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Production of Biosynthesized Gold Nanoparticles from Stem Bark of *Walsura trifoliata* (A. Juss) Harms Characterization and Validation

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A B S T R A C T

The exigency to promulgate the rapid synthesis, non-hazardous, cost- effective and eco-friendly green method for the synthesis of nanoparticles utilizing the plants of great significance. This is a result for alternative method of high toxicity associated with the chemical procedure of synthesizing nanoparticles. The aim of the study was to monitor the potentiality of green synthesized AuNPs on microbial cell proliferation. Green method benign AuNPs were characterized by UV-visible Spectrophotometer, Dynamic Light Scattering (DLS), Zeta Potential and the size, shape and morphology of the AuNPs analysed with the Transmission Electron Microscopy (TEM). Maximum phytochemicals were found with the help of polar and the non- polar solvents in the selected stem bark of *Wasura trifoliata*. The synthesized AuNPs showed good antibacterial activity on selected two gram positive bacteria like Bacillus *subtilis*, *Staphylococcus aureus* and two gram negative bacteria like *Escherichia coli, Klebsiella pneumoniae*. The green synthesized AuNPs should helpful to develop new drug alternative to conventional in pharmaceuticals, pharmacognosy to diagnose various diseases.

Introduction

Indian flora is the suzerain and inexpensive source of medicinal plants and plant products. Medicinal plants were utilized from many decades in the Ayurveda broadly. Plants obtaining the significance due to their unique bio-active constituents and multi faced applicability in the diverse fields of research and development.

Recently, metallic nanoparticles have received much attention because of their distinctive optical, magnetic, biotechnological, chemical and biological sensors and catalytic properties. The size, shape, monodispersity, and morphology of the particles are essential to tune these properties (Suganthy *et al.*, 2017). Biomedical applications using gold nanoparticles (GNPs) have become a very active research area in recent years (Daniel and Astruc, 2004; Mieszawska *et al.,* 2013). Use of biological organisms such as microorganisms, pant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner (Bhattacharya and Rajinder, 2005; Mohanpuria *et al.*, 2008). Phytochemical constituents in the plants and species extract like essential oils (terpenes, eugenols etc.), poly phenols and carbohydrates compounds contain active functional groups, such as hydroxyl, aldehyde and carboxyl units which may play important role for reduction of $HAuCl_4$ to AuNPs. Gold nanoparticles produced by using phytochemicals or other extract component remain stable for certain time (Chandra *et al.,* 2011).

In recent years, several plants have been successfully used and reported for efficient and rapid extracellular synthesis of silver, copper and gold nanoparticles such as both extracts of *Neem* (Shivshankar *et al.*, 2004), *Aloe vera* (Prathap *et al.*, 2006), *Avenasativa* (Armendariz *et al.,* 2004), Wheat (Armendariz *et al.,* 2004), *Alfalfa* (Gardea-Torresdey *et al.,* 2002), Geranium (Shankar *et al.,* 2003) and Lemongrass (Shivshankar *et al.*, 2005). The antimicrobial properties of biomolecules present in the plant species extract have facilitated excellent stability of the nanoparticles (Ankamwar *et al.,* 2005). Chemical methods are the most popular approach for the synthesis of metallic nanoparticles (Thakkar *et al.*, 2010).

