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Eco-friendly Synthesis of Silver Nanoparticles Characterization, Antibacterial, Antioxidants and Effect of Synthesized Silver Nanoparticles on Seedling Growth from Fruit Extract of *Walsura trifoliata* (A. Juss) Harms A Multipurpose Medicinal Tree Taxon

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Abstract

Biogenic synthesis silver nanoparticles (AgNPs) by the helping of plants sources have become a promising substitute to conventional chemical synthesis method. Due to the wide applications of nanoparticles motivates the essential for synthesizing the nanoparticles. Here, a nontoxic, eco-friendly and cost-effective procedure has been followed for the production of silver nanoparticles (AgNPs) using the aqueous fruit extract of *W. trifoliata*. The greener synthesized silver nanoparticles have been characterized by UV-visible spectroscopy (Nano drop), DLS Zeta potential, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM). The UV-visible absorption spectrum of these synthesized silver nanoparticles (AgNPs) exhibited an absorption peak at around 400 nm. FTIR analysis revealed about different functional groups, DLS and Zeta potential studies showed 2.5 nm and -21.4 mV. By the SEM micrographs exhibited about the shape, size and agglomeration pattern of the synthesized silver nanoparticles. The synthesized silver nanoparticles showed excellent antibacterial activity against selected two-gram positive (*B. subtilis* and *S. aureus*) and two-gram negative (*E. coli* and *K.pneumoniae)* bacteria, antioxidants and seedling growth of ground nut.

***Key words*: *Walsura trifoliata*, Characterization, Antibacterial, Antioxidant, Seedling growth**

For the past few years, metal and metal oxides nanoparticles (NPs) contain attracted a plenty of attention owing to their applications as Nano sensors, catalysts, anti-bacterial, antifungal, scavenging agents [1-2]. And due to their size, shape and agglomeration pattern nanoparticles also used in wide diversity of fields like drug delivery, cancer treatment, waste water treatment, DNA analysis and solar power generation [3]. The nanoparticles of noble metals like gold, silver, palladium, platinum and copper are exuberantly utilised in various industrial and pharmaceutical practices because of their spectacular physiochemical, optical and biological properties [4]. Amidst the different metal nanoparticles, silver nanoparticles (AgNPs) have been extensively utilized for the purpose of diverse technological and medicinal fields. AgNPs have been found highly fruitful for the development of worthwhile drug to the candidate for the treatment of different deceases like diabetes, microbial infection, aging, cancer, inflammation as well as for the targeted drug delivery [5-7]. From the source of the literature, there are plenty physical and chemical approaches reported for the synthesis of nanoparticles (NPs), hence the green synthesis method for the synthesis of nanoparticles (NPs) does not incorporate toxic, expensive of huge chemicals and time consumption, this greener method is environmentally friendly and cost effective [8]. Green synthesis protocols utilize extracts through various parts of plant, microbial cells, and biopolymers. The nanoparticles created are biocompatible and have the right level of efficiency for the purpose for which they were created [9]. Silver is a secure antibacterial metal that is reported to destroy more than 650 pathogenic bacteria, and many researchers are taking part in the synthesis of silver nanoparticles (AgNPs) due to their huge antimicrobial ability [10]. Silver is more toxic to the bacteria but nontoxic to the animal cells in little concentrations [11]. The plant extracts contain diverse types of bio reducing constituents like flavonoids, terpenoids, ketones, aldehydes, proteins, enzymes, and DNA, all of these assist the reduction and precipitation of AgNPs [12]. Due to the marvelous properties towards the different biological activities of AgNPs gained unlimited attention through the researchers across the world. The wide use of silver nanoparticles in different fields of study because of relatively low cost of silver, availability and has excellent known antifungal, antibacterial, antibacterial, anti-cancer properties. This makes silver nanoparticles our choice in this study. Furthermore, it was reported that silver is the most

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used nanoparticle with a production rate of five hundred tonnes per year [13]. Several works reported that the plant extracts utilized for the synthesis of AgNPs i.e. *Callicarpa maingay*i [14], *Terminalia chebula* [15], *Trachyspermum ammi* and *Papaver somniferum* [16], *Bauhinia varigata* [17], *Hevea brasiliensis* [18], *Alovera* [19], and tea leaf [20], *Erythrina abyssinica* [21]. In this work the *Walsura trifoliata* extract was selected as a reducing and capping agent.

The tropical reputed medicinal plant species *Walsura trifoliata* A. Juss.). Harms (Meliaceae) are usually cultivated in the gardens of India for its ornamental and medicinal purposes. The plant is well reputed in traditional system of medicine and utilized by tribal peoples to treat various diseases such as astringent, skin allergies and diarrhoea [22]. It is an evergreen tree distributed widely in the tropical areas of Asia, such as Southern China, India, Malaysia and Indonesia. It grows on dry deciduous forests of 200 to 300 m height. The bark of the plant is reported to possess stimulant, expectorant, emmenagogue and emetic properties. The fruit pulp is used as fish poison. The bark extract of *Walsura trifoliata* showed the activity against pathogenic microorganism.

Collection of the plant material

The *Walsura trifoliata* plant material was collected from the medicinal plant garden-1, department of Botany, Sri Venkateswara University, Tirupati.

MATERIALS AND METHODS

Preparation of plant extract

The freshly collected fruit material of *Walsura trifoliata* was washed with running tap water thrice for the removing of admixture on the material, then washed by double distilled water twice and moisture was removed with the help of tissue paper then the fruit was kept in shade dried 14 days. Then the material made ground in to fine powder with the help of electric mixture. 10 mg of the fruit powder was taken in to 250 ml conical flask, 100 ml of double distilled water added to this and rinse well for 5 min, it was kept on water bath for 60 min. After cooled it was filtered through the Whatman no.1 filter paper and it was kept in to 4°C until the synthesis.

