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Phytochemical properties and antimicrobial activities of leaf, bark, fruit extracts and silver nanoparticles of *Samadera indica* Gaertner

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Abstract
The phytochemical and pharmacological studies of leaf, bark and fruit extracts of *Samadera indica* were carried out on aqueous and methanolic extracts, obtained by soxhlet technique. The qualitative tests showed the presence of carbohydrates, proteins, phenolics, alkaloids, saponins, flavonoids, tannins and resins, whereas quantitative estimations of carbohydrate, protein, phenolics and tannins showed that all the parts of plant were rich in proteins and poor in phenolics and have got good quantity of tannins. Among plant parts, bark possessed good quantities of chemical constituents while better extract yield was obtained in aqueous system except for leaf extract. The silver nanoparticles formation were confirmed by change in the colour of the extract from pale yellow to dark brown after two hours of mixing with silver nitrate solution. The UV-Vis spectrum analysis showed the absorption peak of synthesized silver nanoparticle at 500 nm for leaf extract, 350 nm for bark extract and 450 nm for fruit extract. The size of the silver nanoparticles synthesized are of cubic shape with the diameter of 124 nm for leaf, 153 nm for fruit, and 340 nm for bark silver nanoparticle extracts as obtained by SEM. FTIR analysis showed different peaks in three extracts which indicated the presence of functional groups.
Antibacterial activity of silver nanoparticle extracts with *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus* at three concentrations exhibited varied effects, but *S. aureus* showed resistance. Similarly antifungal activity with *Aspergillus fumigatus* and *Candida albicans*, methanolic extract showed good activity against *C. albicans.*

Keywords:*Samadera indica*, antimicrobial activities, silver nanoparticles, phytochemical analysis, characterisation

Introduction
A medicinal plant is any plants which in one or more of its organs contains active ingredients which can be used for therapeutic purposes or contain foundation compounds that can be used for the synthesis of useful drugs. Clearly there is an urgent need for new and efficient drugs currently used to treat infectious diseases are mostly natural products. Medicinal plants are still invaluable source of safe, less toxic, low price, available and reliable natural resources of drugs all over the world.
The tropical rain forest is home to a significant array of rich bioresources and accounts for 25% of plant extractable allopathic medicines used by humans [1, 2]. Medicinal plants accumulate various active compounds used in curing a variety of diseases in human and animals. In modern medicine the importance of herbal plant is increasing[3,4] with pharmaceutical and cosmetic industries increasingly using plant resources from rural or unpolluted areas. Secondary metabolites produced by the plant constitute development of new drugs based on traditional knowledge over 80% for health care as they are safe and efficient [5-7]. India is rich with herbal formulations and plant diversity which were used in different disease treatment from ancient times.
Samadera indica Gaertner belongs to the family Simaroubaceae with vernacular names Lokhandi (Hindi), Niepa bark tree (English), Karinjotta, Karingota (Malayalam). Guccha karanjah (Sanskrit) and Kaduhonge (Kannada) a locally available medicinal plant mainly used by local folklore practitioners and tribal people for various disease treatments.

The various parts of the plant (Plate 1) yield terpenoids and flavonoids in bitter taste which exerts medicinal properties against various disorders and skin diseases. The plant is distributed in Africa - Madagascar, through the Indian Ocean to the Indian subcontinent, Myanmar, through south East Asia to Papua New Guinea and the Solomon Islands. The Western Ghats of India includes Kerala, Karnataka states, among which in the evergreen forests usually rather rare, but locally common, in tidal swamp forest or periodically inundated forest. In lowland mixed Dipterocarp forest it is usually found at elevations below 150 metres along backwaters and moist deciduous forests. Maharashtra: Sindhudurg, Kerala District/s: Thrissur, Kottayam, Alappuzha, Kollam, Kannur, Thiruvananthapuram, Malappuram, Kozhikkode, Kasaragod, Wayanad, Ernakulam.

