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Pharmacological Assessment, Green synthesis and Characterization of Silver Nanoparticles of *Sonneratia apetala* Buch.-Ham. Leaves

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ABSTRACT

The present investigation evaluates phytochemical screening, antibacterial, antioxidant activities and green synthesis, characterization of silver nanoparticles and its antibacterial activity. Three dissimilar solvents viz., hexane, ethyl acetate and methanol were used to prepare crude extracts of *Sonneratia apetala* leaves to study the phytochemicals, antibacterial and antioxidant activities. Green synthesis, characterization of silver nanoparticles (AgNPs) and its antibacterial activity using *S. apetala* leaves were also studied. Antioxidant activity was examined by means of DPPH free radical scavenging method. AgNPs were synthesized by using 1mM $AgNO_3$ solution mixed with leaf aqueous extract of *S. apetala*. The characterization of the prepared AgNPs was done by UV-Vis spectrometry, FTIR spectroscopy and Scanning electron microscopy. Antibacterial activity was studied by agar well diffusion method. The phytochemical screening results unveiled the bearing of different phytochemicals viz., flavonoids, alkaloids, saponins, carbohydrates, terpenoids, steroids, tannins and free anthraquinones particularly with relatively high abundance in methanol extract. Likewise methanol extract too exhibited effective free radical scavenging and antibacterial activities. The characterization results of the prepared AgNPs displayed that the silver nanoparticles are formed and stabilized by plant phyto-constituents and also exhibited virtuous antibacterial property with great antagonism towards *Proteus mirabilis* with 27.3 mm diameter zone of inhibition. Green synthesis process is a pivotal area in nanotechnology and usage of natural resources is the best choice for the making of NPs as a sustainable, eco-friendly, inexpensive and free of chemical contaminant method. These AgNPs have several potential biological and medical applications.

INTRODUCTION

There is an imperative need to explore new warfare schemes to combat against multi drug resistant bacteria and also to subdue the problems of chemical drug consumption in order to control the microbial infections. These drugs from plants are less harmful, side effects are minimal and also cost effective (Harishchandra *et al*., 2012). Mangroves are common salt permissive plants with an origin of tropical and subtropical intertidal regions of the world. With reference to the much potency of mangroves, research is advancing on mangrove plants especially to study their chemical constituents. The undisclosed factors influencing the orchestrated research on mangrove plants ensues from the fact that mangroves belong to a group of tropical forests which can be easily generated. As stated, they can survive where other plants would not have survived. This is because they can withstand seemingly harsh environments including high moisture concentrations, low and high tides as well as in the presence of large population of insects and other living microorganisms (Sachin *et al*., 2014). Mangrove plants possess some compounds with special potential antifungal, antibacterial, and antiviral properties which can be isolated from mangrove plants, and they contain large percentage of disease preventing phytochemicals as they have been widely reported to be viable sources for flavonoids, saponins and alkaloids (Nebula *et al*., 2013). Antioxidant compounds in foods play a critical role as health protecting factors. Scientific evidences indicated that antioxidants decrease the risk of chronic diseases such as cancer and cardiovascular and gastrointestinal diseases (Mardani *et al*., 2016).

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Mangrove plants are rich in natural antioxidants and also contain bioactive compounds which are active against many pathogenic bacteria. Production of NPs is a sort of bottom up approach, with reduction/oxidation as the key reaction which seems as a superior substitute to battle against the multi-drug resistance bacteria. The usage of NPs is attaining impulse in the contemporary period due to their distinct mechanical, optical and chemical properties (Siva kumar *et al*., 2011). Biotic approaches can be employed to synthesize AgNPs without use of toxic, harsh and costly chemical substances. Safety, better accessibility, non-toxicity in most cases, having broad variety of metabolites that can involve in silver ions reduction and faster rate of nanoparticle formation than in microbes are the major advantages of utilizing plants for the purpose of AgNPs synthesis. The key mechanism concerned in the method is plant phytochemical aided reduction. Quinones, flavones and organic acids are the utmost chief water-soluble phytochemicals that are accountable for the quick reduction of the ions. Hence, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increases in the incidence of new and re-emerging infectious diseases (Anand *et al*., 2011). The present study aimed to assess the qualitative nature of the phytochemical constituents and to evaluate the antibacterial and antioxidant activities of *S. apetala* leaf extracts. Green synthesis of plant leaf mediated silver nanoparticles, its characterization and antibacterial activity were also attempted as there is no earlier report on the green synthesis of *S. apetala* leaf AgNPs.

