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Original Article

Pharmacology Section

Characterisation and Evaluation of Antimicrobial, Antioxidant and Antibiofilm Activities of Silver Nanoparticles Biosynthesised from *Harpullia arborea* Bark Extract

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ABSTRACT

Introduction: In recent years, plant-based antibacterial substance has replaced the conventional chemical synthesis method. *Harpullia arborea* belongs to the Sapindaceae family; its bark, fruit, and seeds are used by Indians as leech repellent, hair wash, and antirheumatic agents. Plant-mediated green synthesis of nanomaterials is gaining popularity due to its environmental friendliness and cost-effectiveness.

Aim: To synthesise Silver Nanoparticles (AgNPs) using bark extract of *Harpullia arborea* and evaluate their antibacterial efficacy against food borne pathogens.

Materials and Methods: The in-vitro study of antimicrobial activity of *Harpullia arborea* bark extract was utilised for the synthesis of nanoparticles with 2 mM of silver nitrate. The study was conducted from March 2017 to April 2017. The synthesised nanoparticles were confirmed and characterised using Ultraviolet-Visible (UV-Vis) spectroscopy, while Fourier transform infrared, and electron microscopy were utilised for the determination of shape and size of the synthesised particle. The synthesised AgNPs were subjected to antibacterial activity against food isolates using agar well diffusion method. Furthermore, Minimal Inhibitory Concentration (MIC) and antioxidant were also measured with titre plate and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method, respectively.

Results: The UV-Vis spectra showed conformation of AgNPs with surface resonance peak of 430 nm, and Fourier-transform Infrared Spectroscopy (FTIR) spectra confirmed the involvement of biological molecules in AgNPs synthesis. In addition, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) analysis confirmed AgNPs with a spherical shape with diameters from 26.3-40.6 nm. The well diffusion method showed the antibacterial activity of AgNPs against bacterial isolates. The results showed that AgNPs possess higher antimicrobial potency than non AgNPs. The lowest Minimum Inhibitory Concentration (MIC) was observed against *Staphylococcus aureus* [3.5 mg] and followed by *Enterococcus faecalis* and *Pseudomonas aeruginosa* (4.5 mg). The DPPH method has confirmed that silver nanoparticles have a similar antioxidant activity compared to ascorbic acid.

Conclusion: It can be concluded that *Harpullia arborea* bark extract can be used effectively in the production of potent antimicrobial and antioxidants for commercial use.

Keywords: Energy dispersive X-ray, Scanning electron microscopy, Transmission electron microscopy

INTRODUCTION

In recent decades, there has been emerging significance in the development of new and effective antibiotics against infections caused by antibiotic-resistant bacteria. The use of traditional medicinal plant to regulate and eliminate pathogenic bacteria is becoming increasingly popular. Various techniques are utilised to kill bacteria, among them nanotechnology is now widely utilised in the scientific community and visually perceived as a milestone in the medical world. The Nanoparticles (NPs) fight and destroy many terrible diseases, and they use the body's natural transport pathway and natural means to absorb the drug through diseased cells [1].

In recent years, Silver Nanoparticles (AgNPs) have received much attention from many researchers working in many fields due to their unique features and wide range of applications, especially in medical field [2,3]. It is active against nematodes, microbes, cancer, and inflammation. Furthermore, the use of these NPs is non toxic and environment friendly. Green synthesis methods for synthesising NPs utilising natural products can be acclimated to address the quandary by utilising plants or microorganisms.

Plants offer an excellent base for the synthesis of NPs because they are non toxic and natural capping agents. In addition, the use of plant extracts reduces the use of NPs by microorganisms, and plant extracts can be used to reduce the cost of NPs produced by microbial synthesis [1]. *Harpullia* species are well recognised for being abundant in tannins, flavonoids, sterols, triterpenes, and saponins. Among the various species of *Harpullia, Harpullia arborea* has active phytochemical components, which were proven to be a remedy for a number of human diseases like cardiovascular diseases, brain cancer, and urinary infection [4]. In earlier study, *Harpullia arborea* showed inhibitory activity against clinical isolates of *Proteus vulgaris*, *Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi*, and *Escherichia coli* [5], and there are no reports available on NPs synthesis using *Harpullia arborea*.

In this study, we used the methanol extract of *Harpullia arborea* in the green biosynthesis of AgNPs, and determine the antibacterial and antioxidant activity. To the best of our knowledge, this is the first study regarding the synthesis of AgNPs using *Harpullia arborea* to suppress the food borne bacteria. The size and morphology of prepared NPs were characterised by UV analysis, FTIR, TEM and SEM with EDX according to the previous study by Rautela A et al., [6]. In addition, the in-vitro anti-oxidant activity of *Harpullia arborea* AgNPs was investigated. With this background, the present study was aimed to identify the antibacterial phytoconstituents present in *Harpullia arborea*.

MATERIALS AND METHODS

The present in-vitro study was to identify the antibacterial properties of phytoconstituents in the *Harpullia arborea*, which was collected from Gedamalai, Namakkal District, Tamil Nadu, India. The sample collection and process was during the period of March 2017 to April 2017 and study was conducted in Gadamali, Namakkal, Tamil Nadu, India.

Study Procedure

The bark samples of *Harpullia arborea* were prepared and extracted individually for further analysis as per the method described by Sreelatha S and Padma PR [7]. The collected fresh, healthy barks weighing 1 kg were washed under running tap water to remove debris and damaged portions and were shade dried at room temperature (32-35°C) over a period of five days to avoid loss of essential oil. The dried barks were pulverised separately into coarse powder by mechanical grinder and stored in airtight bottles in a cool dry place until further use.