These methods are based on the reaction of metallic ion solutions with traditional reducing and capping agents such as sodium borohydride, sodium citrate and sodium dodecyl sulphate. Most of these materials are toxic and their use is a threat to human health. In addition, since a small amount of these reagents remaining in the reaction mixture free and non-reactive, the entrance of these materials into the environment leads to environmental pollution (Noruzi *et al.*, 2011). It is difficult to use these nanoparticles in biology and medicine (Shankar *et al.*, 2004). Moreover, the shape and morphology of the metallic nanoparticles affect their properties (Kelly *et al.,* 2003), especially in biomedical applications. It is well known that metallic nanoparticles produce by chemical methods are mainly spherical in shape (Murphy *et al.,* 2008), which limits their applications. For these reasons, in recent years there has been a growing need to develop alternatives to chemical approaches (Shankar *et al.,* 2004). Biological methods (particularly using plants' extracts) which are eco-friendly, cost-effective, rapid and single step process are considered more attention for solving mentioned problems (Hoshyar *et al.*, 2016). Applications of AuNPs important biological mechanisms including drug delivery, cancer diagnosis and treatment due to their unique chemical and physical features (Awwad *et al.*, 2013). Various plant parts (roots, stems, bark, leaves and petals) can be exploited as capping and stabilizing agents in the green synthesis of AuNPs (Babu *et al.*, 2011; Huang *et al.*, 2007). In the present study we have developed the eco-friendly, non-toxic benign AuNPs by green method with using of *Walsura trifoliata* stem bark. The bark of woody vascular plants plays important roles in plant protection because of their content of bio-active compounds with antimicrobial effects (Alfredsen *et al.,* 2008). In recent study, it was found that bark extracts may be a good source of reducing agents in synthesized metallic NPs (Tanase *et al.*, 2019) due to plants contains bioactive compounds.

Walsura trifoliata (A. Juss.) Harms. Belongs to the family Meliaceae (Syn: *Walsura piscidia* Roxb., *Heynea trifoliata* A. Juss). It is an evergreen tree distributed widely in the tropical areas of Asia, such as Southern China, India, Malaysia and Indonesia (Chetty *et al.,* 2008). It grows on dry deciduous forests of 200 to 300 m height. This plant is well reputed in traditional system of medicine and used by tribal peoples to treat various diseases like skin allergies, astringent and diaoerrhea (Pullaiah and Rani, 1999). The bark of the plant is reported to possess stimulant, expectorant, emmenagogue and emetic properties. The fruit pulp is used as fish poison (Anonymous, 1976). The bark extract of *Walsura trifoliate* showed the activity against pathogenic microorganism (Sri Rama Murthy and Nagamani, 2008).

Collection of the Plant Material

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

Materials and Methods

Preparation of Plant Extract

The freshly collected stem bark 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 stem bark was kept in shade dried two to three weeks. Then the material made ground in to fine powder with the help of electric mixture. 10 mg of the stem bark 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 what man no.1 filter paper and it was kept in to 4 ^{0} C until the synthesis.

Preparation of $HAuCl_4$ solution

Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$) obtained from Sigma Aldrich. Then 1 mM of $HAuCl_4.3H_2O$ solution was prepared with Double Distilled water.

Bio-Synthesis of AuNPs

Theprocedure (Banerjee *et al.*, 2014) was acquired in the synthesis of gold nanoparticles. 10 ml of obtained bark extract taken in to 250 sterile conical flask and added to this 100 ml of 1 mMTetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$) solution stirred accordingly at 60-80^{0}C temperature until the brown colour turns in to purple colour that confirms the synthesis of AuNPs.

This was incubated for 24 hours for bio- reduction of gold chloride ($HAuCl_4$) to gold nanoparticles (AuNPs) from stem bark extract, then it was centrifuged at 15000 rpm for 20 min. to removal of presence biological admixture in the reaction mixture, and then it was used characterization and validation.

Characterization of AuNPs

UV- visible absorption spectrum of AuNPs was with the help of Nano drop 800 nm Spectrophotometer. To determine the size of the particle and size distribution in aqueous GNPs solution done by the advanced tool Dynamic Light Scattering (DLS) Malvern-Zeta analyzer. To study the size and shape of the AuNPs performed by the Transmission Electron Microscopy (TEM) HF-3300 advanced 300 kV from Hitachi.

Antimicrobial Studies of AuNPs

Biosynthesized nanoparticles were analysed against for antimicrobial activity two gram Negative like *Escherichia coli* MTCC-443, *Klbsiella pneumoniae* MTCC-741 and two gram positive like *Bacillus subtilis* MTCC-441, *staphylococcus aureus*MTCC-731.Disc diffusion method assay method was carried out using standard protocol (Cruickshank, 1986) was followed for testing antimicrobial activity of AuNPs and comparative studies were made with plant stem bark extract, 1mM $AuCl_4$ as negative controls and streptomycin as standard core bacteria respectively. 7 mm sterile disc were prepared from What man no.1 filter paper and 20 µl of plant extract, 1 mM $AuCl_4$ solution and 10 µg/ disc streptomycin loaded on separate disc and allowed to dry for 60 min at sterile conditions.