a. Leaves b. Flowers

c. Matured fruit d. Dried fruit

Plate 1: Leaf, flowers and fruits of *Samadera indica*

Medicinal uses: The bark is used in the treatment of fevers; the juice of the pounded bark is considered a cure for skin diseases; after maceration, or in decoction, the bark and wood are used as a febrifuge, tonic, stomachic and emmenagogue. A decoction of the leaves is taken to relieve cough; the leaves are bruised and then applied externally as a treatment for erysipelas; the macerated leaves, mixed with coconut oil, are used to kill head lice. The seeds in particular are of medicinal importance and are commonly applied as an emetic and purgative; they are used in the treatment of bilious fevers. The oil from the seeds is applied externally on rheumatic joints, and used as a liniment on bruises; all parts of the plant contain the glucoside samaderin and a bitter substance.

Other uses: A decoction of the leaves is used to kill termites; an infusion of the leaves is used as an insecticide; the macerated leaves, mixed with coconut oil, are applied to the hair for cleansing purposes; the seeds contain about 33% oil, but it is difficult to get a sufficient supply for commercial use; the pale yellow wood is light and soft. It is used for making 'parang' handles; the wood is used for making the handles of knives.

Nanotechnology is the study of manipulating matter on an atomic and molecular scale. In the past few years, there has been an increasing interest in silver nanoparticles on account of the antimicrobial activities that they display. They are even being projected as future generation antimicrobial agents. Silver nanoparticles have been recently known to be promising antimicrobial agent that acts on abroad range of target sites both extracellularly and intracellularly. Biological synthesis of plant silver nanoparticles gaining importance as it is reliable and eco-friendly. The extract of different plant parts got medicinal value even against bacteria but silver nanoparticles of different plants have showed high level of antimicrobial activity [8, 9].

The following are the objectives of the present study:

- To analyse phytochemical constituents in aqueous and methanolic extracts of *Samadera indica.*
- To synthesise silver nanoparticles from leaf, bark and fruits of *S. indica.*
- To characterize silver nanoparticles using UV-Vis Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).
- To study antimicrobial activities of methanol, aqueous and silver nanoparticle extract on selected bacterial and fungal strains.

Materials and methods

Collection of sample: *Samadera indica* leaves bark and fruit were collected from the forests of Kasaragod district, Kerala during the month of March – May 2015. The leaf, bark and fruits were allowed to shade dry for a week. These were then kept in hot air oven at 60˚C for 24-48 hours until it was dried completely. These were then coarsely powdered and stored in a closed container for further use.

Preparation of extracts: The coarse powder of the leaf, bark and fruit (25gms) was extracted by soxhlation process. The mass thus obtained was extracted for 1hour with 150 ml of distilled water. This was followed by the distillation process. The extract thus obtained was dried in hot air oven at 40˚C for a week. The dried extract thus obtained was used for assessment of antimicrobial activities. For the preparation of methanol extract methanol was used.

Synthesis of silver nanoparticles: For the biosynthesis of nanoparticles, 300 ml of 1mM $AgNO_3$ was taken in a conical flask and 9 g of leaf, bark, and fruit were added into the respective conical flasks, centrifuged at 2000 rpm for 30 min. The supernatant were collected and kept in boiling water bath. A change in colour of the solution was observed within 1 hour. The extracts were stored at 4^0C for further use.

Screening of phytochemicals and their quantification in plant extracts: Phytochemical tests for carbohydrates, proteins, alkaloids, phenolics, tannins and resins were carried out on the aqueous and methanolic extracts with the respective solvents using standard protocols. Quantitative analysis of the phytochemical like total carbohydrates by Anthrone method, proteins by Lowry's method, phenols by Folin - ciocalteau method and tannins by Folin- Denis method.

Characterisation of silver nanoparticles

UV-VIS spectra analysis: The reduction of pure Ag^+ ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-VIS spectral analysis was done by using UV-VIS spectrophotometer.

Fourier Transform Infrared Spectroscopy (FTIR): Fourier Transform Infrared Spectroscopy was used to recognize the functional groups bound to the silver nanoparticles. The lyophilised powder sample was used and examined by Infrared (IR) Spectrum at the spectral range of 1000-4000cm^{-1}.