Preparation of the 1 mM Ag $(NO_3)_2$ solution

10 grams of $AgNO_3$ was purchased by Sigma Aldrich, 1 mM Ag $(NO_3)_2$ solution was prepared with the adding of 100 ml of Milli-Q water.

Synthesis of silver nanoparticles

10 ml of aqueous fruit extract was taken into 250 ml conical flask and heated on water bath with 100 ml of silver nitrate with heating between 60-80 °C for 60 min. Colour change from light brown to deep brown indicated formations of silver nanoparticles. Then it was centrifuged at 15,000 rpm for 20 min to remove the presence biological admixture, and it was used for characterization and as well as antibacterial, anti-oxidant activities along with the effect of AgNPs on growth para meters towards ground nut.

Characterization of AgNPs

UV-Vis absorption spectrum of AgPs was using with Nano drop 800nm spectrophotometer. Fourier Transform Infra-Red (FT-IR) spectra of synthesized SNPs were analysed in the range of 4000 to 500 cm1 with an ALPHA interferometer (ECO-ART), Bruker, Ettlingen, Karlsruhe, Germany by KBr pellet method. Crystalline nature of metallic silver nanoparticles were monitored using with an X-ray diffractometer (XRD) from Shimadzu, XRD-6000 equipped with Cu Ka radiation source using Ni as filter at a setting of 30 kV/30 mA. Scanning electron microscopy (SEM) and percentage of silver ions in synthesized sample was done by using FEI Quanta 200 FEG HR-SEM machine equipped with EDAX instrument. Transmission electron microscopy (TEM) analysis was performed with the using HF-3300 advanced 300 kV TEM from Hitachi.

Antimicrobial studies of AgNPs

Antimicrobial studies of fruit extract AgNPs synthesized from *Walsura trifoliata* was analyzed for antimicrobial activity against two-gram positive strains like *Bacillus subtilis* ATCC, *Staphylococcus aureus* ATCC, and two-gram negative bacterial strains like *Escherichia coli* ATCC and *Klebsiella pneumoniae* ATCC. Disc diffusion assay procedure was followed using standard protocol [23]. For this 20 µl of plant extract, Ag $(NO_3)_2$ solutions, SNPs, Streptomycin was applied on each separate Whatman no. 1 filter paper discs (6 mm diameter), allowed to dry before being placed on agar pored Petri plates. Each stain tested triplicate with each extract and incubated at 37°C for 24 hours in incubation chamber. Diameter of the zones was measured with the help of scale and results were tabulated.

DPPH radical scavenging activity

DPPH is a stable free radical that can accept an electron or hydrogen radical and get converted to a stable, diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons or hydrogen atoms taken up. Such a change in the absorbance by this reaction has been extensively adopted to test the capacity of several molecules to act as free radical scavengers. 1 mL of various concentrations of the test compound (25, 50, 75 and 100 µg/mL) was added to a 4 mL of 0.004% (w/v) methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was measured against blank at 517 nm. All tests and analyses were performed with three replicates and the results were averaged. The percent of inhibition (I %) of free radical production from DPPH was calculated by using the following equation. This assay was done according to a slightly modified method of Burits *et al*. [24].

I% = A control – A sample / A blank × 100

H_2O_2 scavenging activity

H_2O_2 free radicals scavenging activity of the different solvent extracts of plant name was determined according to the method of Ruch *et al*. [25]. The H_2O_2 (0.6 ml, 40 mM) solution prepared in phosphate buffer (pH 7.4), was added to the different extracts having the concentrations 25, 50, 75 and 100 µg/mL in 3.4 ml phosphate buffer. In this method also ascorbic acid was used as the standard. The absorbance of the reaction mixture was recorded at 230 nm. The compound without the test compound was used as the control. The reaction mixtures were incubated at 37 °C for 60 min in a water bath. Absorbances were measured at 520 nm. The percent of inhibition (I %) of free radical production from H_2O_2 was calculated by using the following equation:

I% = A control – A sample / A blank × 100

The antioxidant activity is expressed as the 50% inhibitory concentration (IC_{50}).

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by a slightly modified method of Green *et al*. [26]. Nitric oxide

radicals (NO) were generated from sodium nitroprusside. 1 ml of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 µg/mL) of the different extracts of the *plant name* and incubated for 150 min at 25°C and 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthyl ethylene diamine dihydrochloride) was added to the 1 ml of the reaction mixture. The standard antioxidant ascorbic acid was used as the positive control. The absorbance of the resulting reaction mixture was measured at 546 nm. The experiment was conducted in triplicate and mean values were taken as the result. The percent of inhibition (I %) of free radical production from NO was calculated by using the following equation:

I% = A control – A sample / A blank × 100

Growth parameters

Seed germination and seedling growth

Ground nut seeds were procured from Regional Agriculture Research station, S. V. Agricultural College, Tirupati, Andhra Pradesh, India. The seeds were surface sterilized through 0.2% $HgCL_2$ solution for 5 minutes with shaking and washed thoroughly with distilled water. The seeds were pre-soaked in 50 ml of respected treatments up to 12 hours for monitoring the percentage of germination and seeds were shifted and germinated on fluted filter paper towels in bread boxes. The seedlings are now exposed to diverse treatments to observe the seedlings growth.

Seed viability test

The seed viability test was assessed by performing tetrazolium test (TZ) test as per the methods of Eplee and Norris [27]. The good quality seeds were separated from the bulk. 50 seeds were longitudinally bisected and seeds were incubated in 50 ml of 1% (w/v) solution of 235-triphenyl tetrazolium chloride (TTC) prepared in 0.1M Sorensen's buffer (pH7.0) for 24 hours at 28°C. After the incubation, seeds and the embryos were examined. The seeds were in embryos turned reddish pink were scored as viable and seeds that remained no colour were scored as non-viable.