Twenty gram of finely powdered sample was filled in the thimble of the extraction apparatus and dropped in to the soxhlet tube and extracted for 8 hours over heat (Plate 2.1). Extraction was carried out individually using 250 mL of methanol and acetone. The extract (condensed vapour) obtained was subsequently concentrated and dried using rotary vacuum evaporator (Equitron, India) at 40°C under reduced pressure. The solvent was distilled off and then the dried crude residues were aseptically weighed and dissolved in Dimethyl Sulfoxide (DMSO) and stored at 4°C in a sterile, labelled, air tight container until further analysis.

Screening of phytochemicals on bark of *Harpullia arborea*: Qualitative phytochemicals (alkaloids, carbohydrates, flavonoids, phenolics, saponins, tannins, quinones, steroids, terpenoids, and proteins) screening of methanol and acetone extracts of *Harpullia arborea* were carried out using standard procedures described by Jamil M et al., [8].

Preparation of AgNPs: According to qualitative phytochemicals studies, highest counting phytochemicals containing methanol extract was subjected to nano particle synthesis with Silver Nitrate ($AgNO_3$). The 5 mL of $AgNO_3$ 2 mM was prepared by adding the 0.5 mL of extract to these solutions. The colour of solution started to change from light brown to dark brown, due to the reaction of Ag ions and formation of AgNPs [9]. Then, synthesised NPs were subjected to characterisation [6].

Characterisation of Nanoparticles Synthesis of *Harpullia arborea*

A spectrum scan from 300-800 nm of wavelengths carried out using UV-Visible spectrophotometer to determine the absorption maxima of synthesised AgNPs [10]. The reduction of silver ions and formation of AgNPs occurred within an hour of reaction. Control was maintained by using $AgNO_3$.

Fourier-transform infrared spectroscopy: For FTIR measurements, initially the synthesised AgNPs solution was centrifuged at 10000 rpm for approximately 30 minutes. The pellet formed was washed three times with 5 mL of deionised water to get rid of the free proteins and enzymes and the pellet was dried using vacuum drier. The FTIR was recorded with Shimadzu IR-Tracer-100, spectrophotometer on KBr pellets and were analysed in the range of 1500-400 cm^{-1}.

Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray (EDX) analysis: The SEM analysis was carried out in Tamil Nadu Agriculture University, Coimbatore, India. The morphology of the green synthesised AgNPs with methanol extract of *Harpullia arborea* were analysed using SEM coupled with EDX. The AgNPs solution was centrifuged at 10,000 rpm for 20 min and drop coated onto thin glass film fabricating and allowing water to completely evaporate and analysed using Zeiss EVO 18 at a voltage of 20 kV. The elemental identification and quantitative compositional information were obtained using EDX.

Transmission Electron Microscopy (TEM) imaging and Energy Dispersive X-Ray (EDX) analysis: The TEM analysis was carried out in Tamil Nadu Agriculture University, Coimbatore, India. The samples for imaging were prepared by carefully placing a single drop of synthesised AgNPs on a copper coated grid, and the samples were dried for 4 minutes and imaged for their size and shape on (spherical in cluster) operating at a voltage of 200 kV. Diameter (D) was calculated for each nanoparticle sample by averaging 200 particles from the TEM images using Image J software (National Institutes of Health, United States of America). The elemental identification and quantitative compositional information was obtained using EDX.

Antimicrobial activity of plant extract against meat isolates: The loop full of inoculums were taken from crushed meat samples and inoculated into selective media and chromogenic media for isolation of bacterial isolates. Based on the colony morphology and cultural characterisation, isolates were confirmed and stored in nutrient agar slant for further study.

Screening of antibacterial activity: Antimicrobial assay of methanol extract of *Harpullia arborea* was performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates [11]. The overnight broth culture (1.5×108 CFU/mL) was lawn cultured on MHA plates. Six wells of 6 mm were bored in the inoculated MHA media with the help of sterile cork-borer (6 mm). The different concentrations (5, 7.5, 10, 12.5 mg) of bark extracts were filled in each well. The positive control of Chloramphenicol 30 mcg, negative control of DMSO and silver nitrate (50 µL) was added in other wells and incubated at 37°C. After incubation period of 24 hrs, the clear zone was observed around the wells, which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition was observed and measured in mm. Same procedure was carried out for NPs synthesis with bark extract, 50 µL of silver nitrate was added in other well and which was as control. The MIC was done with method of The CH et al., [12]. The lowest concentration at which colour change occurred was taken as the MIC value.

Antibiofilm Activity Determination for the AgNPs

A 96-well microtiter plate (flat bottom, polystyrene) was used to determine the antibiofilm activity of the AgNPs with procedure of Gurunathan S et al., [13] and Barapatre A et al., [14]. The percentage of inhibition of biofilm formation was calculated using following equation:

% Biofilm inhibition=(1–[OD620 of cells treated with AgNPs or plant extracts/OD620 of the non treated control]×100).

DPPH free Radical Scavenging Activity

The percentage of antioxidant activity (AA%) of each substance was assessed by DPPH free radical assay, which was carried out by using Ara N et al., procedure [15]. DPPH is reduced when it reacts with antioxidant compounds that can donate hydrogen. Colour change (from dark purple to light yellow) (absorbance (Abs)) was read at 517 nm after 100 min of reaction using a UV-Vis spectrophotometer.