SEM analysis of silver nanoparticles: Scanning Electron Microscope (SEM) analysis was done using SEM machine. Thin films of the sample was 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 film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes.

Antimicrobial activity studies: For antibacterial studies *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Salmonella typhi* were used as test organisms from the P.G Department of Biotechnology, Alva's College and microscopic examinations was done for the confirmation and were maintained in slants. For antifungal studies *Aspergillus fumigatus* and *Candida albicans* were selected and cultures were maintained in PDA medium.

Preparation of inoculums: A loop full of culture was inoculated into Nutrient broth and incubated at 37^0C for 24 hours to obtain a bacterial culture.

Antimicrobial activity test by agar well diffusion method: Petri dishes were plated with Muller Hinton Agar media/PDA medium and allowed to solidify for 30 min. The test organisms were then spread on the surface of the media using sterile ear buds. Cork borer (4mm) was used to bore wells in media. The aqueous extract of different concentrations viz., 100 µl, 200 µl and 500 µl was dispensed into the wells using a micropipette. A negative control of distilled water and a positive control of streptomycin were kept and the extract was allowed to diffuse for 1 hour at room temperature. Then the plates were incubated at 37°C for 24 hours in case of bacteria and 2-3 days for fungi. Zone of inhibitions were measured. Similarly, methanolic and silver nanoparticle extracts were used for antibacterial activity in which methanol and silver nitrate used as negative control.

Results and discussion

Extraction and analysis of phytochemicals: Solvent extraction method was used to extract the phytoconstituents from leaf, bark and fruits of *Samadera indica*. Aqueous and methanolic systems were used. The per cent yield showed variation within plant parts (Table 1). The plant extracts – leaf, bark and fruit showed a positive results for carbohydrates, tannins, flavonoids, alkaloids, phenolic compounds, proteins, resins and triterpenes in methanolic extract but in aqueous extract some of them showed negative result (Table 2). The results of quantitative estimations of carbohydrate, protein, phenolics and tannins in the leaves, bark and fruits of *S. indica* were given in table 3. Among these, the leaves possessed higher contents of these except tannins which were found higher in the fruits. Viswanad *et al*. (2011) [10] reported the presence of alkaloids, tannins, triterenoids, steroids, carbohydrates, proteins and flavonoids in the leaf extracts of *Samadera indica*.

Kanti Dev *et al*. (2015) [11] found the presence of flavonoids, alkaloids, reducing sugar, saponin, phenolics, tannin, amino acids and proteins in methanolic leaf extracts of *Mikania micrantha*. Phenolics was 45.5mg/g, they revealed that antimicrobial properties was attributed by the presence of phenolic compounds. Dhawale (2013)[12] studied the phytochemical analysis of 8 plants showed the presence of alkaloids, flavonoid, steroin and terpinoids in methanolic extracts of leaf and roots. Similarly Ndam *et al*. (2014) [13] screened 20 medicinal plants for their active medicinal principles which revealed the presence of different substances in all the plants. Qualitative analysis of *Terminalia chebula*, *Terminalia arjuna*, *Xanthium strumrium* and *Heliotropium indicum* leaves showed the presence of alkaloids, phenolics, flavonoids, saponins, tannins [14]. There are number of earlier reports indicates the presence of various medicinal constituents in the herbal plants [15, 16].

Table 1: Per cent yield*of extracts in different parts of *Samadera indica*

Plant parts	Solvent system	
	Water (Aqueous)	Methanol
Leaf	4.8± 0.56	7.4± 0.67
Bark	5.0± 0.73	2.82± 0.088
Fruit	4.7± 0.29	2.15± 0.155

*means of five replicates, mean ± SE

Table 2: Qualitative tests for phytoconstituents of methanolic and aqueous extract of *Samadera indica*

Phytochemicals	Inference	
	Aqueous extract	Methanol extract
Carbohydrates	+	+
Proteins	+	+
Alkaloids	+	+
Tannins	-	+
Flavonoids	-	+
Phenolics	-	+
Resins	-	+
Saponins	-	+
Triterpenoids	-	+