Freshly prepared nutrient agar media poured in to sterile Petri plates and allowed to 30 min for solidification. 60 µl of bacterial cultures were swabbed in Petri plates and placed the previously prepared discs and these plates were incubated at 37^0 C for 24 hours, then the zone of inhibition was measured with the help of scale and results were tabulated. Moreover, nanoparticles also enhance the expression of genes helping in redox processes and thus leading to fungal and bacterial death (Nagy *et al.*, 2011).

Results and Discussion

UV- visible spectroscopy

UV-visible absorption spectrum of AuNPs was with the help of Nano drop 800 nm Spectrophotometer. Brown colour turns in to purple colour that confirms the synthesis of AuNPs. This study is the primary confirmatory tool for the detection of the surface Plasmon resonance (SPR) property of AuNPs. The absorbance peak was obtained at 540 nm in *Walsura trifoliata* and same results found in *Annona muricata* extract from 530-538 nm and stem of *Periploca aphylla* AuNPs (Kim *et al.,* 2011; Massoud *et al.,* 2018).

Dynamic Light Scattering (DLS) Zeta potential studies

Both DLS and Zeta potential measurement were used to study of AuNPs by evaluating attraction or repulsion of effects aroused through fluctuations in charge densities. Not only that, the dissemination and accumulation levels of AuNPs dissolved in liquid medium can also found by using this tool.

To determine the size of the particle and size distribution in aqueous AuNPs solution done by the advanced tool Dynamic Light Scattering (DLS) Malvern- Zeta analyzer. Due to the Brownian moving of AuNPs, light is dispersed different intensities. The synthesized AuNPs in the present study expressed 24. nm average size (Fig. 2.a) and -24.4 mV of Zeta potential value (Fig. 2. B). It shows that the nanoparticles were well settled in poly dispersed junction (Dash *et al.,* 2015).

These results stated that the selected medicinal plant and the protocol are best suited for synthesizing AuNPs in eco-friendly approach. The negative potential value might be due to the capping action of biomolecules present in the bark extract of *Walsura trifoliata. Mimosa pudica* flower extract of AuNPs shows same results among the particle size ~ 24 nm and zeta potential values are -33.4 mV (Krishnaprabha and Manjunatha).

Transmission Electron Microscopy(TEM)

The size and shape of the synthesized**,** optimized *Walsura trifoliata* AuNPs were analysed by HF-3300 advanced 300 kV TEM from Hitachi. To prepare the samples for TEM study of synthesized nanoparticles were suspended in deionized water, and dispersed with ultra-sonication, and then aqueous solution of GNPs solution was drop on 50 micro copper grid and dried at room temperature (Aderonke *et al.,*). TEM image of synthesized AuNPs shows the spherical and monodispersed ranging from 27.72 to 29.43 nm and average size about 28.74 nm (Fig. 4).

Antibacterial studies of AuNPs

The antibacterial activity of green synthesized AuNPs were analysed by using two gram negative and two gram positive bacteria grown on nutrient agar medium. The zone of inhibition of each extract is compared with the standard drug streptomycin and $HAuCl_4$ solution used as negative control. AuNPs of *Walsura trifoliata* show good inhibition zones were observed on both gram negative and gram positive bacteria like *Escherichia coli*(MTTC-443**)**, *Klbsiella pneumoniae*(MTTC-741**)**, *Bacillus subtilis* (MTTC-441**)** and *staphylococcus aureus* (MTTC **-**731**)** and results were tabulated (Table: 2, Fig. 3., Graph. 1).

The previous results were also revealed with the plant based AuNPs on bacterial activity (Francis *et al.,* 2018). The major outcome of nanoparticles as antimicrobial agents was antibiotics, surface coating in flooring and textiles (Mehmood *et al.,* 2017).

In the present study we have reported that the simple, eco-friendly, low cost benign stable Gold nanoparticles (AuNPs) from bark extract of *Walsura trifoliate* it is an important ethno medicinal tree as reducing agent. First and for most method to confirm nanoparticles the colour changed reaction mixture and Surface Plasmon Resonance (SPR) spectra of UV-vis spectroscopy data (540 nm) confirmed the formation of AuNPs.

