Synthesis of silver nano particle from *Gymnema sylvestre*: study of antimicrobial and antioxidant activity

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Abstract: A versatile technique was implemented for the synthesis of silver nanoparticle using aqueous extract of leaves of *Gymnema sylvestre*. A nanoparticle has very interesting properties in different fields such as medicine, nutrition, energy and has remarkably advantages in pharmacological industries to act against bacterial and viral diseases, therapeutics for cancer and tumours. The synthesis of silver nanoparticle from *Gymnema sylvestre* showed very high antimicrobial activity and also showed antioxidant activity by inhibiting DPPH, scavenging Nitric oxide. Significant antioxidant activity of alcoholic extract of *Gymnema sylvestre* was found which might be due to the presence of Acidic compounds, Flavonoids, Phenols, Saponins, Tannis (Phenolic compounds) and Triterpenoids found in the preliminary phytochemical screening.

IndexTerms- *Gymnema sylvestre,* silver nanoparticle, antimicrobial, antioxidant activity.

I. Introduction

Plants have been considered as a valuable source for natural products and explored continuously for therapeuticals for human well being. The use of plant products for pharmaceutical purposes has been gradually increasing. According to World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs. About 80% of individuals from developed countries use traditional medicines, derived from medicinal plants. The use of plant extracts and phytochemicals, with known antimicrobial properties, can be of great significance in treatment of various microbial infections. In the last decade, numerous studies have been conducted in different countries to prove such efficiency in number of medicinal plants. Most of the studies are restricted with crude extracts (Venkatesan Gopiesh khanna and Krishnan Kannabiran., 2008).

In recent years, the use of ethno-botanical information in medicinal plant research has gained considerable attention in some segments of the scientific community. For treatment of various diseases, bioactive components from medicinal plants that are similar to chemical compounds are used. In one of the ethno-botanical surveys of medicinal plants commonly used by the Kani tribals in Tirunelveli hills of the Western Ghats in Tamil Nadu, India, it was revealed that *Gymneme sylvestre* is the most important species based on its use. The use of plant parts and isolated phytochemicals for the prevention and treatment of various health ailments has been in practice for many decades.

Nanotechnology has emerged as a promising area of research owing to its wide range of applications in the field of biology and medicine. Although various methods are available for the synthesis of metal nanoparticles, most of the methods such as chemical reduction of metal ions in aqueous solutions [Liz- Marzan and Lado-Tourino 1996], use of reverse micelles for chemical and photo reduction [Sun *et al*. 2001], thermal decomposition in organic solvents [Esumi *et al*. 1990], and chemical reduction using radiation [Henglein 1998] involve noxious chemicals. These methods apart from being expensive pose a major threat to environment and also prove to be dangerous for living beings. Thus, the growing quest to develop eco-friendly nanoparticles led to the development of biomimetic approach. Biomimetic is an interdisciplinary area involving the synthesis of nanoparticles using biological principles [Forough and Farhadi 2010].

Antioxidants are added as redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule (Lachman, 1986). It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are present in plants and are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexers of prooxidant metals, quenches of singlet oxygen etc (Ebadi, 2002).

G. sylvestre commonly known as "Meshasringi'', is distributed over most of India and has a reputation in traditional medicine as a stomachic, diuretic, and a remedy to control diabetes mellitus. *G. sylvestre* (Karthic R *et al.*, 2012) is a woody, climbing plant that grows in the tropical forests of Central and Southern India and in parts of Asia (Wu X *et al*., 2012). It is a pubescent shrub with young stems and branches, and has a distichous phyllotactic opposite arrangement pattern of leaves which are 2.5–6 cm long and are usually ovate or elliptical. The flowers are small, yellow, and in umbellate cymes, and the follicles are terete, lanceolate, and up to 3 inches in length. In homeopathy, as well as in folk and ayurvedic medicine, *G. sylvestre* has been used for diabetes treatment (Chattopadhyay RR., 1998). *G. sylvestre* has bioactive components that can cure asthma, eye ailments, snakebite, piles, chronic cough, breathing troubles, colic pain, cardiopathy, constipation, dyspepsia, hemorrhoids, and hepatosplenomegaly, as well as assist in family planning (Patel K., Gadewar .,2012). In addition, it also possesses antimicrobial, antitumor, anti-obesity, anti-inflammatory, anti-hyperglycemic, antiulcer, anti-stress, and antiallergic activity (Arun LB *et al*., 2014).The present study was green synthesis of nano particle from *Gymnema sylvestre* tested for in vitro evaluation of antimicrobial and antioxidant activity.

Fig 1 *Gymnema sylvestre*

II. MATERIALS AND METHOD

Plant material

Gymnema sylvestre (Sirukurujan) plant were collected from Intagro farms, Coimbatore. The leaves of *Gymnema sylvestre* were washed with distilled water, shade, dried, powdered and stored in an airtight container until further use.

