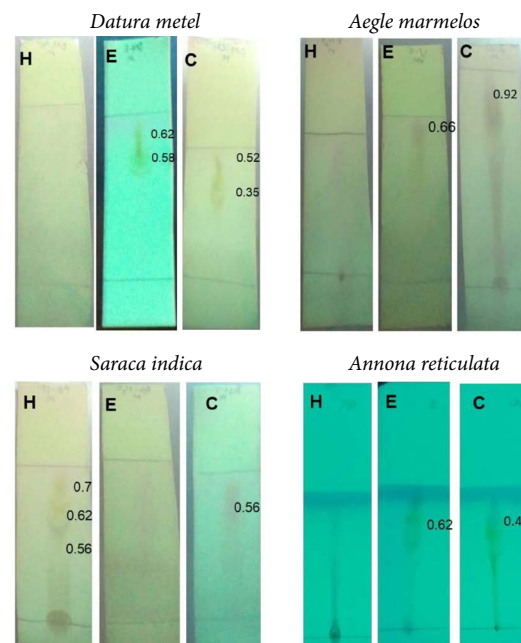


Fig. 2. UV light exposed Thin layer chromatography of medicinal plants chosen for this study. H – aqueous extract, E – ethanol extract, C- Chloroform extract

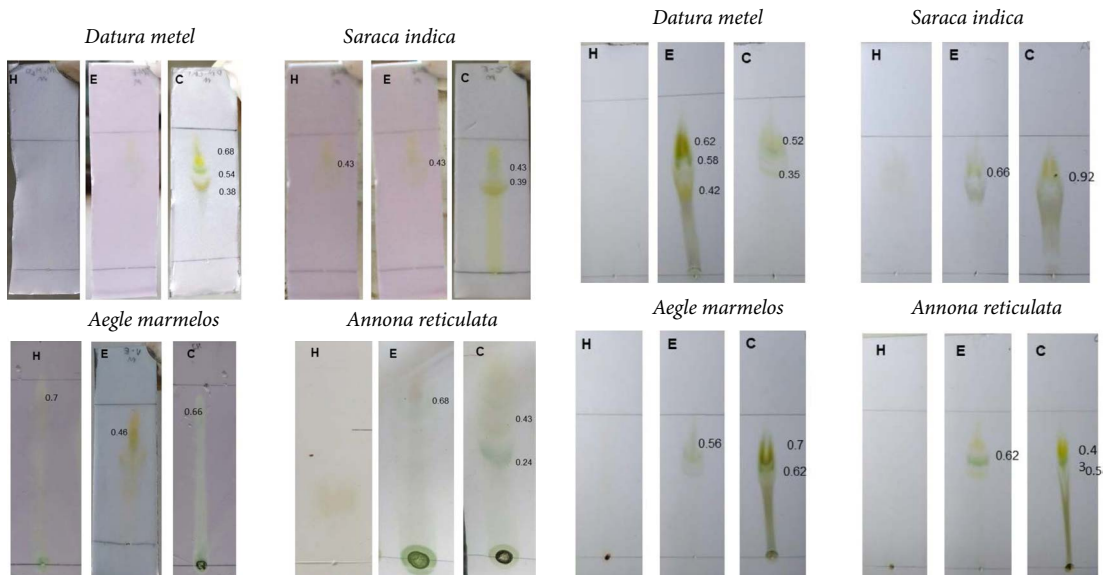
Table 2. Antibacterial activity of various extracts

Components	Water extract Zone of inhibition (diameter in cm)				Ethanol extract Zone of inhibition (diameter in cm)				Chloroform extract Zone of inhibition (diameter in cm)			
	DM	AM	SI	AR	DM	AM	SI	AR	DM	AM	SI	AR
2 µg	-ve	0.1	0.1	0.1	0.2	0.1	0.1	-ve	-ve	0.2	-ve	0.2
4 µg	-ve	0.1	0.2	0.3	0.3	0.1	0.1	-ve	-ve	0.3	-ve	0.2
8 µg	-ve	0.3	0.2	0.4	0.4	0.3	0.2	-ve	-ve	0.3	-ve	0.4
16 µg	-ve	0.5	0.3	0.5	0.8	0.4	0.3	0.2	-ve	0.4	-ve	0.4
Positive Control (Ciprofloxacin)	0.9	1.0	0.9	1.1	1.2	1.2	1.2	0.9	0.9	1.2	0.8	0.9