+ Present, - Absent

Table 3: Quantities* of carbohydrate, protein, phenolics and tannin indifferent parts of *Samadera indica*

Constituents (mg/g)	Leaf	Bark	Fruit
Carbohydrate	2.1± 0.451	1064±0.311	2.289±0.154
Protein	29.22± 2.134	22.08± 2.90	25.44± 2.619
Phenolics	0.0252± 0.0029	0.054±0.003	0.034± 0.0019
Tannin	1.592± 0.0624	1.130±0.173	2.456± 0.0914

*mean of 5 replicates, mean ± SE

Synthesis of silver nanoparticles: Formation of silver nanoparticles by the reduction silver ions during the exposure to fruit, bark and leaf extract of *S. indica* was easily monitored from the colour change in the reaction mixture from yellow to brown in case of leaf extract, pale yellow to deep yellow in case of bark extract and orange to dark brown in case of fruit extract. The change of colour in the reaction mixture after two hours of incubation in hot water bath at 80^0C, which indicated the formation of silver nanoparticles. This formation indicates that the silver ions in the reaction mixture medium have been converted to elemental silver having the size of nanometric range. Similarly, on other studies with the leaves of *Svensonia hyderobadensis* and stem bark of *Boswellia ovalifoliata* revealed the synthesis of silver nanoparticles noted by the reduction of silver ion in silver nitrate solution exposed to plant extract followed by colour change from yellow to dark brown [9, 17, 18]. There are few other recent works supporting the present investigation [19, 20, 21].

Ultraviolet–visible (UV- VIS) spectra analysis of the leaf extract: Silver nanoparticles are known to exhibit UV-Visible absorption spectra with a peak in the range of 350-500 nm. In this study the formation of silver nanoparticles was initially confirmed by using UV-Visible spectroscopy due to surface Plasmon Resonance phenomenon. Absorption peaks were observed at 500 nm for leaf extract, 450 nm for fruit extract and 350 nm for bark extract (Fig 1-3). Similarly, Geetha *et al.* (2012) [22] reported a narrow absorption band at 450 nm and Maribel *et al*. (2009) [23] reported that the absorption peak of silver nanoparticles by chemical reduction method was 412 nm. There are other studies supporting the result with maximum peak at different wave lengths in different plant extracts [19, 20, 21].