Table.1 Phytochemical constituents from *Walsura trifolita* Stem Bark.

Constituents	Bark				
	Aq	Me	Be	Pe	Hex
Proteins	+	-	+	+	+
Fatty acids	-	+	-	-	-
Reducing sugars	-	+	-	-	-
Alkaloids	+	+	-	+	-
Anthocyanins	-	-	-	-	-
Anthroquinons	-	+	-	-	-
Coumarins	-	+	-	-	-
Emodins	+	+	-	-	-
Flavanoids	-	-	-	-	-
Glycosides	-	-	+	+	+
Leucoanthocyanins	-	-	-	-	-
Phenols	+	+	+	-	+
Saponins	+	+	+	+	-
Tannins	+	-	+	+	-
Triterpenoids	+	-	-	-	+
Steroids	-	-	+	+	-

Note: **Aq**– Aqueous **Be** – Benzene **P e** – Petroleum ether
Me – Methanol **Hex- Hexane**
+: Presence **- :** Absence

Table.2 Effect of Synthesized *W. trifoliate* stem bark AuNPs on Bacterial Species. ± SE of Three samples.

S. No.	Name of the organism	Zone of inhibition (mm)			
		Plant extract	$HAuCl_4$ solution	AuNPs	Streptomycine
1	*Escherichia coli*	7.63 ± 0.03	9.04 ± 0.14	17.6 ± 0.04	24.26 ± 0.17
2	*Klebsiella pneumoniae*	8.13 ± 0.81	10.93 ± 0.10	18.73 ± 0.10	26.16 ± 0.14
3	*Bacillus subtilis*	6.76 ± 0.04	6.96 ± 0.14	14.43 ± 0.08	19.6 ± 0.14
4	*Staphylococcus aureus*	7.16 ± 0.10	7.96 ± 0.08	16.53 ± 0.10	22.5 ± 0.12

Fig.1 UV- visible absorption at 540 nm. of AuNPs.

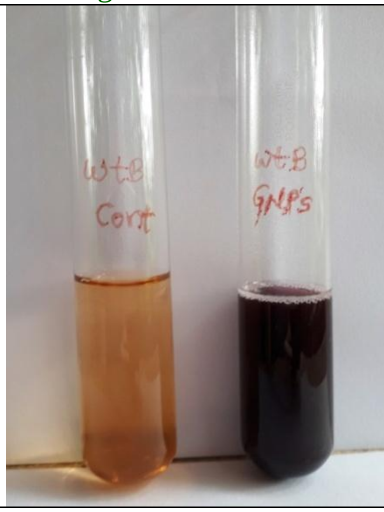
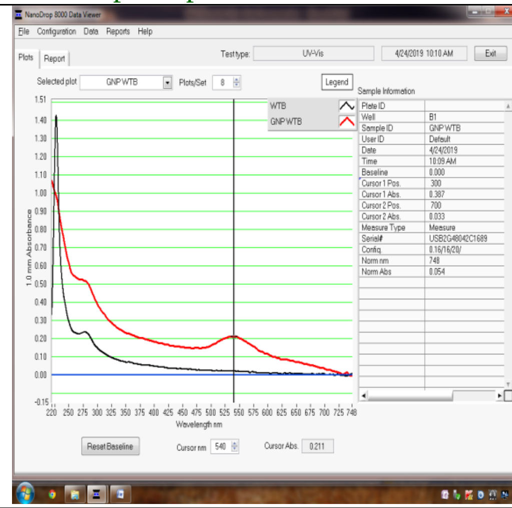
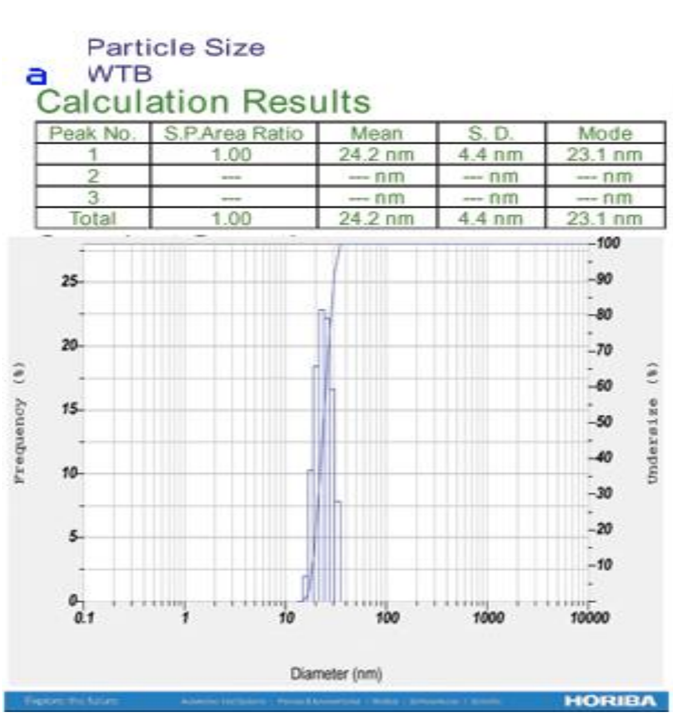
a.Color change of W. t stem bark AuNPs	**b.**UV Obsorption peak of W. t stem bark AuNPs
	