Biogenic synthesis of AgNPs

Aqueous solution of 1 mM $AgNO_3$ was prepared and used for the synthesis of AgNPs. 10 mL of aqueous leaf extract was added to 90 mL of 1 mM $AgNO_3$ solution in a 250-mL flask and incubated at room temperature in dark condition for about 24 hour until the solution turned dark brown in color. The synthesis of AgNPs was primarily detected by observing the solution for color change from yellow to dark brown.

Fig 2 Silver nanoparticle prepared from *Gymnema sylvestre*

Antibacterial activity of *Gymnema sylvestre* produced AgNPs

The antibacterial activity of AgNPs was evaluated against the following pathogenic strains *E. coli, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. These cultures were grown on appropriate medium at 37°C for overnight incubation and maintained at 4°C in a refrigerator. Disc diffusion method disc of 5mM was made for nutrient agar medium and each disc was dipped at different concentration (100, 50 ppm) efficiency of prepared AgNPs. The pure cultures of bacterial pathogens were sub-cultured on an appropriate medium. For comparison, plate of the same diameter with 5mM Ampicillin (30µg) was used. After incubation at 37°C for 24h the zones of bacterial inhibition were measured. The assays were performed triplicate.

In-vitro antioxidant activity of AgNPs

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay

The antioxidant activity of Ag NPs was determined using a DPPH radical method. 3.9 ml of (0.1mM) DPPH was mixed with 0.1 ml of various concentrations (10, 20, 40, 60, 80 and 100 µg/ml) of Ag NPs. The reaction mixture was allowed to stand at room temperature for about 45 min. After the absorbance of reaction mixture was determined by UV–Vis spectra with wavelength at 517 nm and against DPPH for the blank solution. The DPPH radical scavenging activity in the percentage of inhibition has calculated by the equation (1).

% of inhibition = (A blank–A sample/A blank) × 100 -------- (1)

Where

A blank is absorbance of the blank solution (DPPH) only.
A sample is the absorbance of the test sample of Ag NPs.
Ascorbic acid was used as a standard positive control.

Nitric oxide (NO) radical scavenging activity

The Nitric oxide radical scavenging activity of the Ag NPs was measured the standard method with some modification. Sodium nitroprusside (1.5 ml) and phosphate buffer (0.5ml of pH 7.4) were separately added to 1.5 ml of different concentrations (10, 20, 40, 60, 80 and 100 µg/ml) of Ag NPs. All the test samples were incubated at room temperature for an hour. After the incubation period, 0.5 ml of Griess reagent was added to each test sample. The notable wavelength at 550 nm of absorbance was measured. Ascorbic acid was used as the standard. The Nitric acid radical scavenging activity was calculated by equation (2).

% of I effect = (A0–A1/A0) × 100 ---------- (2)

Where

"% of I effect" is the percentage inhibition of Nitric acid radical,
"A0" is the absorbance of all the reagents without Ag NPs and
"A1" is the absorbance of all reagents with Ag NPs.

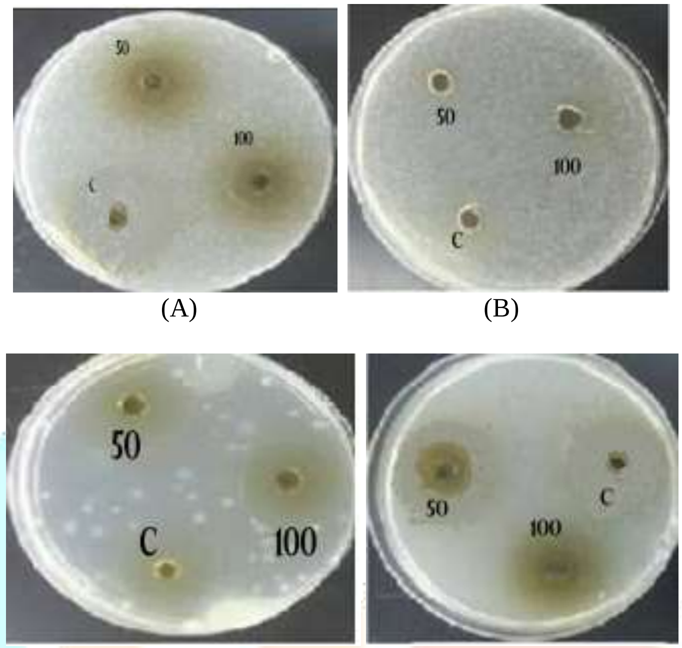
III. RESULT

Antimicrobial activity of *Gymnema sylvestre* extracts mediated silver nanoparticles

It is well- known that silver nanoparticles exhibit brown color, arising due to excitation of surface Plasmon vibrations in the silver nanoparticles. Silver nanoparticles obtained from *Gymnema sylvestre* shown very strong inhibitory action against Gram-positive and Gram-negative bacteria. Two concentrations of nanoparticle (100, 50ppm) were prepared and were applied against an array of bacterial species *viz., Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. The higher concentration (100ppm) of AgNPs showed significant antimicrobial effect compared with other concentrations (50ppm). The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but studies suggest that when bacteria were treated with silver nanoparticles, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane (Auffan *et al*., 2009), leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death (Supraja *et al*., 2015). It is observed that silver nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus- and sulfur-containing compounds such as DNA (He *et al*., 2008). Moreover, *Gymnema sylvestre* AgNPs showed good antibacterial activity (Fig.2) (Table 1). The findings in this study may lead to the development of AgNPs-based new antimicrobial systems for medical applications.