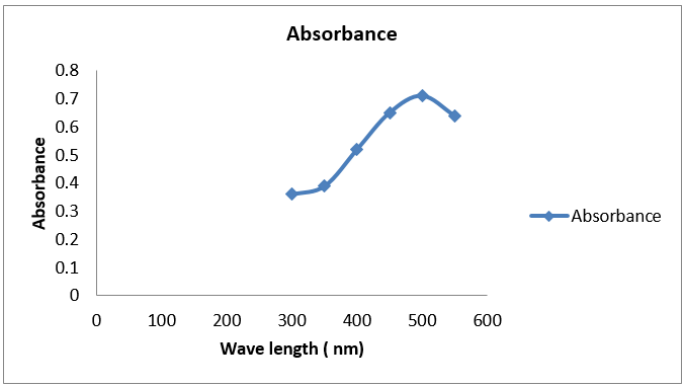

Fig 1: UV- Vis spectrum of leaf extract of *S. indica*

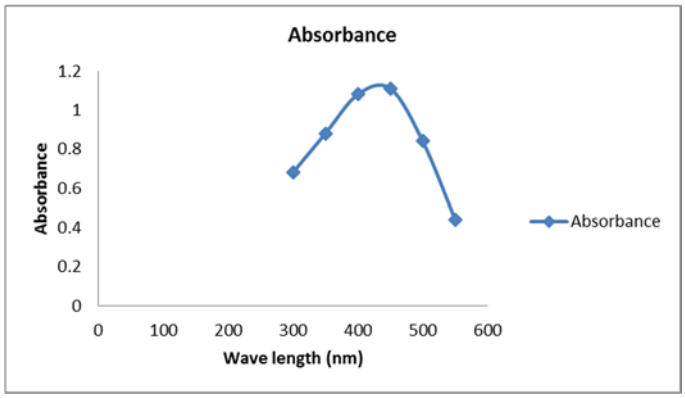

Fig 2: UV- Vis spectrum of fruit extract of *S. indica*

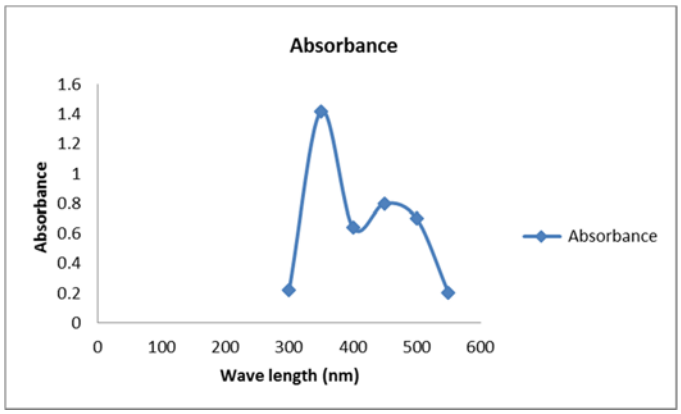

Fig 3: UV- Vis spectrum of bark extract of *S. indica*

Scanning electron microscopic (SEM) study: The scanning electron microscopic image has been employed to characterize the size, shape and morphology of synthesised silver nanoparticles. From the SEM image of synthesised silver nanoparticles (Fig. 4-6), it is evident that the morphology of the synthesised silver nanoparticles are cubic shaped with the diameter range 124 nm for leaf, 153 nm for fruit, and 340 nm for bark extract. Christensen *et al*. (2011) and Charusheela *et al*. (2013) [24, 25] reported spherical shaped nanoparticles of 10-25 nm and Hemanth *et al*. (2010) [26] obtained the nanoparticles of 52-104 nm. In other studies, hexagonal shaped nanoparticles of 80-120 nm and 90-120 nm [19, 20] and oval with a diameter ranged from 553-610 nm [21] were reported. According to Mie theory, only a single surface Plasmon resonance band is expected in the absorption spectra of spherical nanoparticles, whereas the number of peaks increases as anisotropy increases [27].

