GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACTS OF *MOLLUGO PENTAPHYLLA*

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Abstract: Eco-friendly or green synthesis of metallic nanoparticles has emerged as a key nanotechnology branch and due to their extensive applications, there is a growing industrial demand for nanoparticles. Compared to bulk materials, nanoparticles exhibit advanced characteristics due to their size, distribution and morphology and are commonly used in several medical fields, plant-mediated inexperienced nanomaterial synthesis has gained more and more popularity due to its eco-friendly nature and value-efficacy. In this study, we report the synthesis of silver nanoparticles using leaf extract *Mollugo pentaphylla* using an eco-friendly and economical way. It has been investigated for the synthesis of silver nanoparticles (SNPs) using the *Mollugo pentaphylla* leaf extract as a reduction agent from 1mM silver nitrate ($AgNO_3$). The resulting SNPs were characterized by UV–Vis, TEM. Silver nanoparticles were synthesized within 24 hours of the incubation period and synthesized SNPs displayed an absorption peak in the UV-visible spectrum at approximately 424.76 nm. Silver nanoparticles ' morphological analysis using the nanoparticles is spherical in shape. This route is quick, simple without any hazardous chemicals as reducing or stabilizing agents and cost-effective to synthesized SNP.

Key words: *Mollugo pentaphylla*, Green synthesis, Silver nanoparticles, UV-Vis, SEM, FTIR, Antibacterial activity.

1. Introduction

Nanobiotechnology is currently one of the most diverse disciplines for nanoparticles (NPs) combination studies of modern material science by which vegetation and unique plant merchandise identify an authoritative application. Particles smaller than 100 nm in diameter are referred to as NPs. Through these particles, maximum progressive and elevated characteristics such as size, distribution and morphology have been discovered in evaluation to the larger particles of the mass product from which they were formed (Wildenberg, 2005). Noble metal NPs such as gold, silver and platinum are nicely traditional to have substantial mechanical, magnetic, optoelectronic and data processing packages (Okuda, 2005). One such important member of the noble metallic NPs is Silver NPs (Ag NPs). In addition, they can be used to a large extent in shampoos, soaps, detergents, cosmetics, toothpastes and medical and pharmaceutical products and are therefore found directly using human processes (Bhattacharya and Murkherjee, 2008). Plants have been a treasured source of herbal products for an extended period of time in order to maintain human health, particularly within the last decade, with more in-depth research for natural treatment plans. Today, a number of nations have slowly expanded the use of phytochemicals for medicinal purposes. Medicinal flowers would be the best, according to the World Health Organization (WHO).

Of late, they have clearly been admitted to having roles in the protection of human health when their food consumption is high. 4,000 and above phytochemicals are collected and labelled to secure their characteristics, physical and chemical (Meagher and Thomson, 1999). Phytochemicals occur naturally in medicinal plants, leaves, vegetables and roots that have the function of protecting and guarding against various diseases (Krishnaiah, 2007). Plant-based antimicrobials have a high therapeutic potential. Plant-derived antimicrobials have a long history of providing the much-needed novel therapy (Silva *et al*., 2012). Microbes are more unlikely to develop immunity to nanoparticles because they target a wide range of targets allowing the microorganism to undergo a series of mutations simultaneously in order to defend itself (Pal *et al*., 2007).

M. Pentaphylla is closely associated with Glinus and is distinguished by the inclusion of a fili-shaped Glinus appendage. The plant is perennial, annual, tufted with white or brown taproot to erect herb, up to 35 cm tall. Stems erect, glabrous, rounded, stable. There are no provisions. Simple leaves, not lobed or divided, opposite or whorled, sessile or stalked, flat, whole margin, apex acute, base acute, with one distinct vein (midrib) below. Bisexual flora in terminal cymes, unmarried or with few flowers, stalked, tepals 5, red, a pill, beginning with three valves. But, the free cell cycle and culture filtrate biosynthesis of silver nanoparticles have not been investigated yet. We comment on the synthesis of silver nanoparticles in this paper by reducing aqueous Ag+ ion by simultaneously reducing aqueous Ag+ with the medicinal plant leaf extract. We found through our screening process involving a number of plants that were potential candidates for rapid silver nanoparticles synthesis.

2. Materials and Methods

2.1 Collection of Plant Materials

M.pentaphylla's fresh plant leaves were randomly collected from the Yercaurd, Salem District, Tamil Nadu. Under running tap water, plant materials were washed, air dried and then homogenized to fine powder, and stored in refrigerator in airtight bottles.