Percentage of seed germination

Seed germination (%) = (Number of germinated seeds / Number of total seeds) × 100

Root and shoot length

Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root- hypocotyl transmission zone up to the base of the cotyledons. The root and shoot length were measured with the help of a thread and scale.

Seedling vigour index

The seedling vigour index was determined by using the formula given by Abdul Baki and Anderson [28].

Seedling vigour index = Average root length in cms + Average shoot length × Germination percentage.

Fresh and dry weight

The fresh weight of root and shoot of seedlings was determined by weigh the root and shoot separately on electric balance. After the fresh weight taken then the seedlings were placed in a hot air oven at 80°C for 48 hours then the weight of dry matter was note downed.

RESULTS AND DISCUSSION

When the 1 mM $Ag(NO_3)_2$ solution was added to aqueous fruit extract of *Walsura trifoiata*, the colour changed from light brown to deep brown which is primary method to confirm that the synthesized nanoparticles are silver (Fig 1). The colour change is because of the reduction of silver ions with the help of bio active molecules present in the sample [29]. Due to NAD and ascorbic acid present in plant parts at higher levels act as strong reducing agents by donating electrons to Ag^+ to form Ag0 nanoparticles [30]. This may be main reason behind the reduction and colour change pattern of silver nanoparticles.

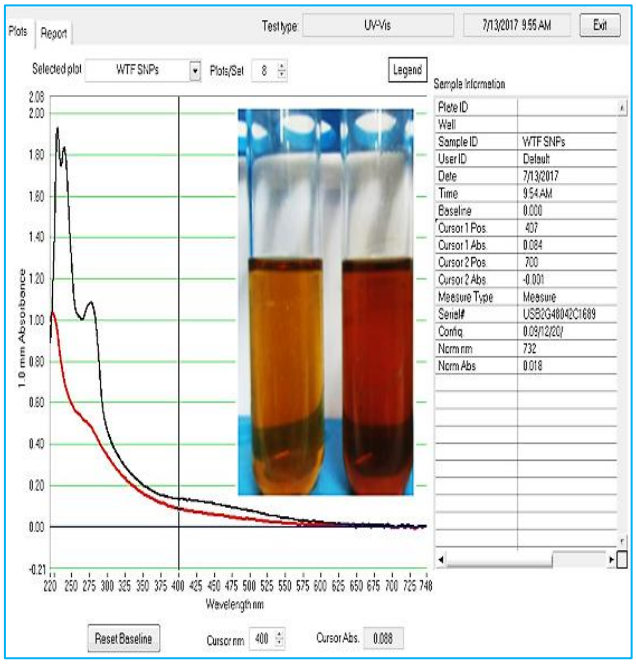


Fig 1 Visible colour change of AgNPs with UV- Vis absorbance peak at 400nm

UV-Vis spectroscopy

In the UV- Vis spectra; a single, strong and broad surface Plasmon resonance (SPR) peak of AgNPs was observed in the range between 190 to 750 nm with the helping of Nano drop. The spectrum displays the crucial role of $AgNO_3$ along with the presence of plant metabolites in the formation of AgNPs. The absorption peak was obtained at 400 nm, which further confirm the reduction nanoparticles are silver.

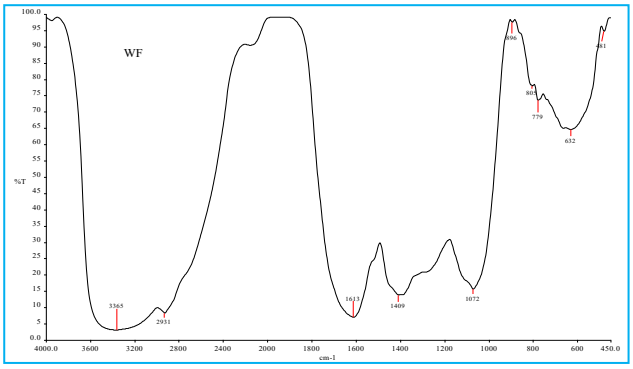


Fig 3 *Walsura trifoliata* FT-IR spectrum peaks in AgNPs mixture

FT-IR (Fourier transform infrared spectroscopy)

FT-IR spectrum of silver nanoparticles carried out to identify the possible bio-molecules responsible for the capping and stabilization of nanoparticles. For this both extract and AgNPs samples were analyzed in the IR spectra range from

4000 to 500 cm^{-1} by the FT-IR. The AgNPs of the fruit exhibited diverse peaks at 3365, 2931, 1613, 1409, 1072, 896, 805, 779, 632 and 481. O-H bond of Alcohols/Phenols, C-H stretching bond Polyphenols, C=C conjugated, -SCN Thiocyanate, N-H bend of Primary amines, O-H alcohol, C=C strong bending alkane group, C-Br halogen (Bromo compound), and -s-s stretch poly sulphides. Many green silver nanoparticles studies have utilized FTIR as a technique for analyzing the structure of AgNPs (Fig 3).