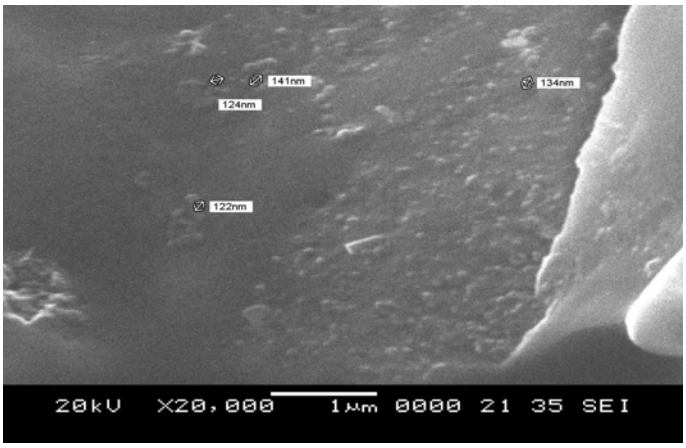

Fig 4: SEM image of silver nanoparticles synthesised from leaf of *S. indica*

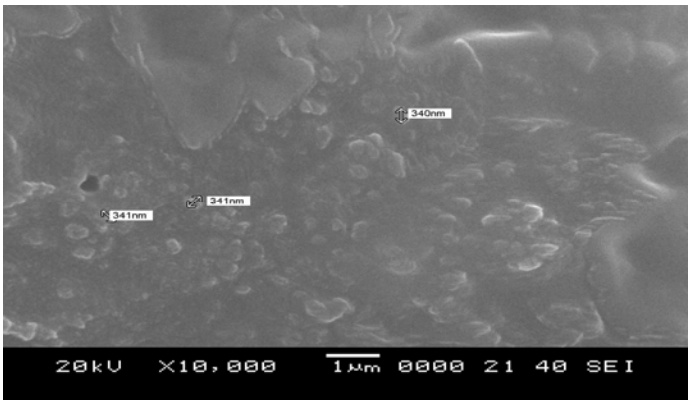

Fig5: SEM image of silver nanoparticles synthesised from fruit of *S. indica*

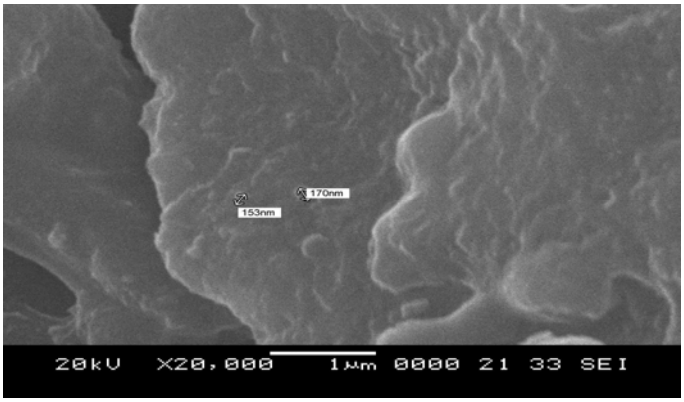

Fig 6: SEM image of silver nanoparticles synthesised from bark of *S. indica*

Fourier Transform Infrared Spectroscopy (FTIR): The lyophilised nanoparticle samples were analysed in FTIR to identify the possible biomolecules responsible for the reduction of the silver ions by cell filtrate. The representative spectra of nanoparticles obtained manifests absorption peaks using the spectral range between 500-4000 cm^{-1}. The absorption peaks for bark extracts were observed at 3631.71cm^{-1}, 3394.48cm^{-1}, 3255.62cm^{-1}, 2360.71cm^{-1}, 1384.79cm^{-1}, 1151.42cm^{-1} and 432.03cm^{-1}. The absorbance peaks for the fruit extracts were observed at 3595.07cm^{-1}, 3078.18cm^{-1}, 2921.96cm^{-1}, 2360.71cm^{-1}, 1384.79cm^{-1}, 1122.49cm^{-1} and 1029.92cm^{-1}. The absorbance peak for the leaf extracts were observed at 3666.43cm^{-1}, 3427.29cm^{-1}, 2929.67cm^{-1}, 2864.09cm^{-1}, 2360.71cm^{-1}, 1737.74cm^{-1}, 1569.95cm^{-1}, 1456.16cm^{-1}, 1392.51cm^{-1}, 1087.78cm^{-1}, 613.32cm^{-1} and 534.25cm^{-1}. These peaks can be assigned as absorption bands of –OH group of alcohol, C-H aromatic stretch of groups, C=C aromatic stretch of group, C=H alkyl Halide stretch of group, C-O alcohol stretch of group, C-H bending of alkene group, and C-Cl halide stretch of alkyl functional groups. The FTIR analysis supported the reducing property of silver nanoparticles by *S. indica* bark extract which in turn imparted the high stability of the synthesised silver nanoparticles. FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. The biomolecules which are involved in the reduction of silver ions can be identified by absorption peaks in the spectral range of 1000-4000 cm^{-1}. Saifuddin *et al*., (2009) [28] observed the FTIR measurements to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized in leaf broth. This study supported by earlier few reports [19, 20, 21].