Table 3. Antibacterial activity of green synthesized silver nanoparticles

Components	Water extract Zone of inhibition (diameter in cm)				Ethanol extract Zone of inhibition (diameter in cm)			
	DM	AM	SI	AR	DM	AM	SI	AR
2 µg	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4 µg	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8 µg	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
16 µg	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

DM – *Datura metel*, AM – *Aegle marmelos*, SI – *Saraca indica*, AR – *Annona reticulata*, -ve – negative



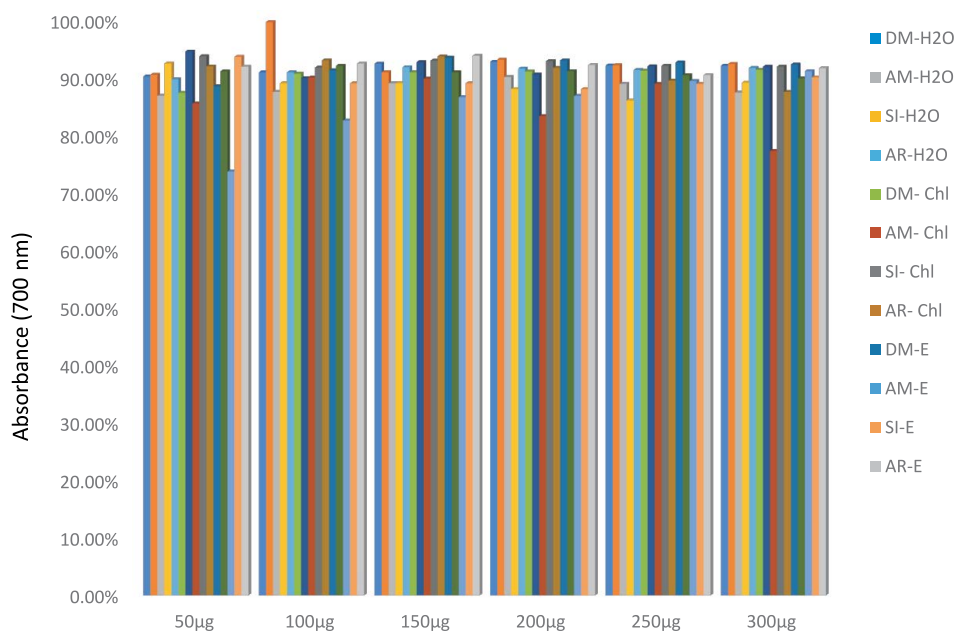


Fig. 5. Iron reducing activity of medicinal plants chosen for this study by FRAP assay. DM-H₂O – aqueous extract of *Datura metel*, AM-H₂O – aqueous extract of *Aegle marmelos*, SI-H₂O – aqueous extract of *Saraca indica*, AR-H₂O – aqueous extract of *Annona reticulata*, DM-chl – chloroform extract of *Datura metel*, AM-chl – chloroform extract of *Aegle marmelos*, SI-chl – chloroform extract of *Saraca indica*, AR-chl – chloroform extract of *Annona reticulata*, DM-E – ethanol extract of *Datura metel*, AM-E – ethanol extract of *Aegle marmelos*, SI-E – ethanol extract of *Saraca indica*, AR-E – ethanol extract of *Annona reticulata*