2.2 Preparation of Extracts

Soxhlet extraction method was used to prepare crude plant extract. Approximately 20gm of powdered plant material was uniformly packed into a thimble and collected separately with 250ml of different solvents, ethanol and ethyl acetate. The extraction process must be continued for 24 hours or until the extractor solvent in the siphon tube is colorless. The extract was taken in a beaker afterwards and placed on a hot plate and heated at 30-40^{o}C until all the solvent was evaporated. Dried extract was kept at 4^{o}C in the fridge for potential use.

2.3 Phytochemical Screening

M.pentaphylla extracts of ethanol and ethyl acetate were subjected to preliminary phytochemical analysis according to the standard methods of Brain and Turner 1975 and Evans 1996.

2.3.1 Detection of alkaloids

The presence of alkaloids in dilute hydrochloric acid was examined from the filtrate prepared by dissolving extracts.

a) **Mayer's test**: The presence of alkaloids confirmed yellow cream precipitate formation that was produced as a result of Mayer's reagent treatment of the filtrates.
b) **Wagner's test**: Brown / reddish brown precipitate formation which treats the filtrate with the reagent of Wagner indicates the presence of alkaloids.

2.3.2 Detection of Flavonoids

a) **Lead acetate test**: Yellow colour formation precipitates the existence of flavonoids by treating the extracts with few drops of lead acetate.
b) H_2SO_4 test: The formation of orange colour indicates the presence of flavonoids by treating extracts with few drops of H_2SO_4.

2.3.3 Detection of Steroids

Liebermann- Burchard test: By adding 2ml of acetic anhydride to 0.5g of extracts, each with 2ml of H_2SO_4 specifies the presence of steroids, the color change from violet to blue or green.

2.3.4 Detection of Terpenoids

Salkowski's test: When 0.2g of the extract mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer, the presence of terpenoids confirmed by reddish brown color.

2.3.5 Detection of Anthraquinones

Borntrager's test: Formation of pink color suggests the presence of anthraquinones produced by adding a few drops of 10 percent NH_3 to the mixture and heating about 0.2g of the extract was boiled for a few minutes in a water bath with 10 percent HCl, while the same volume of $CHCl_3$ was applied to the filtrate, filtered and allowed to cool.

2.3.6 Detection of Phenols

a) **Ferric chloride test**: Phenol presence is indicated by the formation of Bluish black colour produced by treating few drops of 5 percent ferric chloride solution.

b) **Lead acetate test**: The presence of phenol is demonstrated by the formation of yellow colour precipitate as a result of treating few drops of lead acetate solution.

2.3.7 Detection of Saponins

Froth test: The production of saponins by shaking 0.2g of the extract with 5ml of distilled water.

2.3.8 Detection of Tannins

Ferric chloride test: A dark green color created by combining a small amount of extract with water and heating in a water bath, filtering and adding 0.1% ferric chloride ensures the presence of ferric chloride.

2.3.9 Detection of Carbohydrates

Fehling's test: Confirmed presence of sugar by forming red precipitate on boiling 0.2gm filtrate in water bath with 0.2ml each of Fehling solutions A and B.

2.3.10 Detection of Oils and Resins

Spot test: The transparent appearance of filter paper by applying the sample solution to filter paper indicates the presence of oils and resins.

2.4 Antimicrobial susceptibility test

The antimicrobial activity was tested using the disk diffusion method (Bauer *et al*., 1966). Muller Hinton Agar (MHA) obtained from Himedia was used to test antimicrobial activity in vitro. By pouring 15 ml of molten media onto sterile Petri plates, the MHA plates are prepared. After 5 minutes allowed the plates to solidify and 0.1 percent inoculum suspension was swabbed uniformly and 5 minutes allowed the inoculum to dry. The extract concentration on the 6 mm sterile disk is 40 mg / disk packed. The packed disk was placed on the medium surface and the extract was allowed to spread for 5 minutes and the plate was held at 37ºC for 24 hours for incubation. Inhibition zones formed around the disk were measured in millimeter with transparent ruler at the end of the incubation. Chloramphenicol is the normal disk.