Fig. 1: *Sonneratia apetala* plant.

MATERIALS AND METHODS

Sonneratia apetala (Fig-1), member of Lythraceae family is a fast-growing evergreen mangrove tree with a columnar crown. It rises up to 20 m tall, but more commonly to between 12 and 15m height. The tree produces pneumatophores up to 1.5 m tall. *Sonneratia* is generally called as mangrove apple in English. This plant is normally introduced as a fast-growing tree for reforestation of mangrove communities.

Phytochemical screening

Preparation of the plant solvent extracts

Leaves of *S. apetala* plant were collected from Coringa Mangrove Forest near Kakinada, Andhra Pradesh, shade dried and powdered using mechanical grinder. Crude extracts were made using different solvents viz., hexane, ethyl acetate and methanol by Soxhlet extractor. The resulted solvent extracts were evaporated using Roto evaporator. The dried and/or semi dried extract material was used for the phytochemical screening. Standard phytochemical methods were employed for the qualitative analysis of different phytochemicals viz., Flavonoids (Ferric chloride test, Shinoda's test, Sodium hydroxide test, Lead acetate test), Alkaloids, Saponins, Terpenoids, Steroids (Salkowskii test, Keller-Killiani test, Liebermann-Burchard test), Carbohydrates (Molisch's test, Fehling's tests for free reducing sugars and combined reducing sugars, Barfoed's test for monosaccharides), Tannins (Borntrager's test, Phlonatanins test) and soluble starch (Harborne, 1973).

Green synthesis and Characterization of silver nanoparticles

Fifteen grams of powdered leaf material of *S. apetala* was added to 100 ml of double distilled water and boiled for 15 minutes at 60°C. Later allowed the solution to cool to room temperature, the extract was filtered using Whatman No.1 filter paper and stored at 4°C for further analysis. Then, the filtrate (20 ml) was added with 80 ml of aqueous 1 mM silver nitrate ($AgNO_3$) solution and heated at 60^{o}C temperature and observed for the development of brown-yellow solution that indicates the formation of AgNPs. For further confirmation of AgNPs formation, the brown-yellow solution was subjected to Ultraviolet-Visible Spectrometry.

The so formed brown-yellow solution was centrifuged at 20, 000 rpm for 30 minutes and the obtained pellet was washed thrice with double distilled water and then dried at 60^{o}C. After drying, powdered form of NPs was preserved for further characterization. The infrared spectra of absorption and emission of the formed AgNPs was acquired by FTIR. The morphology of the prepared AgNPs was examined with SEM.

Antibacterial activity of leaf extracts and AgNPs

Test organisms used

The antibacterial activity of the crude extracts was tested against both Gram +ve and Gram –ve bacteria. Nine Gram +ve bacteria including *Staphylococcus aureus* MTCC 737, *Micrococcus luteus* MTCC 106, *Enterococcus faecalis* MTCC 439, *Bacillus subtilis* MTCC 441, *Arthrobacter protophormiae* MTCC 2682, *Bacillus megaterium* MTCC 428, *Rhodococcus rhodochrous* MTCC 265, *Lactobacillus acidophilus* MTCC 10307 and *Streptococcus mutans* MTCC 497 and six Gram –ve bacteria including *Proteus vulgaris* MTCC 426, *Alcaligens faecalis* MTCC 126, *Salmonella enterica* MTCC 3858, *Enterobacter aerogenes* MTCC 10208, *Proteus mirabilis* MTCC 425 and *Pseudomonas aeruginosa* MTCC 1688 were used in the study.

Antibacterial activity

Antibacterial activity was checked by agar well diffusion method (Nagababu and Umamaheswara Rao, 2012). Bacterial suspensions of different test organisms were prepared using 24 hrs

old bacterial cultures and cultivated (100 µl) on agar medium. With a sterile cork borer, 6 mm diameter wells were made in solidified agar plates. Streptomycin (10µg/ml in DMSO) was used as positive control. A minute quantity of sterile agar suspension was placed at the bottom of the well to prevent the leakage and 100 µl of the crude elicit sample made by dissolving 100 mg of crude in 1 ml of DMSO was added to each well. DMSO was taken in separate well as the control. Then, plates were incubated at 37^{o} C for 24 hrs. After incubation, inhibition zone diameter was measured.