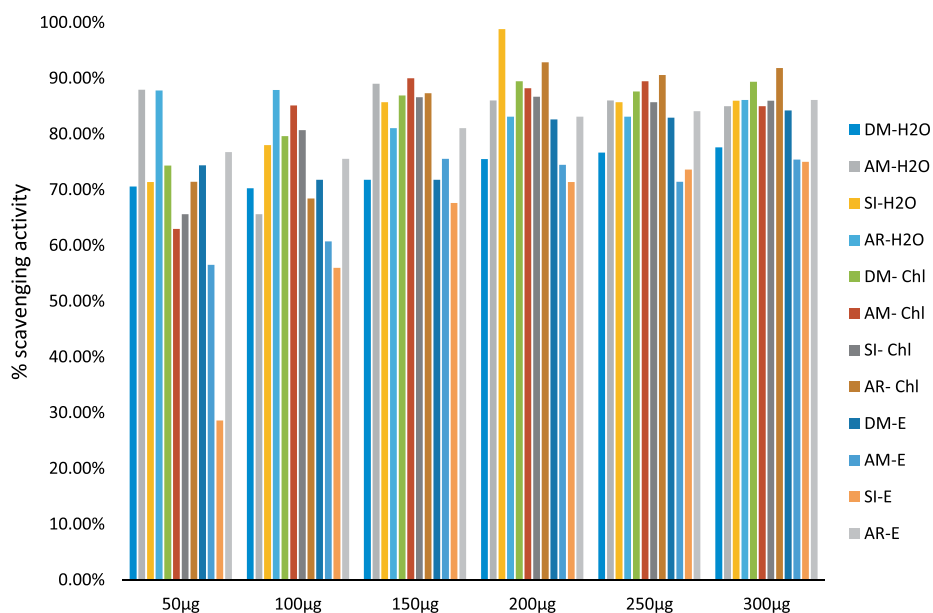


Fig. 6. Free radical scavenging activity of medicinal plants chosen for this study by DPPH assay. DM-H₂O – aqueous extract of *Datura metel*, AM-H₂O – aqueous extract of *Aegle marmelos*, SI-H₂O – aqueous extract of *Saraca indica*, AR-H₂O – aqueous extract of *Annona reticulata*, DM-chl – chloroform extract of *Datura metel*, AM-chl – chloroform extract of *Aegle marmelos*, SI-chl – chloroform extract of *Saraca indica*, AR-chl – chloroform extract of *Annona reticulata*, DM-E – ethanol extract of *Datura metel*, AM-E – ethanol extract of *Aegle marmelos*, SI-E – ethanol extract of *Saraca indica*, AR-E – ethanol extract of *Annona reticulata*