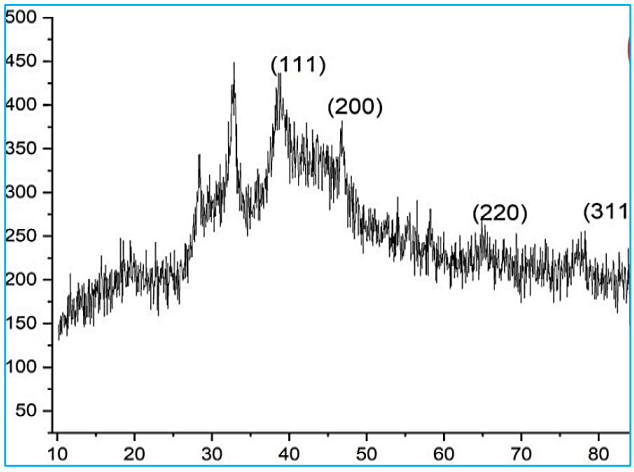


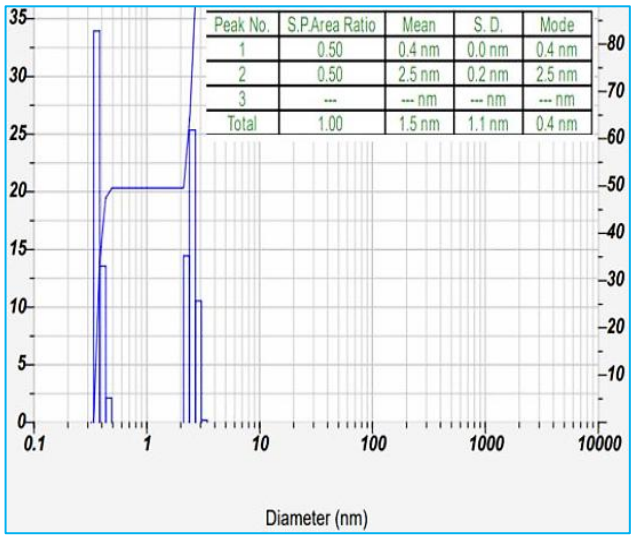
Fig 4 XRD pattern of AgNPs shows Bragg reflection

XRD analysis

The crystalline nature and size of the synthesized AgNPs were studied by XRD. AgNPs of *Walsura trifoliata* exhibited different peaks in XRD at 2θ = at 38.60°, 47.40°, 66.20° and 78.80° of 2θ of X-axis which corresponds to 111, 200, 220 and 311 'hkl' integer plans respectively. These respective XRD peaks confirmed that biosynthesized AgNPs nanocrystal and crystalline in structure. The peaks seen in XRD can be attributed to the planes (122), (111), (200) and (311) facet of silver crystal respectively [31]. This was carried out using with the Shimadzu XRD- 6000/6100 model with 30 kV, 30 mA with Cuk α radians at 2θ angle. This was carried out using Shimadzu XRD-6000/6100 model with 30 kV, 30 mA with Cuk radians at 2h angle (Fig 4).

DLS particle size and Zeta potential

Dynamic light scattering technique was performed to measure the particle size and zeta potential value of the biosynthesized AgNPs. The stability of biosynthesized AgNPs depends on both size and surface charge (Zeta potential value) present on the AgNPs. The data procured through DLS measurements divulged that the particle size of AgNPs was 2.5 nm, with the Zeta potential value of was found to be -21.4 mV**,** which is accordance with previous results. The high negative value of AgNPs supports their good colloidal nature, high dispersity, and long-term stability because of negative-to-negative repulsion (Fig 2).



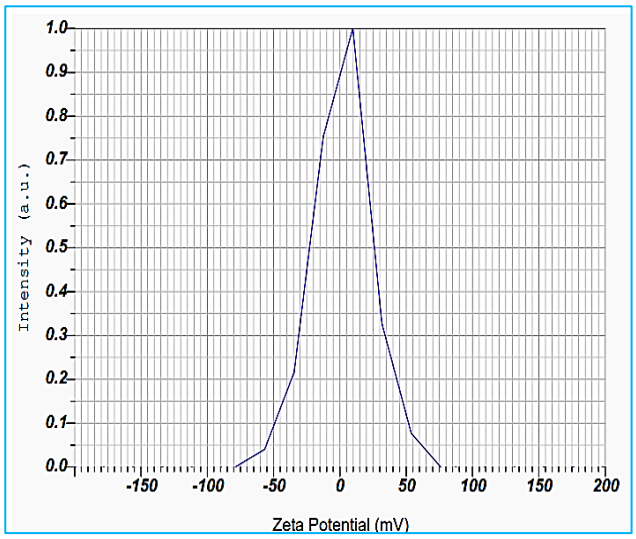
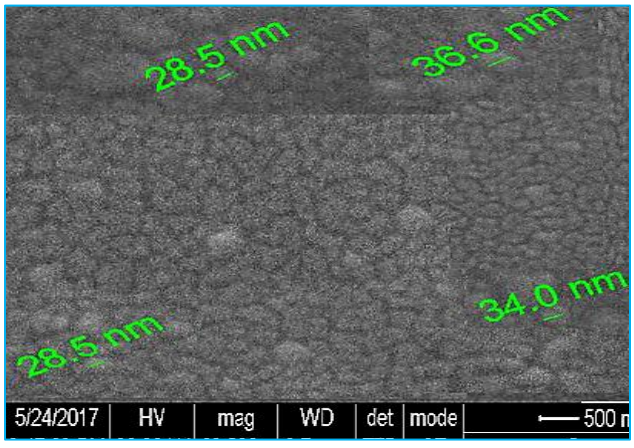


Fig 2 *Walsura trifoliata*- Fruit pulp AgNPs (a). Zeta potential (b). particle size

SEM image at 500nm

EDAX



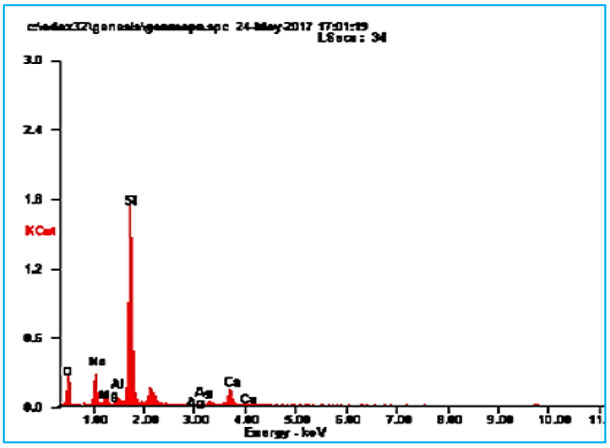


Fig 5 SEM images: (a) and (b) average size of the AgNPs 31.9nm from Walsura trifoliata fruit pulp and EDAX analysis in the weight percentage of silver in mixture