The percentage was calculated using the following equation:

$$\text{Radical scavenging activity [\%]} = \frac{[\text{Absorbance of Control} - \text{Absorbance of Sample}]}{\text{Absorbance of Control}} \times 100$$

STATISTICAL ANALYSIS

All the observations were tabulated in Microsoft (MS) Excel. The tests were carried out in triplicates. Data were presented as mean±SD.

RESULTS

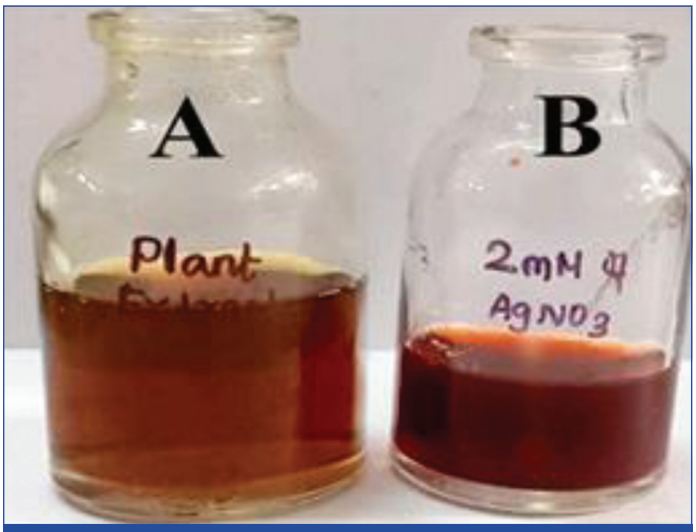
In the present study, the extraction yield was evaluated using methanol and acetone. The findings revealed that maximum yield was obtained with methanol (84.2 mg/5 g); compared with acetone (67.5 mg/5 g). Henceforth the present study demonstrated the maximum extractive value percentile with 1.68% methanol than with 1.35% acetone when compared to other solvent extracts.

The results of the qualitative analysis of phytochemicals of both solvents from bark extract of *Harpullia arborea* is given in [Table/Fig-1]. The results revealed that methanol bark extract of *Harpullia arborea* demonstrated positive reactions for carbohydrates, phenolics, saponins, tannins, steroids, and terpenoids and revealed negative reactions for alkaloids, flavonoids, quinones, and proteins. In case of acetone extract, five phytochemicals of alkaloids, carbohydrates, phenolic compounds, quinones, and proteins were observed.

S. No.	Phytochemicals	Test name	Observation	Methanol	Acetone
1	Alkaloids	Wagner's	Reddish brown colour	-	+
2	Carbohydrates	Molisch's	Purple ring at the junction	+	+
3	Flavonoids	With NaOH	Yellow colour	-	-
4	Phenolic compounds	Ferric chloride	Deep chloride	+	+
5	Saponins	Foam test	Froathing	+	-
6	Tannins	Braymer's test	Greenish colour	+	-
7	Quinones	With HCl	Yellow precipitate	-	+
8	Steroids	Salkowski test	Bluish red	+	-
9	Terpenoids	Salkowski test	Reddish brown	+	-
10	Proteins	Millon's test	Red colour	-	+

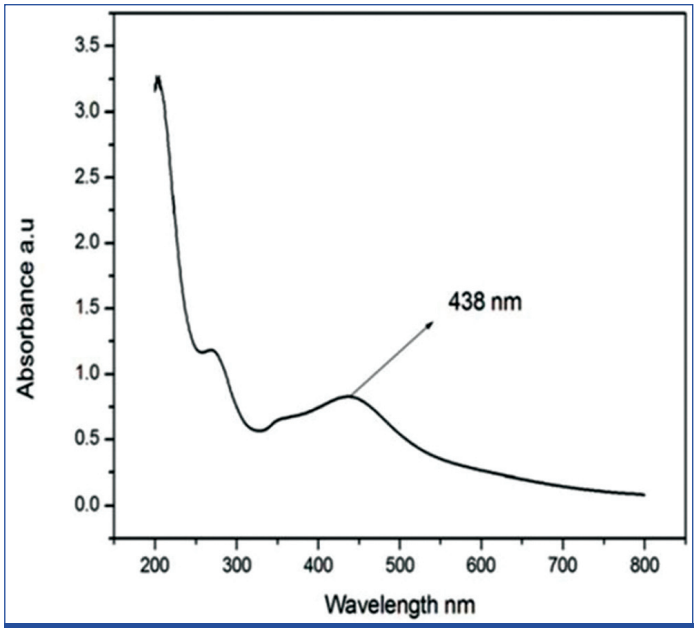
[Table/Fig-1]: Preliminary phytochemicals screening on bark extracts of *Harpullia arborea*.

According to phytochemicals results, methanol extract was subjected to AgNPs synthesis with 2 mM of silver nitrate. The colour change of the colourless $AgNO_3$ solution to a brown suspension of AgNPs, as shown in [Table/Fig-2], is one approach for confirming the creation of NPs. The UV–Vis spectrum of the biosynthesis of AgNPs using the *Harpullia arborea* showed a peak at 438 nm corresponding to the plasmon absorbance of AgNPs for the tested sample [Table/Fig-3].