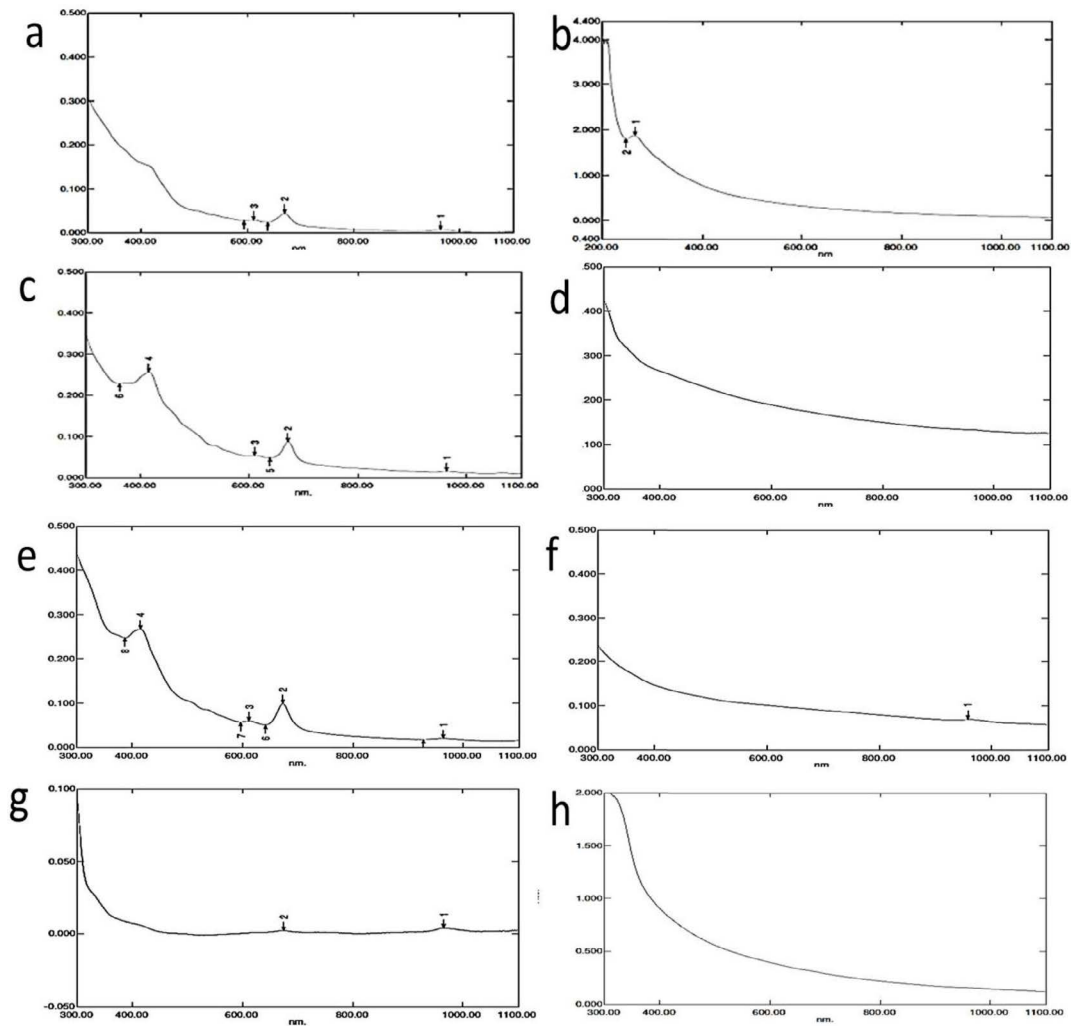


Fig. 7. UV-visible spectroscopy analysis of silver nanoparticles
 a) *Saraca indica* (ethanol) b) *Saraca indica* (water) c) *Annona recticulata* (ethanol) d) *Annona recticulata* (water) e) *Datura metel* (ethanol) f) *Datura metel* (water) g) *Aegle marmelos* (ethanol) h) *Aegle marmelos* (water)

Scanning Electron Microscopy

Size ranging from 45 to 120nm was found for silver nanoparticles produced. Nanoparticles produced using *Aegle marmelos* ranged from 47 to 52nm (Fig. 8h) and was recorded to be the smallest in the group where *Saraca indica* induced nanoparticles were ranging from 90 to 110nm recorded to be the largest nanoparticle in the group. (Fig. 8a). 35nm - 60nm sized particles were produced using *Aegle marmelos*^{41,39}. Using methanolic extract of *Annona muricata*, 22nm sized nanoparticles were made³⁸.

Antibacterial activity

There was no activity against the bacteria used (Table 3), which is contradictory to earlier reports⁴², where it was found that nanoparticles produced using *Aegle marmelos* to have antibiofilm activity. There are more evidences that silver nanoparticles synthesized using plant extracts have antibacterial activity⁴³. Extract of *Annona squamosa* has been reported to produce silver nanoparticles with anti-microbial activity⁴⁴.