Table.1 In vitro antibacterial studies against bacteria using *Gymnema sylvestre* extract mediated silver nanoparticles as inhibitors

S.No	Bacteria	*Gymnema sylvestre* aqueous extract mediated synthesis of silver nanoparticles		
		50ppm±0.8ppm	100ppm±1.1ppm	Ampicillin30µg
1.	*Escherichia Coli*	2.3±0.5	3.4±0.4	1.1.3±0.02
2.	*Staphylococcus aureus*	1.8±0.02	2.7±0.5	0.4±0.03
3.	*Pseudomonas aeruginosa*	1.1±0.08	3.0±0.4	0.6±0.04
4.	*Bacillus subtilis*	2.0±0.5	3.2±0.4	0.9±0.02



(A) (B)

(C) (D)

Fig 3 Anti bacterial activity of synthesized silver nanoparticles from *Gymnema sylvestre*

(A)*Escherichia Coli* (B) *Staphylococcus aureus*
(C) *Pseudomonas aeruginosa* (D) *Bacillus subtilis*

DPPH Radical Scavenging Activity

Fig.4 illustrates a significant ($p < 0.05$) decrease in the concentration of DPPH radicals due to the scavenging ability of gymnema extract. This activity was dose dependent. Maximum scavenging activity was observed at 100 µg/ml concentration and the IC50 value of gymnema extract were found to be 45.41µg/ml respectively.

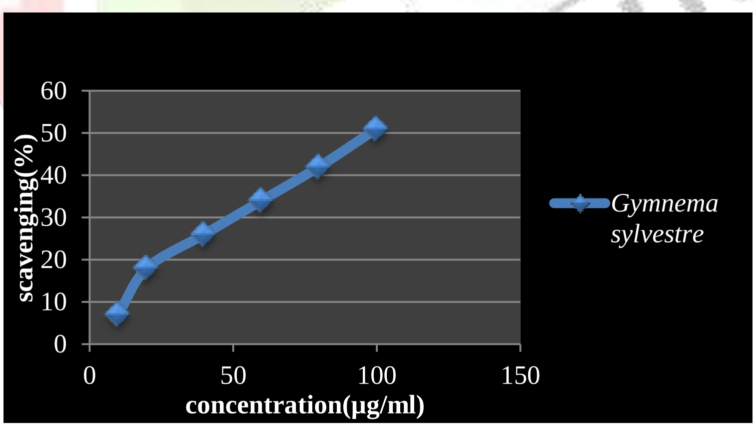


Fig 4: DPPH radical scavenging activity of gymnema extract.

Nitric oxide (NO) radical scavenging activity

The Nitric oxide radical scavenging activity was determined by the different concentration of Ag NPs using the nitrating agent of sodium nitroprusside and Griess reagent. An increase in concentration of nitroprusside increases the Nitric oxide radical scavenging activity (Fig. 5). This result reveals that the Ag NPs shows superior scavenging activity as compared to the standard with similar such inhibition was respectively reported.

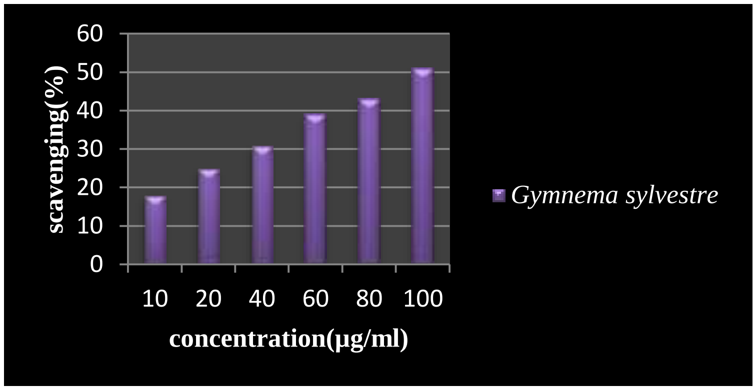


Fig 5: Nitric oxide scavenging activity of gymnema extract

IV. CONCLUSION

In conclusion, the silver nanoparticles were biologically synthesized from *Gymnema sylvestre* was economical, nontoxic and environmentally benign. Due to the reducing and capping nature of the bioactive phytocompounds, present in the aqueous extracts. a cap was formed around the silver ions of the biofunctionalized SNPs which were stable. The gymnema extract showed good antimicrobial and antioxidant activity by inhibiting DPPH, Nitric oxide scavenging activity which may be due to presence of Flavonoids, Phenols, Tannis (Phenolic compounds) and Triterpenoids found in the preliminary phytochemical screening. So *Gymnema sylvestre* is a good plant for further studies in alternative medicine due its multifunctional medical properties.

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