[Table/Fig-2]: Synthesis of AgNPs and its identification by the colour change; a) Methanol extract of bark of *Harpullia arborea*; b) AgNPs of bark of *Harpullia arborea*.
AgNPs: Silver nanoparticles

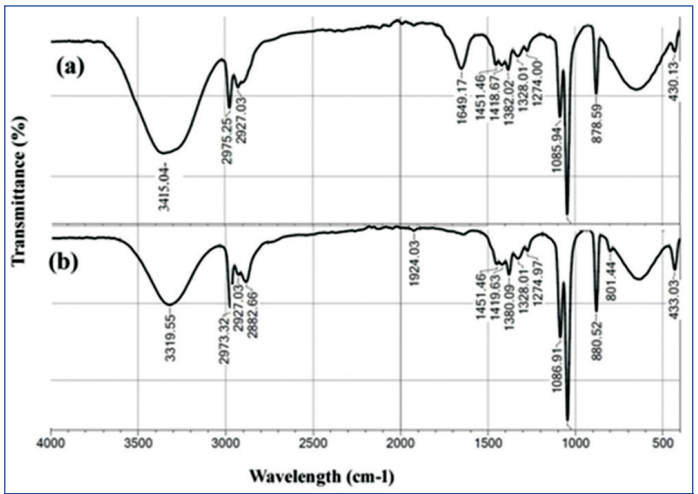
The FTIR analysis was used to determine the organic functional groups like C=O,-OH of the plant extract, which linked to the



[Table/Fig-3]: Characterisation of AgNPs using Ultaviolet-Visible (UV-Vis) spectroscopy.
AgNPs: Silver nanoparticles

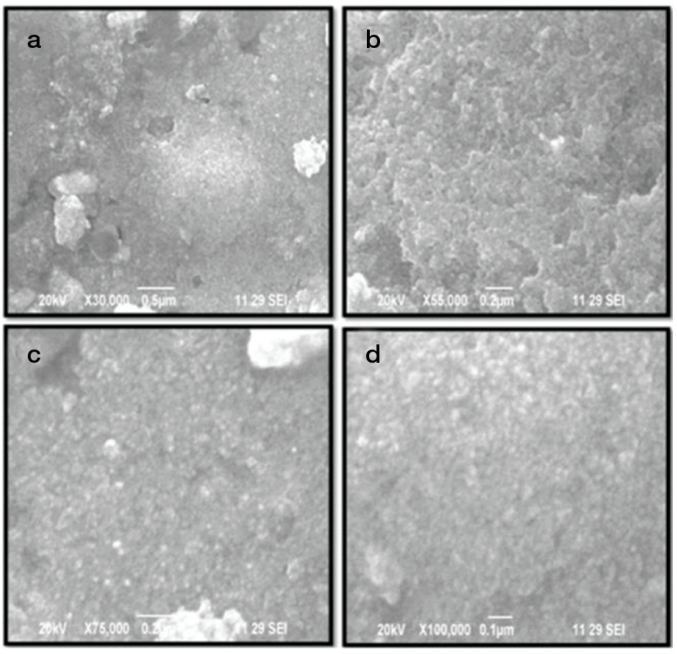
surface of AgNPs [Table/Fig-4a]. The FTIR spectra of the methanol extract of plant *Harpullia arborea* demonstrated an absorption peak at 3415.04 cm^{-1}, which assigned to-OH stretching frequency in phenolic compound and alcohols with strong hydrogen bonds. The absorption bands at around 2975.25, 2927.03 cm^{-1} were related to-CH stretching frequency. The peak at 1649.17 cm^{-1} indicated the presence of stretching vibration of the C=O bond of an ester. Moreover, the absorption bands in the range of 1451.46-1085 cm-1 were related to C-O, C-C stretching vibrations.

The FTIR of synthesised $AgNO_3$ in [Table/Fig-4b] represented a slight difference in the intensity and positions of the peaks. The displacement of peak at 3319.55 cm^{-1} and the disappearance of peak at 1649.17 cm^{-1} might be due to the breakdown of hydrogen bond that plays a vital role in the reduction of silver ions into AgNPs. The SEM and TEM analysis revealed the predominance of spherical nature of synthesised AgNPs and the average size of particle recorded was 30.78 nm. The EDX analysis coupled with both SEM and TEM demonstrated the presence of silver accounting to 30.42% [Table/Fig-5-8].

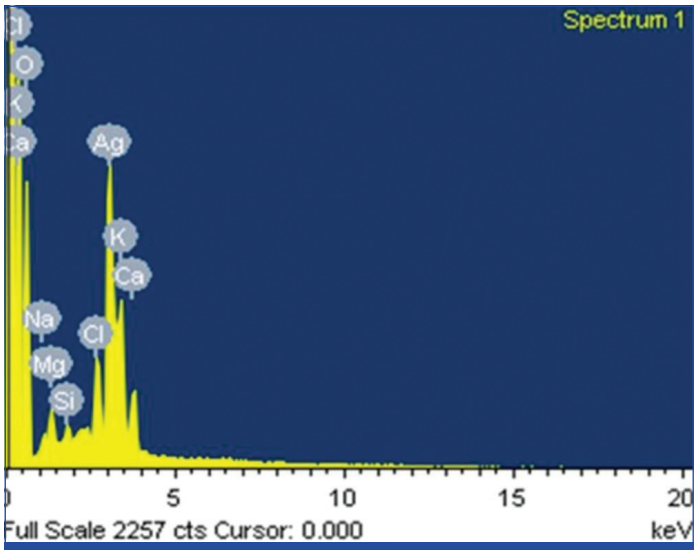


[Table/Fig-4]: FTIR analysis; a) FTIR analysis of *Harpullia arborea*; b) FTIR analysis of plant extract of AgNPs.
AgNPs: Silver nanoparticles; FTIR: Fourier-transform infrared spectroscopy