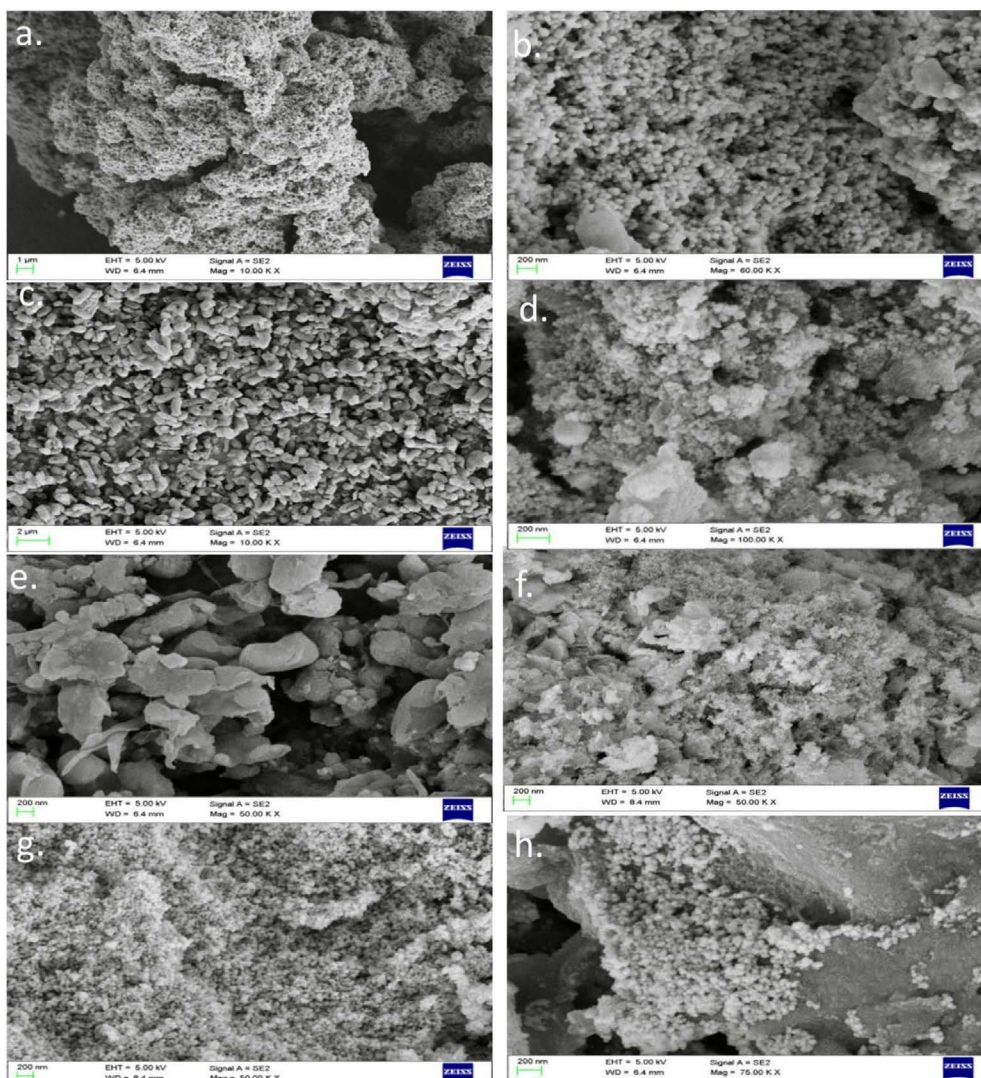


Fig. 8. SEM analysis of silver nanoparticle produced using different extracts
 a) *Saraca indica*(ethanol), b) *Saraca indica*(water), c) *Annona reticulata* (ethanol), d) *Annona reticulata* (water),
 e) *Datura metel*(ethanol), f) *Datura metel* (water), g) *Aegle marmelos* (ethanol), h) *Aegle marmelos* (water)

CONCLUSION

Datura metel, *Aegle marmelos*, *Annona reticulata* and *Saraca indica* leaves were collected and subjected to solvent extraction. Extracts were analysed for its phytoconstituent and bioactivity study. All plants were found to possess antibacterial as well as antioxidant activity. Plant mediated silver nanoparticles did not show any antibacterial activity against *Pseudomonas aeruginosa*.

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None

CONFLICT OF INTERESTS

The author declares that there are no conflict of interest.

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