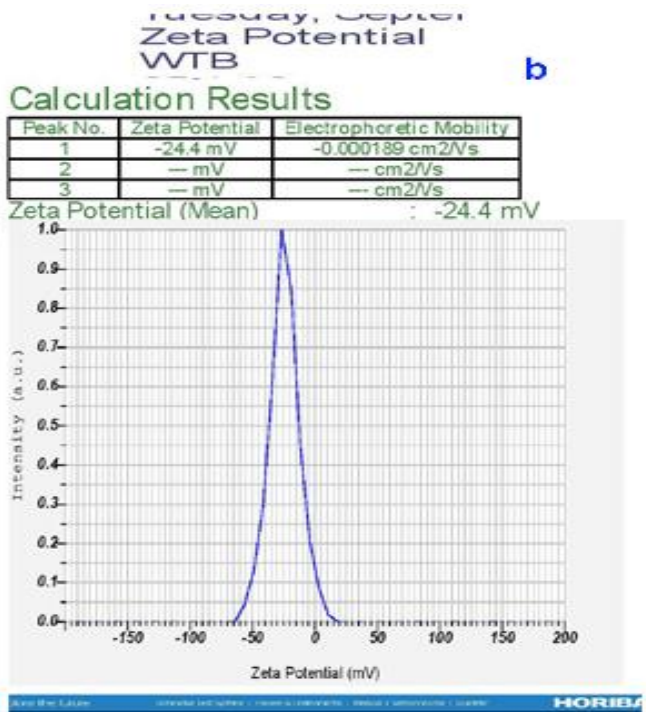
Fig.2 DLS particle size and Zetapotential of AuNPs from *W.trifoliata*

a). Particle size of *W. trifoliate* AuNPs



Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	24.2 nm	4.4 nm	23.1 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	24.2 nm	4.4 nm	23.1 nm

b). Zetapotential of *W. trifoliate* AuNPs



Peak No.	Zeta Potential	Electrophoretic Mobility
1	-24.4 mV	-0.000189 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Fig.3 TEM images of stem bark AuNPs of *Walsura trifolita*

a). At 50 nm Average size of the AuNPs 28.74 nm b). At 200 nm and
c). At 50 nm AuNPs in Spherical shape d). At 51 nm beam of TEM