In the present study, isolates were identified from meat samples, which were grouped under 6 generae. The gram-negative isolates were identified as *Escherichia coli* accounting to 21.43% and *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Proteus*



[Table/Fig-5]: SEM analysis of AgNPs synthesised by *Harpullia arborea*; a) 30000 X magnification, B.55000X magnification; c) 75000X magnification, d) 100000X magnification.
AgNPs: Silver nanoparticles; SEM: Scanning electron microscopy

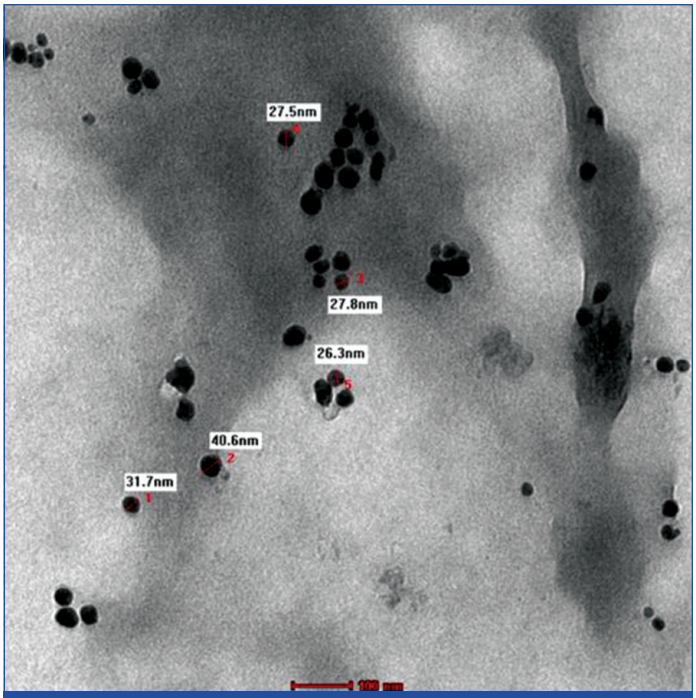


[Table/Fig-6]: SEM EDX analysis of AgNPs synthesised by *Harpullia arborea*.
AgNPs: Silver nanoparticles; SEM: Scanning electron microscopy

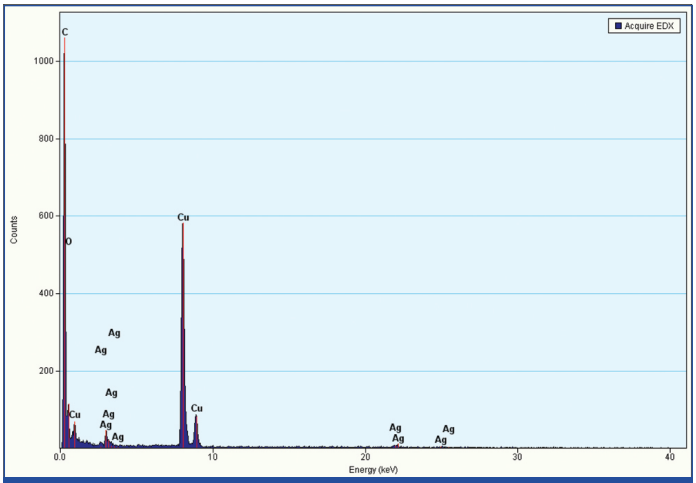
mirabilis each accounting to 14.29%. The gram-positive isolates were identified as *Staphylococcus aureus* and *Enterococcus faecalis* and accounting for 21.43 and 14.29%, respectively.

Presently, methanol bark extract of *Harpullia arborea* was exhibited antibacterial activity against all the test pathogens. Plant extract at 5 and 7.5 mg concentration showed no antibacterial activity for *K. pneumoniae, P. mirabilis,* and *S. aureus*. Among the various concentrations, 12.5 mg was active against all six isolates. The highest zone of inhibition was recorded against *E. coli* (20.6±1.24 mm) with maximum antibacterial activity, whereas least activity was found against *K . pneumoniae* (14±1.63) at 12.5 mg concentration. This methanol bark extract have demonstrated substantial antibacterial activity towards gram negative than gram positive bacteria. Negative control of methanol and positive control antibiotic showed no zone of inhibition activity [Table/Fig-9].

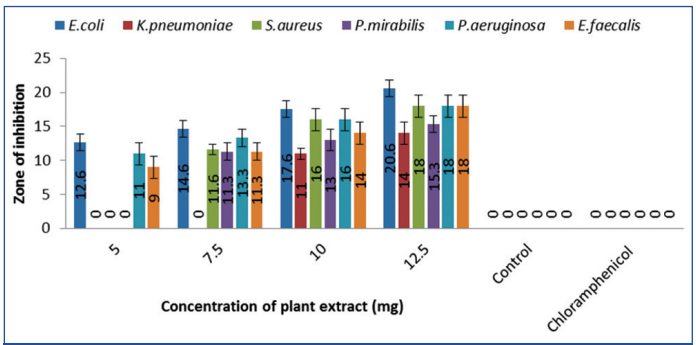
Furthermore methanol extract of *Harpullia arborea* bark was utilised for the silver nanoparticle synthesis. The inhibitory activity was improved while using the AgNPs than non AgNPs. The zone of inhibition ranged from 12.66±2.054 mm to 21.66±1.247 mm. This AgNPs suppressed all bacterial isolates. Among the 6 generae,