For each crude extract sample and bacterial species, triplicates were maintained. Antibacterial activity of synthesized AgNPs was tested against both Gram +ve and Gram –ve bacteria (Nagababu and Umamaheswara Rao, 2016). Into each agar well, 100 µl of sample prepared by dispersing 100 µg of nanoparticle material in 1 ml of dimethyl sulfoxide (DMSO) was placed. In a separate well, DMSO was placed to maintain the control. After 24 hrs incubation (at 37°C), the diameter of the clear zone was measured. For each sample and bacterial species, triplicates were maintained.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were determined at different concentrations viz., 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml on those bacterial strains which showed sensitivity towards crude extracts of the leaves, by following the broth dilution and plating method (Umamaheswara Rao and Nagababu, 2014). Control tube was maintained for each test concentration. The lowest concentration of the extract that developed no visible bacterial growth in comparison with the control tubes was regarded as MIC. However, MBC was ascertained by sub culturing the test dilution with no growth on to a fresh drug-free solid medium and incubated further for 18–24 hours. The highest dilution that gave no individual bacterial colony on agar medium was confirmed as MBC.

***In vitro* antioxidant assay [DPPH (2, 2-diphenyl-1-picryl hydrazyl) Free radical scavenging activity]**

The antioxidant activity of different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml) crude extracts was measured by following the established method (Chew *et al*., 2012). One ml of each concentration was added with 4 ml of the 0.004% (w/v) solution of DPPH prepared in methanol. The reaction mixture was kept for incubation in dark for 30 minutes. Methanol and Ascorbic acid were used as control and positive control, respectively. The absorbance was measured at 517 nm.

The DPPH scavenging activity (%) was calculated by the following formula

$$\text{DPPH scavenging activity (\%)} = [(AO - AS)/A] \times 100$$

Where, AO - absorbance of the control,
AS - absorbance of the plant sample

RESULTS

Phytochemical screening

Phytochemical screening of the leaf extracts disclosed that the solvent extracts contain almost all phytochemicals tested including alkaloids, terpenoids, flavonoids, saponins, carbohydrates, steroids, tannins and free anthraquinones (Table-1). Methanol crude extract was positive for all the phytochemicals tested relatively with high abundance.

Table 1: Phytochemical analysis of *Sonneratia apetala* Leaf extracts of different solvents.

S.No.	Phytochemicals	H	E	M
1.	Carbohydrates	--	+	+
2.	Monosaccharides	--	+	++
3.	Free reducing sugars	--	--	++
4.	Combined reducing sugars	--	--	++
5.	Tannins	--	+	++
6.	Free anthraquinones	--	+	++
7.	Steroids	+	++	++
8.	Cardiac glycosides	+	++	++
9.	Terpenoids	+	+	+
10.	Saponins	--	+	++
11.	Flavonoids	--	++	++
12.	Soluble starch	--	++	++
13.	Alkaloids	--	++	++

H – Hexane; E – Ethyl Acetate; M – Methanol.
-- Negative; + Positive.

For all phytochemicals tested, ethyl acetate extract was positive except the free reducing sugars and combined reducing sugars. Only cardiac glycosides, steroids and terpenoids were found in hexane extract. The phytochemical results of our present study are in good concurrence with the reports of these earlier studies.

Antibacterial activity of leaf extracts

The antibacterial activity of different solvent extracts of *S. apetala* leaves is given in Fig-2. The results showed that the crude extracts of *S. apetala* leaves own the antibacterial activity against both Gram +ve and Gram –ve bacteria. The antibacterial activity of methanol extract was noticed to be comparatively higher than that other solvent extracts.

The greater zone of inhibition was 8.6 mm and 8.0 mm against *Micrococcus luteus* and *Arthrobacter protophormiae*, respectively. The methanolic extract exhibited activity against almost all the test organisms but not for *Salmonella enterica*. Hexane extract's antibacterial action was evidenced with *Rhodococcus rhodochrous*, *Micrococcus luteus* and *Staphylococcus aureus*.

Ethyl acetate extract exhibited antibacterial activity against *Micrococcus luteus*, *Streptococcus mutans* and *Proteus mirabilis*. The crude methanol extract demoed more antibacterial activity towards *Arthrobacter protophormiae*, *Staphylococcus aureus* and *Alcaligens faecalis* than that of Streptomycin which is considered as positive control. On the whole, differences in antibacterial activities between the extracts were observed.