SEM (Scanning electron microscopy)

Scanning electron microscopy was used to determine the size and morphology of the synthesized AgNPs. Structural analysis completed by SEM exhibited the presence of phenolic compounds, amino acids, waxes, antioxidants vitamins, and flavonoids. Observation revealed that the particles are of spherical in shape, the particle size range of 28.5 nm and the average size is 36.6 nm displayed at the 500 nm scale bar. EDAX analysis of synthesized AgNPs exhibited 0.030 weight percentage of Ag metal along with 28.55% of potassium, 10.35% of sodium, 3.09% of magnesium, 1.46% of aluminium and 49.62% of Silicon and this was furtherly confirmed presence of silver nanoparticles in the reaction mixture and also presence of carbon suggesting that the silver nanoparticles must be capped by the organic compounds present in the plant extract (Fig 5). SEM is a surface imaging approach capable of discerning varied particle size, size distributions, Nano material forms, and surface morphology of manufactured particles at the micro and nanoscales, among other electron microscopy techniques [32].

Antibacterial activity

Disc diffusion method was carried out for the evaluate the bacterial potentials of the bioactive ingredients of *W. trifoliata* fruit AgNPs. Biologically synthesized nanoparticles found to possess better activity than Ag $(NO_3)_2$ solution and the plant aqueous extract against two gram negative (*E. coli* and *K. pneumoniae*) and two-gram positive bacteria (*B. subtilis*, *S. aureus*) and streptomycin used as standard. The diameter of inhibition zone around each disc measured and each disc have 20 µl of AgNPs solution. Among the two kinds of bacteria gram-negative bacteria zone of inhibition showed higher due to containing thick layer of peptide glycan's (together with polypeptide contains proteins). Because of this penetration of AgNPs through cell membrane is not easy in case of inhibition growth not possible than gram negative strains it leads to cell death. Thus, by comparative analysis, it is clear that AgNPs produced by *W. trifoliata* possess an excellent bacterial activity against selected bacteria. Based on these results, one can conclude that the biologically synthesized AgNPs showed highest antibacterial activity against gram negative bacteria specially *E. coli* and *K. pneumoniae*. The results were correlated with the previous reports (Fig 6-7, Table 1).

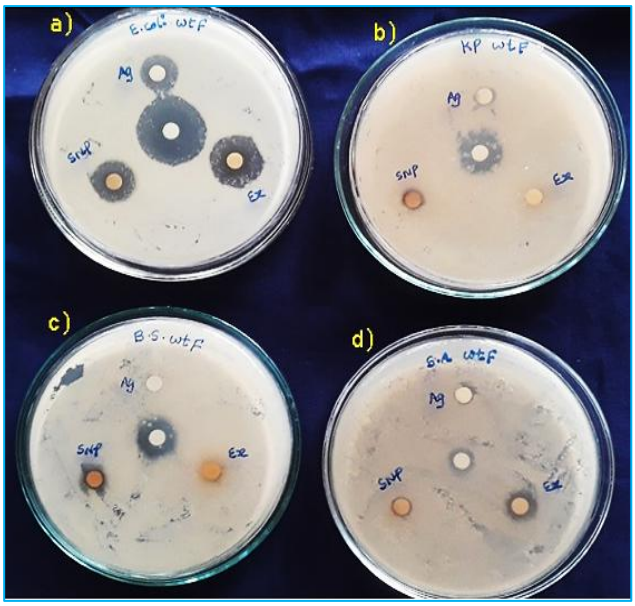


Fig 6 Antibacterial studies of AgNPs synthesized from fruit pulp extract of *Walsura trifoliata*

a) *E. coli* b) *K. pneumonia* c) *B. subtilis* d) *S. aureus*

Table 1 Comparison table of antibacterial activity of *Walsura trifoliata* fruit pulp

		Zone of inhibition in mm			
S. No.	Name of the organism	Plant extract	$Ag(NO_3)_2$ Solution	Ag NPs	Streptomycin
1.	*Bacillus subtilis*	8.3 ± 0.03	6.8 ± 0.12	12.2 ± 0.11	19.8 ± 0.06
2.	*Staphylococcus aureus*	8.6 ± 0.12	8.0 ± 0.06	15.0 ± 0.08	20.1 ± 0.13
3.	*Escherichia coli*	10.1 ± 0.11	6.3 ± 0.08	15.2 ± 1.25	20.6 ± 0.06
4.	*Klebsiella pneumoniae*	8.9 ± 0.06	12.8 ± 0.05	17.1 ± 0.12	21.8 ± 0.03