[Table/Fig-7]: TEM analysis of AgNPs synthesised by *Harpullia arborea*.
TEM micrographs provided additional insight into the morphology and particles size distribution profile of the green synthesised AgNPs. The analysis of data obtained from TEM micrographs of silver nanoparticles revealed that the particles are spherical and size ranged from 26.3-40.6 nm with an average particle size of 30.78 nm; AgNPs: Silver nanoparticles; TEM: Transmission electron microscopy



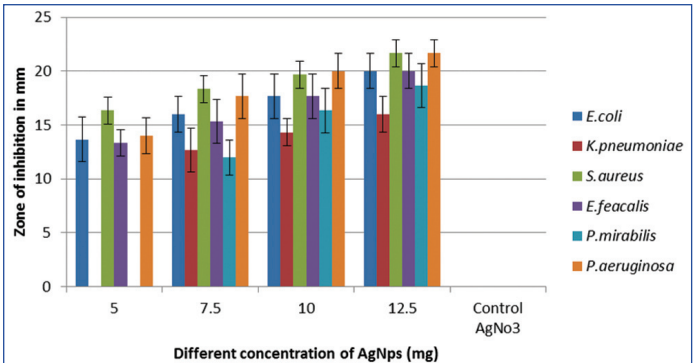
[Table/Fig-8]: TEM EDX analysis of AgNPs synthesised by *Harpullia arborea*.
TEM EDX imaging confirms the accumulation and dissolution of AgNPs and the spectrum demonstrates the presence of silver



[Table/Fig-9]: Graphical representation of antimicrobial activity of methanol bark extract of *Harpullia arborea* against bacterial isolates.

S. aureus was highly suppressed, exhibiting the zone of inhibition ranging from 16.33±1.247 mm to 21.66±1.247 mm [Table/Fig-10].

The MIC also subjected with AgNPs of methanol extract of *Harpullia arborea*. The lowest MIC was observed against *S. aureus* (3.5 mg) followed by *E. feacalis* and *P. aeruginosa* (4.5 mg). In the present study, the in-vitro anti-biofilm activity of AgNPs was evaluated against



[Table/Fig-10]: Graphical representation of antimicrobial activity of AgNPs of *Harpullia arborea* bark against bacterial isolates.

all bacterial isolates. Treatment of *P. aeruginosa* for 24 hrs with AgNPs (7.5 mg/mL) synthesised using *Harpullia arborea* plant extract, reduced biofilm formation by 79.8%, simultaneously while using non AgNPs, 27.6% of biofilm formation was reduced [Table/Fig-11].

S. No.	Isolates	AgNPs-% of inhibition of biofilm formation	Plant-% inhibition of biofilm formation
1	*E. coli*	33	20.8
2	*K. pneumoniae*	47	29
3	*S. aureus*	37	15
4	*E. feacalis*	40.5	15.5
5	*P. mirabilis*	56.5	14.4
6	*P. aeruginosa*	79.8	27.6

[Table/Fig-11]: Antibiofilm activity of nanoparticles of *Harpullia arborea* extract.

In our present study, the DPPH radical scavenging increased with increasing concentration of *H. arborea* bark extracts. The maximum scavenging activity was observed at 5 mg/mL concentration for both ascorbic acid being the highest [85.14±6.52%] followed by methanol [75.14±1.75%]. The IC50 value of ascorbic acid was found to be 2.8 mg/mL while that of the bark extract was 3.6 mg/mL. In case of AgNPs, 78.6±1.75% of scavenging activity was shown and IC50 value was 3.0 mg/mL [Table/Fig-12].

Concentration (mg/mL)	Ascorbic acid (%)	Plant extract (%)	AgNPs of plant extract (%)
1	15±1.14	10.2 ±0.78	12.3±1.62
2	30.42±2.33	21.21±1.62	26.4±1.62
3	55.11±4.2	41.01±1.12	50.0±1.12
4	76.34±5.84	59.13±1.53	66.2±1.12
5	85.14±6.52	75.14±1.75	78.6 ±1.75

[Table/Fig-12]: Antioxidant activity of AgNPs of *Harpullia arborea* and non AgNPs of plant extract.

DISCUSSION

In recent decades, there has been increasing interest in developing new, effective antimicrobial agents to combat infections caused by antibiotic resistant bacteria. Currently, the medical world realises that medicinal products derived from plants are less harmful than synthetic drugs. Numerous studies are now being conducted for this purpose, citing which the present study aims to identify various plant phytochemicals that impart antimicrobial activity.

When plant material is used for medicinal purposes, its extraction method, solvent type used as well as the extraction yield, plays major role. in the present study, methanol solvent extract showed higher yield than acetone extract. This can be attributed due to the increased polarity of methanol than acetone and at elevated extraction temperature, methanol has more dielectric constants than acetone [16].

The results revealed that methanol bark extract of *Harpullia arborea* demonstrated positive reactions for carbohydrates, phenolics, saponins, tannins, steroids, and terpenoids. In case of acetone extract, alkaloids, carbohydrates, phenolic compounds, quinones, and proteins were observed. In the present study, tannins were present in both solvent extracts of *Harpullia arborea*. Tannins are of great importance in human health, and which are considered to be cardio-protective, anti-inflammatory, anticarcinogenic and antimutagenic. Tannins enhance glucose uptake and inhibit adipogenesis, thus being potential drugs for the treatment of non insulin-dependent diabetes mellitus [17].