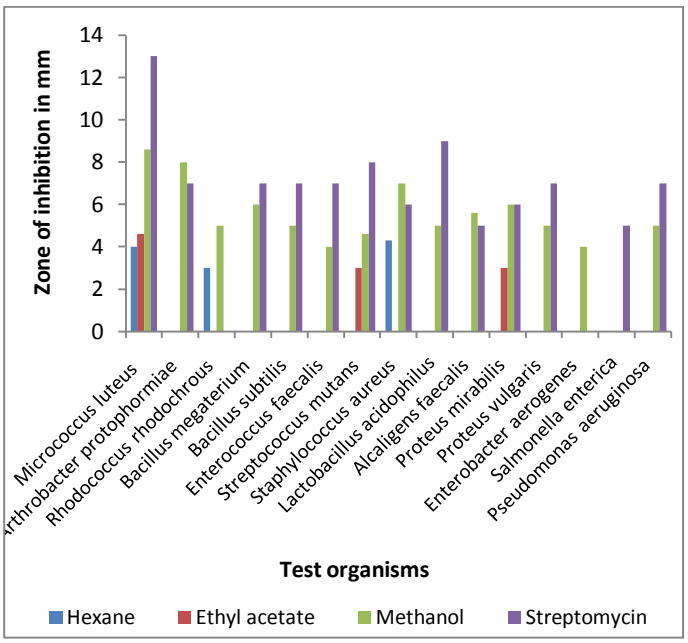

Fig. 2: Antibacterial activity of various solvent extracts of *Sonneratia apetala* leaves.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values (Table-2) were recorded for methanol extract against the susceptible bacteria. The MIC value ranged from the lowest of 12.5 mg/ml against *Micrococcus luteus* to highest of 75 mg/ml against *Streptococcus mutans*, *Bacillus subtilis*, *Lactobacillus acidophilus* and *Pseudomonas aeruginosa*. The range of MBC values found was 25mg/ml (*Micrococcus luteus*) to 100 mg/ml (*Bacillus subtilis*, *Streptococcus mutans*, *Lactobacillus acidophilus* and *Pseudomonas aeruginosa*).

Table 2: MIC and MBC (mg/ml) values of *Sonneratia apetala* leaf methanol extract.

Test organisms	Methanol	
	MIC	MBC
Micrococcus luteus MTCC 106	12.5	25
Arthrobacterprotophormiae MTCC 2682	25	50
Rhodococcusrhodochrous MTCC 265	50	75
Bacillus megaterium MTCC 428	50	75
Bacillus subtilis MTCC 441	75	100
Streptococcus mutans MTCC 497	75	100
Staphylococcus aureus MTCC 737	50	75
Lactobacillus acidophilus MTCC 10307	75	100
Alcaligensfaecalis MTCC 126	50	75
Proteus mirabilis MTCC 425	25	50
Proteus vulgaris MTCC 426	50	75
Pseudomonas aeruginosa MTCC 1688	75	100

In vitro antioxidant assay [DPPH (2, 2-diphenyl-1-picryl hydrazyl) Free radical scavenging activity]

DPPH is a free radical compound usually used to test free radical scavenging ability of different types of samples. The antioxidant activity of various extracts of *S. apetala* leaves (Fig.3) unveiled the free radical scavenging potency of the methanolic extract. At all the concentrations tested, the methanol extract showed greater scavenging activity than the other extracts as well as the positive control i.e., ascorbic acid. The percent DPPH free radical scavenging activity of methanol extract ranged from 65.31% (100 µg/ml conc.) to 92.12% (500 µg/ml conc.). However, the scavenging activity of all the extracts and positive control increased with the rise in the concentration of the samples.