'±' indicates standard error

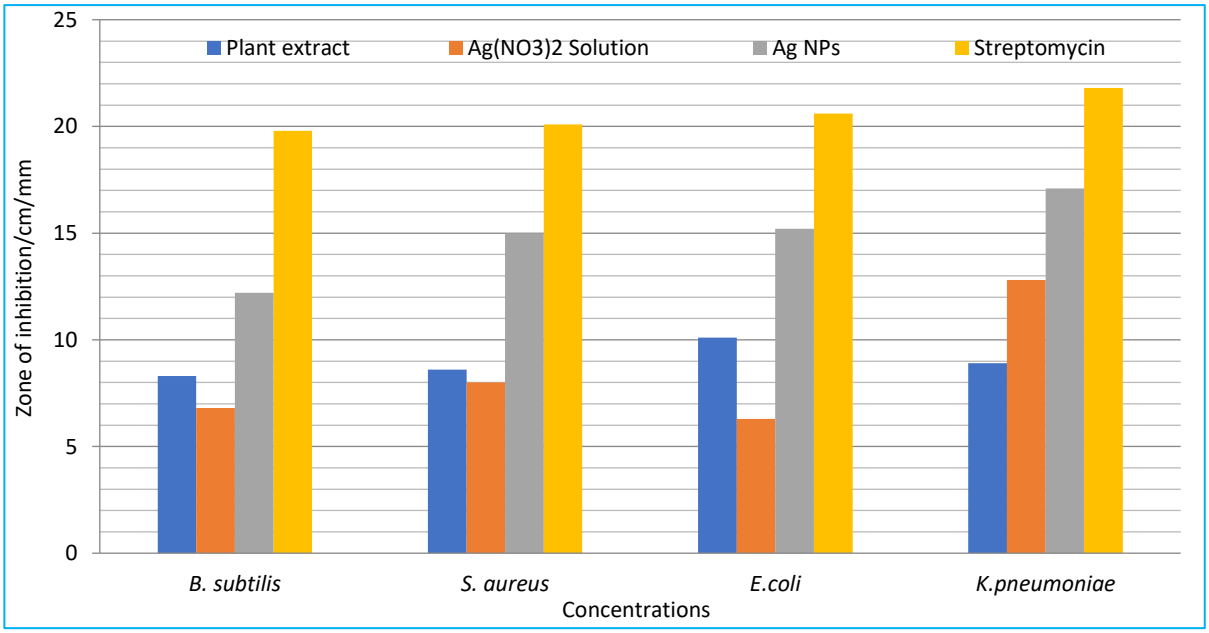


Fig 7 Antimicrobial activity of AgNPs from *Walsura trifolita* fruit pulp

Table 2 Comparison tables of DPPH, H_2O_2, and Nitric oxide scavenging activities of AgNPS s from fruit pulp of *W. trifoliata*

DPPH activity					
Compounds	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	IC50
WF-Ag	57.1	60.4	66.2	85	21.8
WF-Ex	35.8	63.6	72	97.08	39.3
Vitamin-C	48.4	56.5	72.8	86.6	20.6
H_2O_2 Scavenging activity					
WF-Ag	38.5	47.9	58.2	62.3	52.1
WF-Ex	25.5	38.8	49.2	57.6	76.2
Vitamin-C	44.7	52.2	70.5	80.4	47.8
Nitric oxide Scavenging activity					
WF-Ag	46.2	53.8	61.3	68.7	46.4
WF-Ex	27.3	49.8	58.2	66.2	50.2
Vitamin-C	46.8	53.4	74.2	83.5	46.8

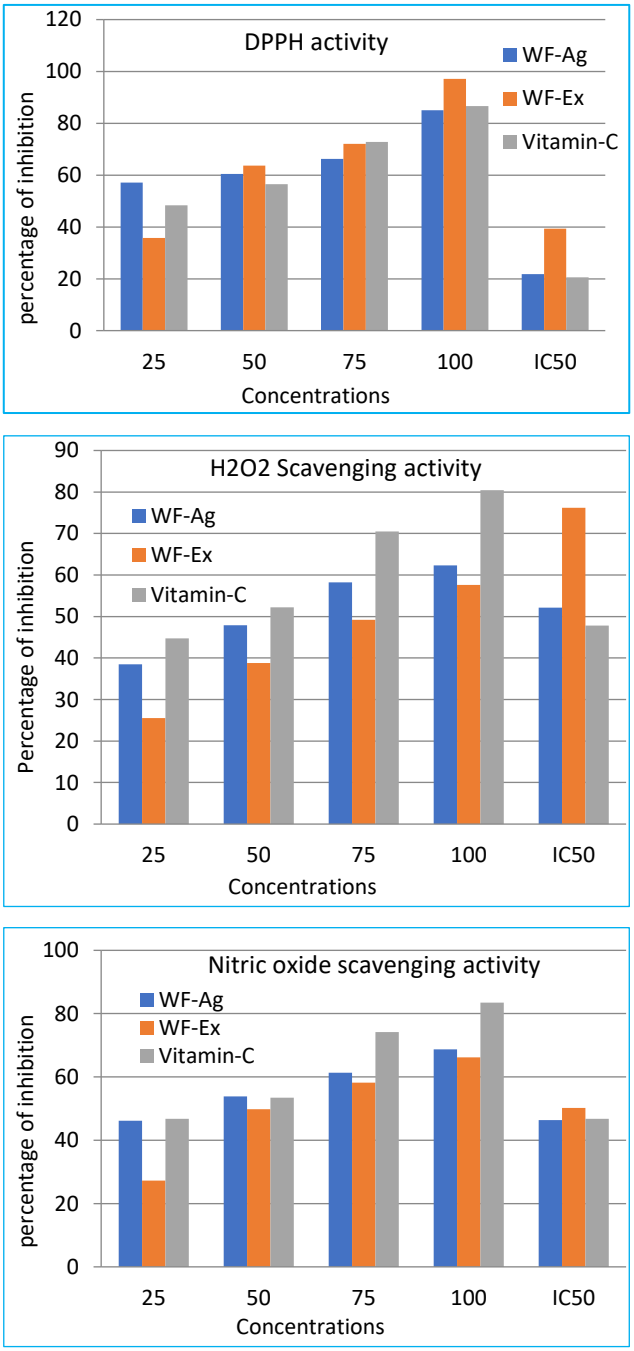


Fig 8 Graph of DPPH, H_2O_2, and NO- activity (µg/ml) by synthesized AgNPs synthesized from fruit pulp of *W. trifoliata*