These medicinally bioactive components exert antimicrobial action through different mechanisms. For several years, there has been great interest in researching natural materials as sources of new antibacterial agents. Many reports indicate that traditional herbs have antimicrobial effects, and plants are one of the foundations for obtaining new principles in modern medicine [18]. Recent developments in nanoscience and nanotechnology have revolutionised the way the evaluators detect, treat, and prevent diseases across the board.

The preparation of NPs can be achieved by three ways namely chemical, physical, and biological methods. The biological methods of NPs synthesis using microorganisms, enzymes, and plant or plant extract have been suggested as possible ecofriendly alternative to chemical and physical methods [19]. With regard to metal NPs, AgNPs are a market leader due to their good properties, including chemical stability, excellent conductivity, catalytic activity, and most importantly which are antimicrobial as well as anti-inflammatory actions [20]. Presently, methanol extract of *Harpullia arborea* bark was utilised for the silver nanoparticle synthesis.

The colour change of the colourless $AgNO_3$ solution to a brown suspension of AgNPs is one approach for confirming the creation of NPs. A change in colour of the mixture indicates the reduction of silver ions [Ag+] to atomic silver [Ag0], which coalesce to form SNPs [21]. The formation of AgNPs was examined by UV-Vis spectrophotometer with surface plasmon resonance at 438 nm. There is an increase in intensity caused by surface plasmon vibration (SPR) in the AgNPs, in agreement with previous statements [22], suggesting reduction of silver nitrate into AgNPs.

In FTIR analysis, the spectral analysis reveals the number of functional biological groups responsible for stabilisation of NPs, which acts as capping or stabilising agents. The displacement of peak at 3319.55 cm^{-1} and the disappearance of peak at 1649.17 cm^{-1} might be due to the breakdown of hydrogen bond that plays a vital role in the reduction of silver ions into AgNPs. As reported in many studies these functional groups have role in capping/stability of AgNPs [23].

The presence of oxygen at higher concentration of 52.46% in the present study might be coming from the chamber of EDX [24]. The characterisation of AgNPs by SEM, TEM, and EDX outline evidenced physically powerful signals for Ag atoms. Both SEM and TEM analyses revealed the predominance of spherical nature of synthesised AgNPs and the average size of particle recorded was 30.78 nm. The results of the present study was in accordance with Khanra K et al., [24], who recorded the prominence of spherical shaped *Scoparia dulcis* extract synthesised AgNPs and a size range between 15-25 nm. The small size of approximately 26.3-40.6 nm in the present study provides the NPs a large surface area, which subsequently increases their catalytic, reactivity, and penetrative properties of microbial membranes that can be utilised in various applications [25]. The different sizes of the nano-particles may be attributed to various shapes and to clumping/aggregation of the smaller particles [26]. Otunola GA et al., [27], have reported a wide range in AgNPs sizes ranging from 5-100 nm and different

morphologies viz., spherical, hexagonal, and rods among different phyto extracts.

The EDX analysis coupled with both SEM and TEM demonstrated the presence of silver accounting to 30.42%. Similar results of 29.38% of elemental silver were reported by Hebeish A et al., [28]. In contrast to present study, Puchalski M et al., [29] have reported higher silver contents (74%) in the examined samples followed by carbon and oxygen, accounting to 21% and 5%, respectively.

In the present study, isolates were identified from meat samples which were grouped under 6 generae. All isolates were confirmed with morphological and cultural characterisation with chromogenic media and selective media. The gram-negative isolates were identified as *E. coli* accounting to 21.43% and *P. aeruginosa, K. pneumoniae,* and *P. mirabilis* each accounting to 14.29%. The gram-positive isolates were identified as *S. aureus* and *E. faecalis* and accounting for 21.43 and 14.29%, respectively. Findings of the present study, corroborate with those of other studies conducted by Ghosh A et al., [30], Adesiji YO et al., [31] and Manguiat LS et al., [32], which equally reported a high prevalence of *E. coli*, *S. aureus*, or both from meat samples.

Multidrug resistance isolates were not to easily eradicate, because may be those were strong for biofilm production. During the formation of biofilm, bacteria are enclosed in an extracellular matrix composed of proteins, polysaccharides, nucleic acid, and lipids. This matrix protects against a variety of stressors, such as antimicrobial exposure and immune cell attack [33]. The worrying prevalence of biofilm producing strains represents a serious challenge to clinicians in the treatment and care of hospitalised patients.

Therefore, researchers urgently need to find a new way to reduce the problem and develop research for new drugs in a natural way. Plants have long been studied as potential sources of new agents. In this way, the objective was to prevent dietary isolation by the bark extract of *Harpullia arborea*.

Several studies have demonstrated the antimicrobial activity of *Harpullia sp*. Khan MR et al., [34] reported antibacterial effect of *H. ramiflora* and their research study concluded that the ethyl acetate fraction of flower exhibited highest activity. According to the research of Khan MR and Omoloso AD [35], solvent fractions such as dichloromethane, ethyl acetate, butanol, and methanol extract of *H. petiolaris* leaves, stem and root barks and hardwoods exhibited antibacterial activity.