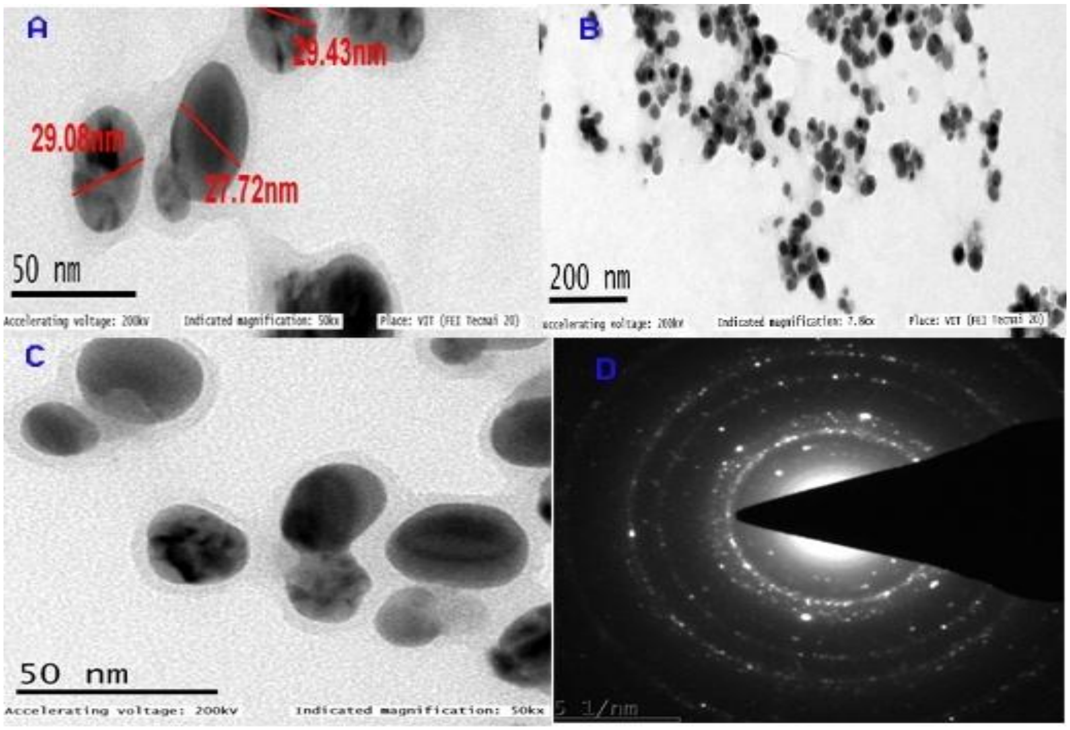
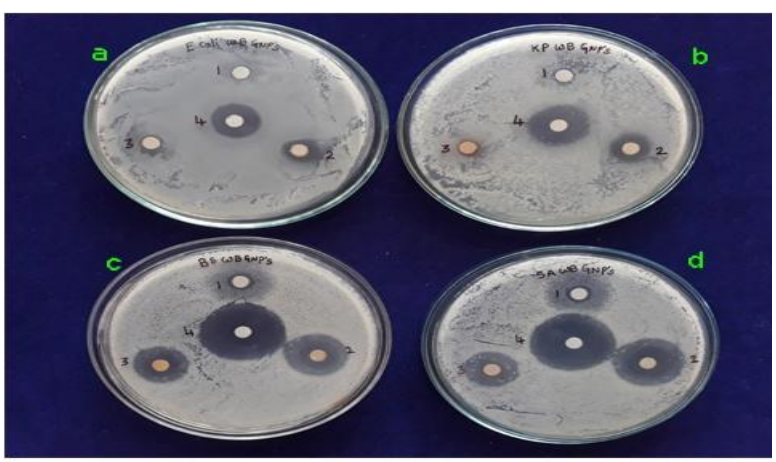
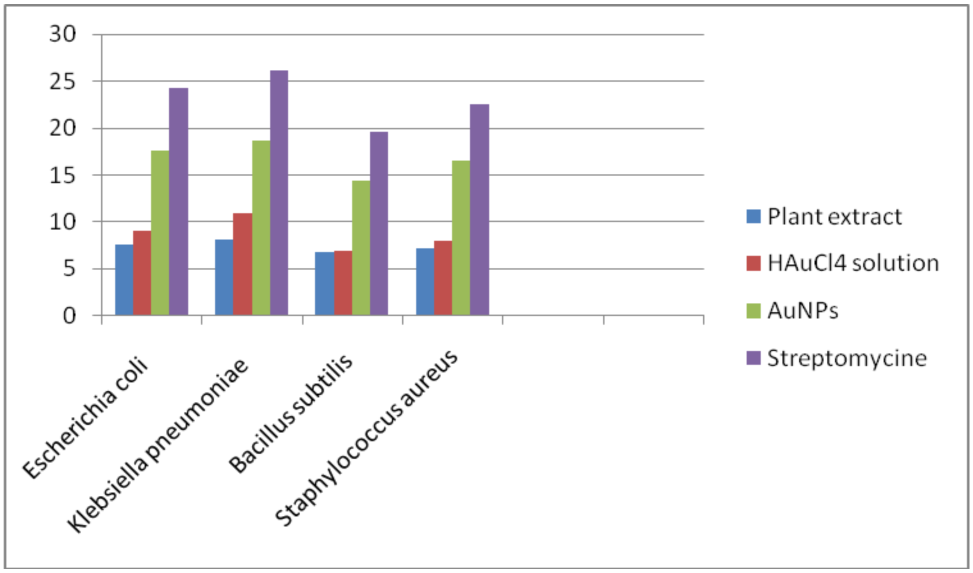