Antioxidant activity

Anti-Oxidant activity of AgNPs was evaluated by DPPH (2, 2-Diphenyl-1-picry Hydrazyl) assay. Among the activity exhibited concentration dependent scavenging activity against DPPH scavenging. In which concentration of AgNPs was increased from 25-100 µg/ml. Following 30 minutes incubation at room temperature, we noticed the absorbance against blank at 517 nm. The inhibition rate (%) of radical generation by DPPH was calculated through the following assimilation. The assay was executed triplicates. Therefore, the activity was showed by increased from 57.1µg/ml to 85µg/ml. IC_{50} of AgNPs against DPPH was found to be 21.8 µg/ml, this inhibition percentage and IC_{50} values clearly indicated that AgNPs are efficiently exhibited antioxidants activity. The activity of antioxidants was exhibited because of high amount flavonoids, polyphenols, proteins, and tannins presence and signed in the bio reduction as well as stabilization of AgNPs Abdel-Aziz *et al.* [33]. The disproportion between antioxidants and oxidative system results in the manufacturing of oxidative stress. Oxidative stress is accompanying with diverse disorders including hypertension, atherosclerosis, cardiovascular, neurodegenerative disorders, diabetes, Cancer and aging [34] (Table 2, Fig 8).

$$RSA\ (\%) = [(' AC' - ' AS') / (' AS')] \times 100$$

Where 'AC' stands for the absorbance of the control reaction and 'AS' stands for the absorbance of the test compound.

H_2O_2 scavenging activity

The hydrogen peroxide scavenging activity evaluation showed that excellent free radical scavenging activity was observed through the plant extract and AgNPs. The highest radical scavenging activity was exhibited at 62.3% at 100 µg/mL concentrations by the biologically synthesized AgNPs and fruit extract showed 57.6% at 100 µg/mL. The lowest activity was observed with AgNPs 38.5 at 25 µg/mL and 25.5 at 25 µg/mL in aqueous fruit extract of the selected plant. In this case the scavenging activity was increased when the concentrations increasing (Table 2, Fig 8).

Nitric oxide scavenging activity

Nitric oxide scavenging activity biosynthesized AgNPs and extract of the multipurpose plant *Walsura trifoliata* detected the highest scavenging activity of 68.7% at 100 µg/mL and 66.2% at 100 µg/mL concentrations. The observed results divulged that from lower (25 µg/mL) to higher concentrations (100 µg/mL) showed excellent scavenging activity, where AgNPs expressed higher activity than the aqueous extract (Table 2, Fig 8).

Seed germination and seedling growth

Dharani ground nut seeds treated with 1mM Ag $(NO_3)_2$ solution, 5mM $CuSO_4$ solution, plant extract and various

concentrations (1ppm, 10 ppm, 20 ppm, 40 ppm, 50 ppm and 100 ppm) of the both kind of biosynthesized Silver nanoparticles (AgNPs) using with fruit pulp of the *W. trifoliata* showed remarkable outperformed on seedling growth when compared to control (Water) in terms of root length, shoot length, wet weight, dry weight and vigour index. Seedling growth was increased from lower concentrations to higher concentration. The highest root length was observed 16.9 ± 0.15 cm at 50ppm and lowest root length was 12.1 ± 0.06 cm at 1ppm, while the control showed 8.7±0.14cm, with silver nitrate root length is about 12.5 ± 0.06cm, and the plant extract was showed 9.6 ± 0.08cm. The highest Shoot length recorded 10.2 ± 0.6 cm at 40ppm, control was showed lesser growth 6.1 ± 0.08cm, which is higher than the plant extract (5.0 ± 0.0.05 cm) and $Ag(NO_3)_2$ (5.3 ± 0.03cm). The Leaf length of control revealed as 0.8 ± 0.06cm, which is lesser leaf length was recorded when compared with the fruit pulp assisted AgNPs 1.3 ± 0.12cm at 40ppm; which indicated slightly less growth than plant extract (1.1 ± 0.03), and little higher growth was seen when compared to 1mM silver nitrate solution. The Leaf width was 0.9 ± 0.05cm through control, 0.8 ± 0.06 cm by selected plant extract, 0.4±0.03 cm with silver nitrate respectively, but plant material assisted AgNPs exhibited higher growth 1.1 ± 0.06 cm at 40ppm. The seedling wet weight exhibited 1.11 ± 0.01cm with water, 1.07 ± 0.01 cm through plant extract, 1.06 ± 0.02 cm with silver nitrate, and 1.93 ± 0.015 at 40ppm of AgNPs. The highest seedling dry weight was observed as 0.53±0.014 at 40ppm which is higher than the control (0.32 ± 0.014), Plant extract (0.37 ± 0.012), and 1mM silver nitrate (0.46 ± 0.006) (Table 3, Fig 9-10).