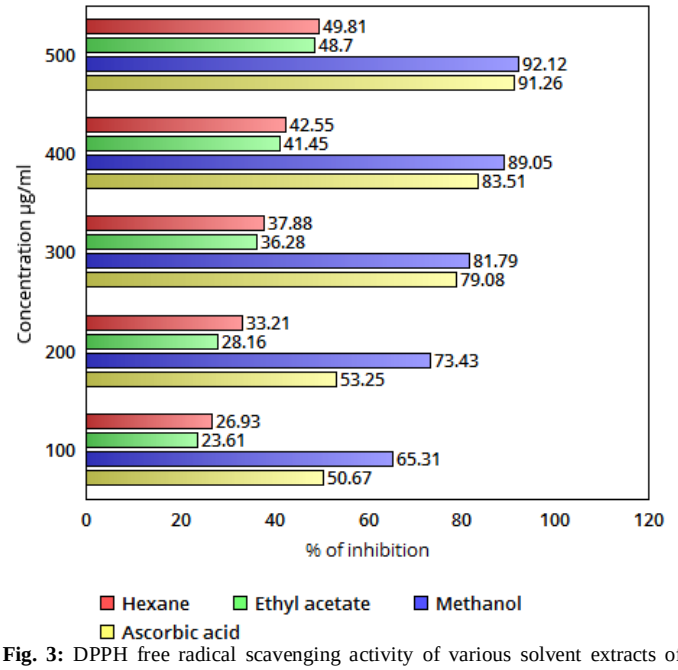

Fig. 3: DPPH free radical scavenging activity of various solvent extracts of *Sonneratia apetala* leaves

UV-Visible, FTIR and SEM

The reduction of AgNPs synthesized with leaf extract of *S. apetala* was indicated by a color change of brown to dark brown solution as shown in the Fig-4.

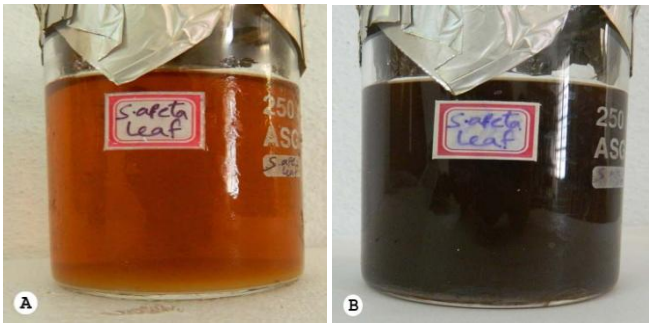

Fig. 4:
A) Plant extract with $AgNO_3$ solution before reaction.
B) Dark brown solution after reaction with $AgNO_3$ solution.

Synthesis of the AgNPs in aqueous solution was checked by recording the absorption spectrum at a wavelength range of 200-700 nm. The UV-Vis absorption spectrum of AgNPs (Fig-5) obtained showed the absorption maxima ranged from 425 to 475 nm which gives the confirmation for the AgNPs synthesis. The FTIR analysis (Fig-6) of *S. apetala* leaf AgNps exhibited peaks at 1619 cm-1 and 3417 cm-1 indicating the possible interaction between proteins and silver nanoparticles. The remaining peaks

found in the spectrum viz., 2926.16, 1383.36, 1095.29 and 595.70 are possibly reflecting the CH stretching, -C=C- stretching, C–N stretching vibration of the amine and CH bending vibration, respectively. AgNPs that gave the spectrum were examined under Scanning Electron Microscope. The SEM image silver nanoparticles (Fig-7) clearly showed the well dispersed particles that are almost spherical.

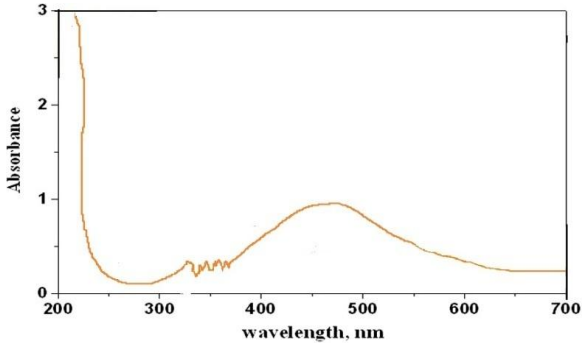

Fig. 5: UV-Vis spectra of *Sonneratia apetala* leaf extract AgNPs.