2.5 Synthesis of Silver Nano-Particles

The solution of silver nitrate 1 mM was prepared in a flask of 100 ml. 1 ml plant extract was blended with a silver nitrate of 9 ml of 1 mM. *M.pentaphylla* leaf and silver nitrate solution aqueous plant extracts have been used throughout the experiment as a monitor (Smetana *et al*., 2005). The final solution was 200 ml and for 25 min at 18,000 rpm centrifuged. The pellets obtained have been stored at -4^0 C. At 50^0 C to 95^0 C, the supernatant was cooled. During the heating process, a shift in solution color was observed.

2.6 Characterization of Silver Nanoparticles:

2.6.1 UV-Vis Analysis:

UV-Vis spectrophotometer Perklin-Elmer, Lamda 35, Germany, determined the optical property of Zinc oxide. The spectra are taken in different time intervals up to 24 hours after the introduction of synthesized extract. From 350 nm to 500 nm. Instead they took the spectra after 24 hours. Synthesized from zinc oxide.

2.6.2 FT-IR analysis:

An FT-IR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer) was used to analyze the chemical composition of the synthesized silver nanoparticles. The solutions were dried at 75^o C and in the range 4000–400 cm^{-1} using KBr pellet method the silver synthesized was characterized.

2.6.3 SEM Analysis:

Scanning Electron Microscope (JSM-6480 LV) has analyzed the morphological characteristics of synthesized silver nanoparticles from *M.pentaphylla* plant extract. Following 24 hours. The SEM slides are prepared from the introduction of $AgNO_3$ by making a smear of the solutions on the slides. To make the samples conductive, a thin layer of platinum has been coated. Then at an increasing voltage of 20 KV, the samples are defined in the SEM.

3. Result and Discussion

Silver nanoparticles green synthesis by plant extracts was performed in two separate samples. The production of *Mollugo pentaphylla* extracts of ethanol and ethyl acetate. The yield in the extract of ethanol (13.86 percent) was higher than the yield in *Mollugo pentaphylla* ethyl acetate extract (4.94 percent).

3.1 Qualitative Phytochemical Analysis

Mollugo pentaphylla's qualitative phytochemical analysis of ethanol and ethyl acetate extracts was performed. Alkaloids, phenols, hormones, tannins, and carbohydrates are present in the ethanol extract. The most active hormones, proteins, oils and resins are contained in the ethyl acetate extract. As a result, *Mollugo pentaphylla's* ethanol extract showed better phytochemical value in Table 1. These secondary metabolites make a significant contribution to medicinal plant biological activities, including hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, anti-leprosy, etc. (Negi *et al*., 2011). Tannins have stringent properties that are amazing. They are believed to promote wound healing and inflamed mucous membranes (Rio *et al*., 1997; Salah *et al*., 1995). In addition, alkaloids are a group that affects the central nervous system, reduces appetite, and functions as a diuretic (Agriculture Department of the United States, 2010).

3.2 Synthesis of Silver Nanoparticles:

By using visible observation, the green synthesis of silver nanoparticles by plant extracts was performed and confirmed. The color changed into greenish brown coloring due to the reduction of silver ions (Jain *et al*., 2009) also found that the leaf extracts were mixed with the aqueous solution of the silver ion complex, transformed into reddish brown coloring due to the excitation of surface plasmon vibrations, indicating that Ag nanoparticles were produced. Because of plasma levitation on vibrations in silver nanoparticles, it was widely recognized that silver nanoparticle shows greenish brown coloration in aqueous solution.

As a product of surface plasmon vibrations, silver nanoparticles (AgNPs) tend to be yellowish brown in aqueous medium (Krishnaraj *et al*., 2010). The color of the solution changed from mild to yellowish brown to reddish brown and subsequently to colloidal brown suggesting AgNP formation (Lalitha *et al*., 2013; Simghal *et al*., 2011; Philip and Unni, 2011), thus confirming the completion of the leaf extract and $AgNO_3$ reaction. The silver nanoparticles are synthesized in plant sample crude extract as well as ethanolic extract. The observed color changes suggested the formation of silver nanoparticles.