Fig 9 Seedling growth of ground nut using with AgNPs synthesized from fruit pulp of *Walsura trifoliata*

Table 3 Comparison of Seedling growth of Dharani ground nut variety by using AgNPs synthesized from *Walsura trifoliata* fruit pulp

Plant part		Concentrations	Root length	Shoot length	Leaf length	Leaf width	Seedling wet weight	Seedling dry weight
		Control (Water)	8.7±0.14	6.1±0.08	0.8±0.06	0.9±0.05	1.11±0.01	0.32±0.014
		Plant extract	9.6±0.08	5.0±0.0.05	1.1±0.03	0.8±0.06	1.07±0.01	0.37±0.012
		1mM $Ag(NO_3)_2$	12.5±0.06	5.3±0.03	0.5±0.08	0.4±0.03	1.06±0.02	0.46±0.006
	Ag	1 ppm	12.1±0.06	6.8±0.12	0.9±0.17	1.0±0.08	1.71±0.011	0.46±0.016
Fruit	NPs	10 ppm	13.8±0.06	7.3±0.05	0.8±0.06	0.7±0.11	1.73±0.016	0.51±0.013
		20 ppm	14.6±0.12	7.8±0.17	1.1±0.11	1.0±0.15	1.85±0.015	0.50±0.023
		40 ppm	16.8±0.06	10.2±0.6	1.3±0.12	1.1±0.06	1.93±0.015	0.53±0.014
		50 ppm	16.9±0.15	10.0±0.11	1.1±0.08	0.8±0.05	1.76±0.016	0.47±0.012
		100 ppm	11.8±0.06	8.5±0.13	0.9±0.16	0.7±0.03	1.67±0.014	0.45±0.021

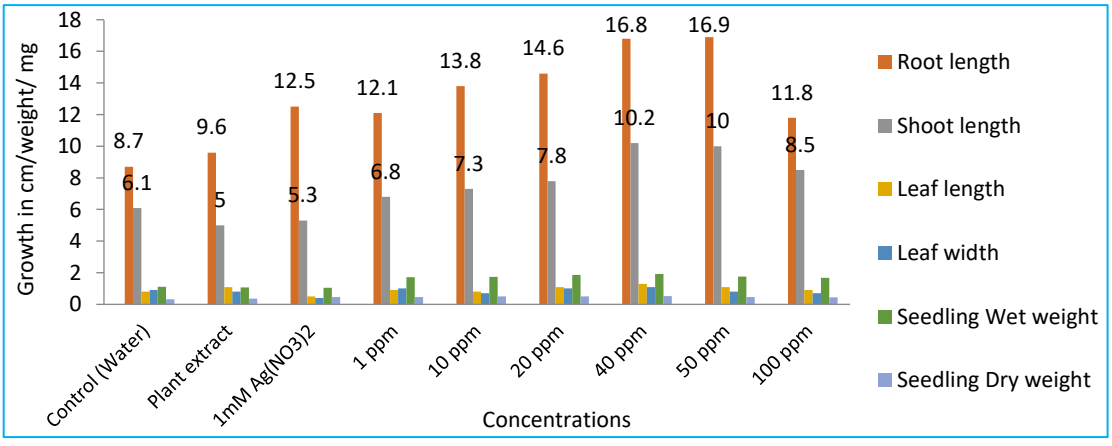


Fig 10 In-vitro study on Dharani ground nut by using AgNPs synthesized from fruit pulp of *Walsura trifoliata*

CONCLUSION

In this study, we present a ground-breaking, cost-effective, and environmentally friendly approach for synthesizing biogenic stable silver nanoparticles using an aqueous extract of *Walsura trifoliata*, a revered traditional medicinal plant, as the reducing agent. This innovative method not only underscores the potential of harnessing natural resources for nanoparticle production but also reflects a commitment to sustainable practices in scientific research. The resulting silver nanoparticles possess remarkable properties that can be harnessed for various applications, particularly in medicine and agriculture, promising to revolutionize how we approach these fields. The colour change pattern and surface Plasmon resonance (SPR) spectra UV- Vis data (400 nm) confirm the presence of silver nanoparticles in the sample. Alcohols/Phenols, Polyphenols, SCN Thiocyanate, Primary amines, poly sulphides, and C-Br halogen are mainly responsible for reduction and stabilization of these AgNPs recorded by FT-IR. The different intense XRD peaks confirmed that biosynthesized AgNPs nanocrystal and crystalline in structure. The DLS zeta potential study revealed about stability, dissemination and aggregation levels of bio-synthesized nanoparticles and the size of the nanoparticles around 2.5 nm evenly distributed and -21.4 mV zeta potential values. The SEM micrograph effectively revealed the presence of small-sized nanoparticles, measuring 31.9 nm, when observed at a high resolution of 500 nm magnification. This finding provides valuable insights into the structural characteristics of these nanoparticles. The TEM analysis confidently identified spherical-shaped nanoparticles ranging from 29.08 nm to 29.80 nm at a resolution of 50 nm, with no evidence of agglomeration. The exceptional resolution and magnification capabilities of TEM allowed for the observation of nanoparticles below 100nm. The average size of the nanoparticles exhibited 29.44 nm. Additionally, TEM is widely regarded as a superior tool compared to SEM and other microscopy techniques. All above microscopic studies reveal that the nanoparticles small size, well dispersed without any agglomeration. The synthesized silver nanoparticles (SNPs) from *W. trifoliata* demonstrate outstanding antibacterial activity against both gram-positive and gram-negative bacteria; and significant antioxidant activity. The biologically synthesized AgNPs are showed excellent seed germination than control (Water). These biogenic nanoparticles are not only eco-friendly but also serve as powerful antimicrobial agents. Moreover, they exhibit superior quality while requiring only a minimal quantity of plant extract for production. This plant is esteemed in traditional medicine, renowned for its potent healing properties. Tribal communities have long utilized it to address a wide range of ailments, including various skin allergies, where its soothing characteristics provide relief. Additionally, it serves as an effective astringent, helping to tighten tissues and reduce secretion, while also being relied upon to manage diarrhea, showcasing its versatility and significance in natural healthcare practices. The fruit pulp of the selected plant *W. trifoliata* is used as fish poison.

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Author contribution

Practical design and manuscript preparation were supervised by my research supervisor Prof. N. Savithramma, Department of Botany, Sri Venkateswara University, Tirupati. The first author, K. Venkata Subbaiah, conducted field and laboratory work assisted by Prof. N. Savithramma throughout the research.

Conflict of Interest statement

The authors declared that they have no interest of conflicts.

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