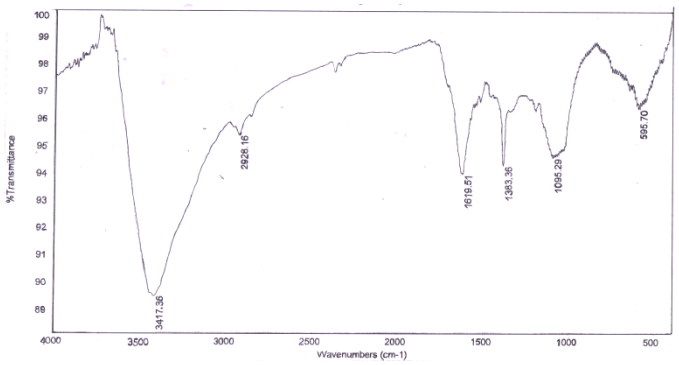

Fig. 6: FT-IR spectrum of AgNPs synthesized by reacting $AgNO_3$ with *Sonneratia apetala* leaf extract.

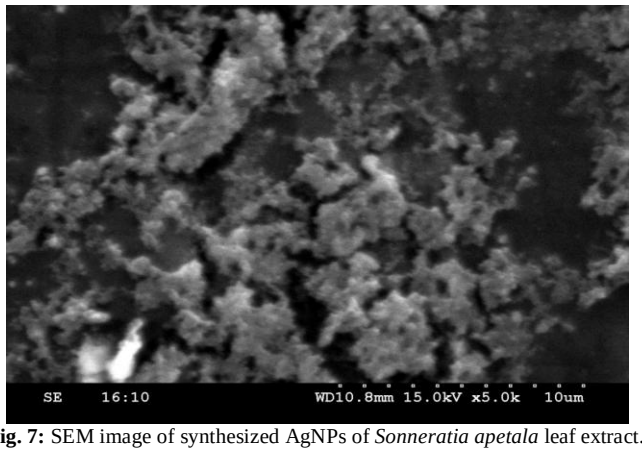

Fig. 7: SEM image of synthesized AgNPs of *Sonneratia apetala* leaf extract.

Antibacterial activity of AgNPs

The results on the antibacterial activity of AgNPs (Fig-8) clearly indicated the promising role of nanoparticles as antibacterial agents. Few photographs showing the clear zones formed by *S. apetala* leaf silver nanoparticles are presented in Plate-1. The silver nanoparticles displayed antibacterial activity against all the tested bacteria except *Salmonella enterica*. However, *Proteus mirabilis* and *Arthrobacter protophormiae* showed more susceptibility towards the AgNPs when compared to the streptomycin, with inhibition zones measuring 27.3 mm and 23.3 mm, respectively. More antibacterial activity of AgNPsthan the streptomycin positive control was also observed against *Micrococcus luteus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Alcaligensfaecalis*. Surprisingly, *Rhodococcus rhodochrous* and *Enterobacter aerogenes* that were found totally resistant to streptomycin showed considerable susceptibility to silver nanoparticles.

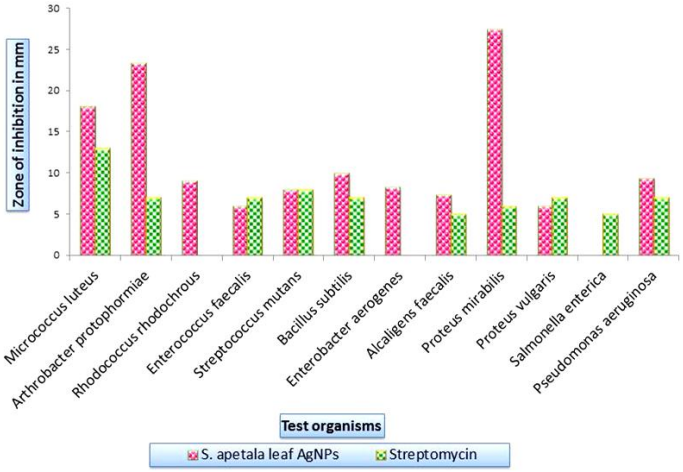

Fig. 8: Antibacterial activity of Sonneratia apetala leaf extract AgNPs against various bacteria.