Antibacterial activity for aqueous extract: The bacterial strains used in the present experiment showed varied levels of sensitivity towards different concentrations of aqueous, methanolic and silver nanoparticle extracts. *Staphylococcus aureus* showed high sensitivity for the extract of the plant. Among different plant part extracts methanolic extract showed good response. The antibacterial activity of plant leaf, bark and fruits in aqueous, methanolic and silver nanoparticles varied at different concentrations (Tables 4 - 12). The present result was comparable with works of Viswanad *et al*. (2011) [10]who reported both Gram negative and Gram positive bacterial strains used in their experiment showed good activity. The antibacterial activity of methanol and aqueous extracts from seeds of *Areca catechu* resulted in highest inhibitory zone with *Pseudomonas aeruginosa* and minimum zone of inhibition by *Klebsiella pneumoniae*[21]. Chin and Fernandez (2013) [29] examined the antimicrobial performance of methanolic extract of *Areca catechu* seeds against mixed oral flora from tooth scum and Gram negative laboratory isolates. Varying concentrations of *A. catechu* ethanol extract was tested for antimicrobial activity against 0.5 Mc Farl and of mixed oral flora and eight Gram negative clinical isolates by agar well diffusion method. All concentration showed to inhibit oral flora models with zone of inhibition about 7-18 mm. In the present study of silver nanoparticles synthesized using areca nut seed extract exerted a significant antibacterial activity compared with positive control and other bacterial strains which symbolised that the extract have antibacterial activity. By increasing the dose of the extract higher inhibitory zone was found. The antibacterial activity can also be compared with other silver nanoparticles synthesized from leaf extracts of *Svensonia*, which also showed activity against *E. coli, Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumoniae* [9]. Maria *et al.* (2012) [30] also reported the antimicrobial effect of areca nut extract on oral pathogens which are of other bacterial strains but *Candida albicans* showed positive inhibitory effect. On the other hand, Marium and Tuslima (2015) [31] obtained inhibitory effect against Gram negative bacteria when carbon tetrachloride extract was used compared with methanol extract of areca seeds. Similarly, Kabbashi (2015)[32] tested ethanolic and methanolic fruit extract of *Balanites aegyptiaca* against two Gram positive, two Gram negative and two fungal species, among which the methanolic extract exhibited high activity only against *Aspergillus niger*, *Bacillus subtilis* and *Staphylococcus aureus*, whereas it was intermediately active against *E. coli, Candida albicans* and, *Pseudomonas aeruginosa*. He concluded that the ethanolic extract proved to be had high activity on all bacteria and fungi. Kanti Dev *et al*., (2015) [11] reported antibacterial and antioxidant properties of *Mikania micrantha* leaves and revealed the activities due to presence of phenolic compounds. Some of the recent studies showed that extracts of *Euphorbia hirta* and *Albizia richardiana* exhibited pronounced antibacterial activities against human pathogens like *Salmonella* spp., *Enterococcus faecalis, Shigella* spp., *Klebsiella pneumoniae, Bacillus* spp., *Vibrio* spp., *Staphylococcus aureus* and *Proteus. mirabilis* [33,16,34].
Smith (2015) [34] studied the antioxidant and antimicrobial activities of ethanolic extracts of *Euphorbia hirta* leaves. The antimicrobial effects was determined by evaluating its inhibitory effects against some microorganisms like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp., *Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis* and *Shigella dysenteri.* This study demonstrated the significant free radical scavenging and antimicrobial potential of the leaves of *Euphorbia hirta* and could be explored for the treatment of various infections caused by these microbes and also in the treatment of free radicals mediated ailments. Ethanolic extract showed mild inhibitory effect, more pronounced against *Proteus mirabilis, E. coli* and *B. subtilis*.

Table 4: Antibacterial activity for the aqueous bark extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of aqueous extract of bark				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	13	15	26	27	-
Klebsiella pneumoniae	-	-	11	28	-
Escherichia coli	-	6	12	30	-
Salmonella typhi		7	14	22	-
Bacillus subtilis	-	6	14	27	-

Table 5: Antibacterial activity for the aqueous leaf extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of aqueous extract of leaf				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	-	12	18	25	-
Klebsiella pneumoniae	-	6	15	20	-
Escherichia coli	-	-	8	20	-
Salmonella typhi	-	8	13	22	-
Bacillus subtilis	-	9	11	27	-

Table 6: Antibacterial activity for the aqueous fruit extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of aqueous extract of fruit				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	-	13	25	25	-
Klebsiella pneumoniae	-	4	11	20	-
Escherichia coli	-	-	-	20	-
Salmonella typhi	-	8	12	22	-
Bacillus subtilis	-	5	10	27	-