Fig.4 Antibacterial activity of *Walsura trifolita*stem bark AuNPs on selected four bacteria against standard drug streptomycin.

a). *Escherichia coli* b). *Klebsiella pneumonia*
c). *Bacillus subtilis* d). *Staphylococcus aureus.*
1). Au solution 2).AuNPs 3).Plant extract 4).Streptomycin



Graph.1 Graphical representation of zone of inhibition for bacterial species against Synthesized AuNPs.



The DLS particle size and zeta- potential values analysed with the Dynamic Light Scattering (DLS) Malvern- zeta analyzer the results are 4.2 nm average size and -24.4 mV of zeta potential value of the AuNPs.

In TEM (Transmission Electron Microscopy) analysis (20 nm scale bar) 27.72 to 29.43 nm sized (28.74 nm) AuNPs were recognized. The TEM is very advance too than SEM and AFM due to the higher magnification and higher resolution in TEM analysis possible to gives small particles. By this microscopic studies revealed that the well dispersion showing no agglomeration and mostly spherical shape with size range from 27.72 to 9.43 nm. The synthesized AuNPs of *Walsura trifoliata* were exhibited excellent anti-microbial activity against four bacteria.

Therefore these biologically synthesized Gold nanoparticles (AuNPs) eco-friendly antibacterial agents and high quality production of nanoparticles with the little amount of plant extract is highly considerable ethno medicinal tree taxon because of plants have huge source for the production of AuNPs due to the wide potential application and desired shape and size. The synthesis of NPs using crude plant bark extracts and purified compounds are novel substrates for industrial production. In the future, plant bark has a wide potential for the synthesis of NPs in health care and commercial products. Due to the plant sourced AuNPs possess wide spread applications in targeted drug delivery, imaging, diagnosis and therapeutics due to their extremely small size, high surface area, stability, non-toxicity and tuneable optical, physical and chemical properties, gold nanoparticles have revolutionized the field of medicine (Ganeshkumar *et al.*, 2012; Huang and El-Sayed, 2010). Synthesis of gold nanoparticles using plant extract is useful not only because of its reduced environmental, but also because it can be used to produce large quantities of nanoparticles. Plant extracts may be both as reducing agents and stabilizing agents in the synthesis of nanoparticles. Synthesis of gold nanoparticles using plant extract is useful not only because of environmental, but also because it can be used to produce large quantities of nanoparticles.

A detailed study needed to crystal clear mechanism of biosynthesized AuNPs using biomolecules present in various plant extracts which will be valuable to improve the properties of this plant based eco-friendly nontoxic simple method.

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Author contribution

Practical design and manuscript preparation was carried out by our research supervisor Prof. N. Savithramma Department of Botany Sri Venkateswara University, Tirupati. The first author K. Venkata Subbaiah has done field work and practical performance and assisted to our research supervisor Prof. N. Savithramma.

Conflict of Interest statement

The authors declared that they have no interest of conflicts.

Abbreviation

mM- Milli- Molar, nm- Nanometre, Aq– Aqueous, Be – Benzene, Pe– Petroleum ether**,** Me – Methanol, Hex- Hexane, **+:**Presence, **- :** Absence, mm- Milli meter

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