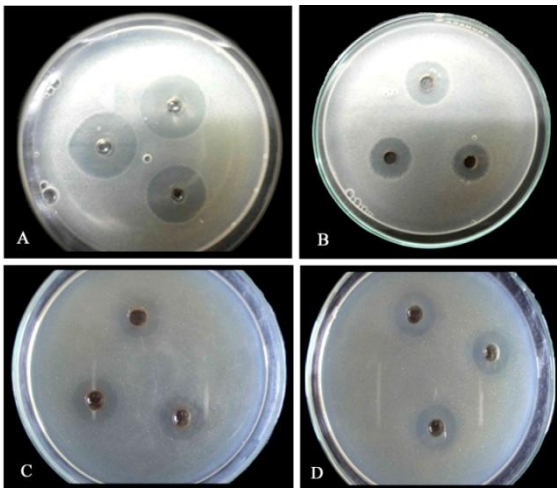

Plate-1. Antibacterial activity of *Sonneratia apetala* leaf extract AgNPs
A) AgNPs activity against *Proteus mirabilis*
B) AgNPs activity against *Arthrobacter protophormiae*
C) AgNPs activity against *Bacillus subtilis*
D) AgNPs activity against *Micrococcus luteus*

DISCUSSION

The current investigation revealed the presence of several phytochemicals, good antibacterial and antioxidant activity. These activities are may be due to the presence of different phytochemical classes. Moreover, these phytochemicals are

responsible for the stabilization of AgNPs. Several earlier reports correlated the plant phytochemicals with their bioactive attributes and reported the antibacterial and free radical scavenging activities of the phytochemical constituents (Ali *et al*., 2002; Dahija *et al*., 2014; Medini *et al*., 2014; Mohammed *et al*., 2014; Lunga *et al*., 2014). The quantity of phytochemical components may vary with the plant part, leaf and stem (Patra *et al*., 2011). Saponins are liable for precipitation and coagulation of red blood cells, cholesterol binding, haemolytic activity, froth formation in aqueous solutions and bitterness. On the other hand, flavonoids are cogent water soluble antioxidants and free radical scavengers which can avert oxidative cell damage. Besides, they are also known to have strong anticancer activity. Steroidal components are of great prominence in pharmacological aspect for the reason that of their association with sex hormones (Okwu, 2004). Alkaloids are in use as primary medication agents for antispasmodic, analgesic and antibacterial effect (Okwu, 2001). Phenolic constituents generally observed in plants are stated to have potential antioxidant and antimicrobial activities. Different phenolic compounds such as tannins existing in plant cells are effective inhibitors of hydrolytic enzymes used by plant pathogens. A number of studies have been concentrated on the biological activities of phenolic compounds, which are acting as powerful antioxidants and free-radical scavengers (Devi *et al*., 2011). The healing properties of medicinal plants are well recognized at worldwide level, particularly for antibiotic development. Hence, the research of alternative and efficient medicines from plants against resistant bacteria has become an important concern all over the world (Wikaningtyas and Yulinah, 2016). PrabhuTeja and Ravishekar (2013) prepared the ethanol extracts of *S. apetala* plant parts viz., leaves, bark and pneumatophores and reported the incidence of alkaloids, flavonoids, tannins, saponins, phytosterols and carbohydrates. Ethanol extract of *S. apetala* fruit also found to contain alkaloids, reducing sugars, tannins, steroids, glycosides, flavonoids and acidic compounds (Anha *et al*., 2014). Patra *et al* (2015) have screened leaves and bark of *S. apetala* using acetone, ethanol, methanol and aqueous extracts and reported the alkaloids, cardiac glycosides, gums, anthraquinones, mucilages, tannins, steroids, flavonoids, carbohydrates, proteins and amino acids and terpenoids. The appearance of saponins, tannins, flavonoids, glycosides and terpenoids was also reported in the ethanol, methanol and aqueous extracts of *Borassus flabellifer* (Muthukumar *et al*., 2014). Several of the molecules fitting to various categories of secondary metabolites are noticed as active on pathogenic organisms (Awouafack *et al*., 2013; Cowan, 1999; Ndhlala *et al*., 2013; Tsopmo *et al*., 2013) and also the distinct mechanisms of action of their bioactive constituents. Phenols are the precise crucial plant components with diverse biological roles including antioxidant activity and their radical scavenging ability is reasoned to their OH groups (Vinayagam and Sudha, 2011). Several earlier reports stated that the mangrove plants are being used as traditional as well as modern systems of medicine, because mangroves contain bioactive compounds that may be of potential use in the long-term treatment of major disorders and diseases. In our present investigation, in addition to molecules already reported we found the presence of terpenoids. Hossain *et al* (2013) have reported that the antioxidant potential of fruit of *S. apetala* and Patra *et al* (2015) reported the antioxidant activity of *S. apetala* leaf and bark which confirms our findings. The absorption maxima of AgNPs in that range perhaps the end result of surface plasmon resonance of AgNPs (Priyabenarjee *et al*., 2014). The absorption peak at 1619 cm-1 might be owing to the C=O amide bond of a protein (Macdonald and Smith, 1996) and the absorption peak at 3417 cm-1 could be of OH group existing in phenolics and alcohols (Ali *et al*., 2011; Jilie and Shaoning, 2007; Theivasanthi and Alagar, 2012). It is well known that the change in the shape of metal nanoparticle is a substantial change of their electronic and optical properties (Jana *et al*., 2000). The larger AgNPs might be formed by the aggregation of smaller ones that is owed to SEM measurements (Devaraj *et al*., 2013). The AgNPs own the capability to bind to the bacterial cell wall and eventually penetrate, and leads to modification cell membrane structure. As a result, the permeability of the plasma membrane changes that may cause the death of the cell. There is a chance for the occurrence of cavities and assembly of nanoparticles on the cell surface (Sondi and Salopek-Sondi, 2004). AgNPs forms free radicals which can be considered as another reason for the death of cells. Some Electron Spin Resonance Spectroscopy studies demonstrated that free radicals may be formed by the AgNPs when they are in contact with the bacteria, and these free radicals are able to disrupt the cell membrane and make it permeable which can finally lead to cell death (Danilcauk *et al*., 2006; Kim *et al*., 2007). These nanoparticles have been shown to accumulate inside the membrane and can subsequently penetrate into the cells causing damage to cell wall or cell membranes. It is of thought that silver atoms bind to thiol groups (-SH) of enzymes forming stable (S-Ag) bonds with thiol containing compounds and then it causes the deactivation of enzymes in the cell membrane that involve in trans membrane energy generation and ion transport. It was proposed that Ag(I) ion enters the cell and intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule. Bacterial cell lysis could be one of the reasons for antibacterial property of plant extract mediated silver nanoparticles (Ahmed *et al*., 2016).