Study by Chung PY et al., [36] demonstrated that among the various microbes examined only *S. aureus* was sensitive to bark extract while other bacteria were not inhibited by bark as well as leaf extract of *H. arborea*. Gowri SS et al., [37] findings on the seed extract of *H. arborea* exhibited antimicrobial activity by disk diffusion method. Presently, methanol bark extract of *H. arborea* was exhibited antibacterial activity against all the test pathogens. This methanol bark extract have demonstrated substantial antibacterial activity towards gram negative than gram positive bacteria. The control agent of methanol and antibiotic were not showed zone of inhibition activity.

Furthermore methanol extract of *Harpullia arborea* bark was utilised for the silver nanoparticle synthesis. The inhibitory activity was improved while using the AgNPs than non AgNPs. The zone of inhibition was ranged from 12.66±2.054 mm to 21.66±1.247 mm. The MIC also subjected with AgNPs of methanol extract of *Harpullia arborea*. The lowest MIC was observed against *S.aureus* and followed by *E.feacalis* and *P. aeruginosa*. From the literature survey, this is the first to report the antibacterial activity of AgNPs of bark extract of *Harpullia arborea*. The inhibition activity was based on the dose depending of AgNPs. Previous reports also showed that AgNPs have a concentration-dependent inhibitory effect on a wide range of pathogenic bacteria [38].

There are various mechanisms about the antibacterial activity of AgNPs, for example Sondi I and Sondi BS et al., [39] studied the interaction of AgNPs with *E. coli* and found that AgNPs adhere to the bacterial cell wall during the initial stage of interaction. After stable adhesion, AgNPs penetrate the bacteria and induce cell death by destroying the cell membrane. Another mechanism is that AgNPs bind to the surface of the cell membrane, thereby reduce permeability and respiration.

Current research supports all proposed mechanism, but confirms that highest bactericidal activity involves may be the absorption of AgNPs, not to mention the bactericidal activity available through plant extracts. A substantive finding of this examine is that AgNPs inhibited the biofilm formation of bacterial isolates with control, indicating that inhibitory impact of AgNPs is partly due to the disruption of flagella and interference with the biofilm formation. Presently, the nanoparticle synthesised using *Harpullia arborea* extract was reduced the biofilm formation by 79.8%, same time while using non AgNPs, 27.6% of biofilm formation was reduced.

The adhesion of bacteria to any surface is the first step for the formation of biofilms. The bacterial adhesion can be influenced by a variety of parameters including growth environment, bacterial viability, and material surface properties. In this study; we tested the inhibitory effect of AgNPs on bacterial adherence. Several studies have also indicated that AgNPs can decrease swarming ability and biofilm formation, resulting in diminished pathogenicity [40]. Notably, plant-synthesised AgNPs have additional advantages over unsynthesised AgNPs, as plant metabolites are able to act as capping agents and stabilisers, thereby enhancing the antibacterial activity of AgNPs overall.

Biological activity of NPs is size dependent; small particles are more likely to interact with the cell surface and are more likely to be incorporated into the cytoplasm. Additionally, smaller NPs are more efficient since they have a bigger surface area to interact with microbes or biological components. The small size (26.3-40.6 nm) of our AgNPs is another contributing factor to its antibacterial effect. Similarly Singh P et al., [41] utilised the 15 nm size of AgNPs for inhibition of biofilm. They reported that AgNPs with a particle size of less than 100 nm were showed high anti-biofilm activity. The significant inhibition in biofilm formation was observed at 7.5 mg/mL of AgNPs. Goswami SR et al., [42] also investigated biofilm eradication with AgNPs, and found that 15 mg/mL of AgNPs inhibited biofilm formation by 89% in *S. aureus* and 75% in E. coli. According to the findings here, AgNPs could affect the biofilm formation.

In the present study the DPPH radical scavenging increased with increasing concentration of *H. arborea* bark extracts, which can be attributed to the presence of various phenolic compounds in the plant extracts. Rahman K et al., [43], attributed the antioxidant effect of plant products primarily to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins.

The maximum scavenging activity was observed from AgNPs of plant extract compared than non AgNPs of plant extract. Similar line of result was observed by Bhakya S et al., [44]. They obtained antioxidant activity from AgNPs of plant extract than non nanopartilces of plant extract.

The leaf extract of *H. arborea* was found to scavenge radicals in a dose-dependent manner, with an IC50 value of 27.26 g/mL, according to research by Raghavendra HI et al., [45]. At extract concentrations of 50 µg/mL, scavenging of >50% was observed.

Radicals were scavenged at a rate of 66.66% at 100 g/mL. However, *H. arborea* bark extract scavenged DPPH radicals to a lesser extent than ascorbic acid; nonetheless they were near ascorbic acid values. Therefore, the bark extract of *H. arborea* possesses hydrogen donating potential.

Limitation(s)

This study has some limitations, the pharmacology of compounds derived from medicinal plants was not studied here and the availability of quality plant species is limited to a specific geographical area; therefore, whether this plant is ubiquitous has not been investigated.

CONCLUSION(S)

Hence, based on this study findings it can be concluded that the bark extract of *Harpullia arborea* have multiple advantageous therapeutic properties viz., antimicrobial and antioxidant (preventing the free radical formation and related diseases). The presence of various bioactive phytoconstituents, identified in this study and its synergistic activity would be of greater therapeutic benefits in the management of diseases. Especially prepared NPs can be used as antibacterial agents, due to this application in the medical field; this method excites large volumes of NPs. However further studies should be conducted in isolating and purifying the crude phytochemicals obtained in the study into an active ingredient and a clinical study should be performed in human population thereby further developing into an active pharmaceutical formulation.

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