Table 7: Antibacterial activity for the methanolic bark extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of methanolic bark extract				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	10	12	15	28	-
Klebsiella pneumoniae	-	-	-	20	-
Escherichia coli	-	-	-	22	-
Salmonella typhi	-	–	-	24	-
Bacillus subtilis	-	-	-	24	-

Table 8: Antibacterial activity for the methanolic leaf extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of methanolic leaf extract				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	-	10	12	28	-
Klebsiella pneumoniae	-	-	-	24	-
Escherichia coli	-	-	-	26	-
Salmonella typhi	-	-	-	22	-
Bacillus subtilis	-	-	-	21	-

Table 9: Antibacterial activity for the methanolic fruit extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of methanolic fruit extract				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	10	11	13	28	-
Klebsiella pneumoniae	-	-	-	20	-
Escherichia coli	-	-	-	22	-
Salmonella typhi	-	-	-	28	-
Bacillus subtilis	-	-	-	24	-

Table 10: Antibacterial activity for the silver nanoparticles of bark extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of silver nanoparticles of bark extract				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	8	13	20	30	-
Klebsiella pneumoniae	-	8	12	22	-
Escherichia coli	-	8	13	29	-
Salmonella typhi	-	-	10	26	-
Bacillus subtilis	-	-	9	22	-

Table 11: Antibacterial activity for the silver nanoparticles leaf extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of silver nanoparticle of leaf extract				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	-	11	13	30	-
Klebsiella pneumoniae	-	-	-	22	-
Escherichia coli	7	11	15	29	-
Salmonella typhi	-	-	7	26	-
Bacillus subtilis	-	-	6	22	-

Table 12: Antibacterial activity for the silver nanoparticles fruit extract of *S. indica* at different concentrations

Test organisms	Zone of inhibition (mm) for different concentrations of silver nanoparticle of fruit extract				
	100µl	200µl	500µl	Control 1	Control 2
Staphylococcus aureus	8	11	16	30	-
Klebsiella pneumoniae	-	-	-	22	-
Escherichia coli	-	11	16	29	-
Salmonella typhi	-	-	11	26	-
Bacillus subtilis	-	-	10	22	-

Control 1- streptomycin, Control 2- Distilled water for aqueous, methanol for methanolic and $AgNO_3$ for silver nanoparticles

Antifungal activity by poison drop method: The antifungal activity of the various concentrations of methanolic extracts of *S. indica* was carried out against fungi such as *Aspergillus fumigatus* and *Candida albicans*. The methanolic extract of *S. indica* showed antifungal activity against *C. albicans* only, but no clear zone of inhibition was showed against *A. fumigatus* (Table 13). Hence the extract is resistant to *A. fumigatus*. Similarly, Viswanad *et al.* (2011) [10] in their experiment on methanolic leaf extract of *S. indica* showed resistant against *Aspergillus niger* and *A. fumigatus* but *Candida albicans* was susceptible. Kabbashi (2015) [32] tested ethanolic and methanolic fruit extract of *Balanites aegyptiaca* against two fungal species, among which the methanolic extract exhibited high activity only against *Aspergillus niger*, whereas it was an intermediately active against *Candida albicans*. Similarly, Rahman *et al.* (2015) [16] observed moderate antifungal activity of *Albizia richardiana* extract against *Aspergillus niger* and *Saccharomyces cerevisiae* where as low activity showed against *Candida albicans.*

Table 13: *In vitro* antifungal activity of extracts of *S. indica*

Fungi	Zone of inhibition (mm)			
	Methanolic extract – 500µg/ml			
	Bark	Fruit	Leaf	Control
Aspergillus fumigatus	–	–	–	–
Candida albicans	9	12	14	20

As the present investigation proved the medicinal properties of leaf, bark and fruits of *Samadera indica* which further confirmed its use as antioxidant and antimicrobial agent. In addition to aqueous extract, the silver nanoparticles further improved in its activity against some of the human pathogens used in the present study.

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