It is also observed that nanoparticles are able to regulate the signal transduction in bacteria. It is a fact that the phosphorylation of protein substrates in bacteria determines bacterial signal transduction. Nanoparticles may alter the phosphotyrosine profile of bacterial peptides and dephosphorylation is realized in the tyrosine residues of Gram –ve bacteria. It was reported that the nanoparticles dephosphorylate the peptide substrates of tyrosine residues, leading to signal transduction inhibition and thus the termination of growth (Shrivastava *et al*., 2007). The variation in the level of antibacterial activity between Gram +ve and Gram –ve bacteria in this study may be the outcome of the arrangement pattern of cell

wall components. AgNPs are especially known for their antibacterial properties. The widespread cases of multidrug resistant bacteria against the standard antibiotics have led researchers to potentially incorporate AgNPs and other nanomaterials as ingredient to boost the antibiotic effects (Ali *et al*., 2016). Morones *et al* (2005) reported the different facets of the AgNps and proposed three ways of bactericidal effect of AgNPs which include- disturbing the proper function of the bacterial cell, causing damage by interaction with phosphorus and sulfur containing groups in DNA molecule of the bacterial cells, release of silver ions from nanoparticles resulting in additional contribution to the bactericidal effect. Ag^+ mainly impacts the function of membrane bound enzymes, such as those in the respiratory chain (Mc Donnell and Russell, 1999). DNA loses its replication ability by treating bacteria with Ag^+ ions (Feng *et al*., 2000) and also inhibits expression of ribosomal subunit proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated (Yamanaka et al., 2005). By using these diversified bioactive chemical components with the AgNPs, there is a scope for the development of new medicine for the dreadful diseases.

CONCLUSION

The present investigation involved the screening and evaluation of *S. apetala* leaf crude extracts for the phytochemicals, antibacterial activity and antioxidant activity. Green synthesis of AgNPs using aqueous leaf extract, Characterization and antibacterial activity of synthesized nanoparticles were also carried out in the study. Outcome of all the experiments carried out suggests the existence of most of the phytochemicals in the leaves and are having some important biological activities. Further work is needed to isolate, purify and identify the exact active principle which is the cause for the biological activities. The Green synthesis is a simple, low cost and ecofriendly approach without any huge inputs in terms of energy. This is the first report of Green synthesis of silver nanoparticles for this plant. Being exhibiting greater antibacterial activity, phytochemical based nanoparticles may stand as a potential remedy in developing drugs against antibiotic resistant bacteria.

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