3.3 Antimicrobial activity

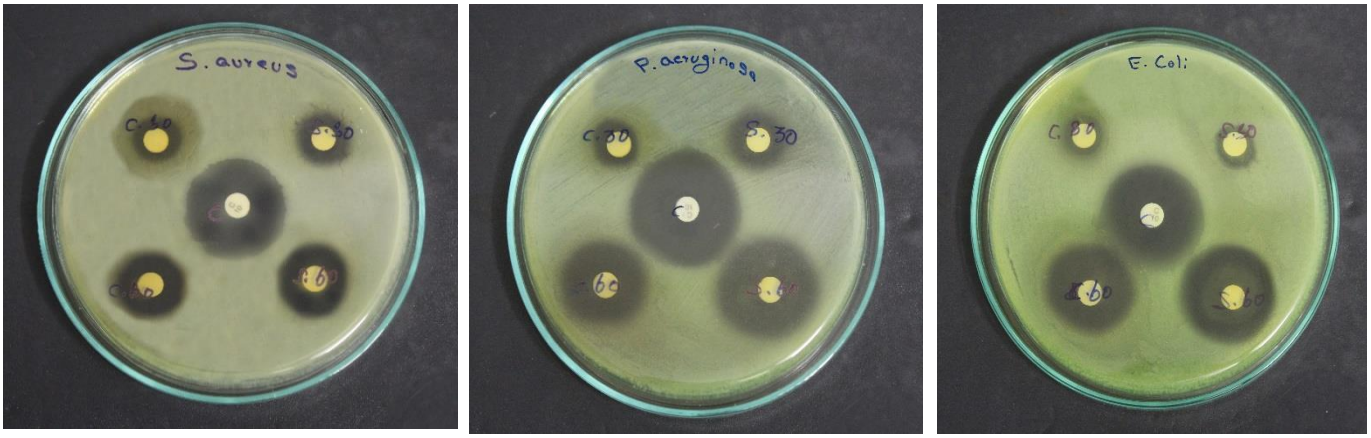
Mollugo pentaphylla ethanol and ethanol nano-synthesized antibacterial activity performed to test the action against *S.aureus, P.aeruginosa, E.coli, B.subtilis*, and *S.typhi*. Antibacterial activity against the species was confirmed by each sample. *Mollugo pentaphylla* ethanol synthesized extract reported higher activity than the extract of ethanol (Table 2 and Figure 1). The antimicrobial activity testing of plant products has shown that the higher plants are a potential source for novel antibiotic prototypes (Afolayan, 2003). In recent years, numerous resistances have occurred in human pathogenic microorganisms, largely due to the indiscriminate use of industrial antimicrobial capsules typically employed inside the infectious disease remedy. This forced scientists to search for new antimicrobial materials from different sources such as medicinal plants (wu *et al*., 1999).

Table 1: Qualitative phytochemical analysis of *Mollugo pentaphylla*

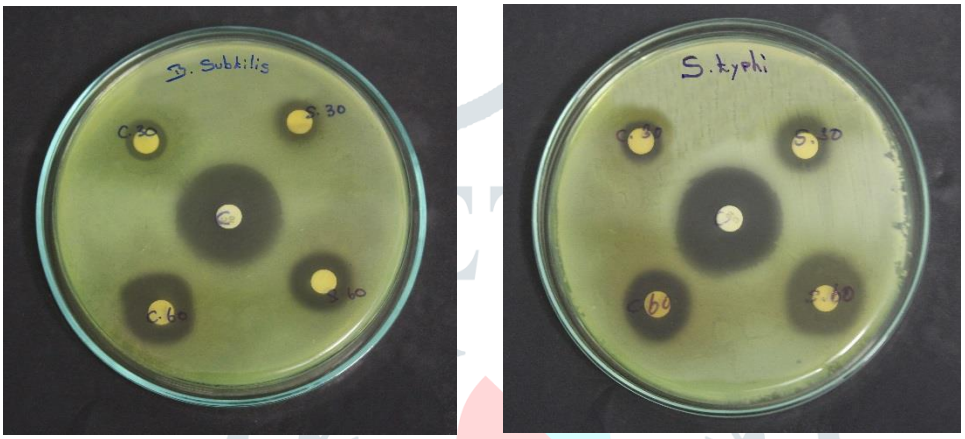
Phytochemicals	Observations	Extracts	
		Ethanol	Ethyl acetate
Alkaloids Mayer's test Wagner's test	Cream color Reddish brown solution/ precipitate	+ +	- -
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange color precipitate	- -	- -
Steroids Liebermann-Burchard test	Violet to blue or Green color formation	+	+
Terpenoids Salkowski test	Reddish brown precipitate	-	-
Arthroquinone Borntrager's test	Pink color	-	-
Phenols Ferric chloride test Lead acetate test	Deep blue to Black color formation White precipitate	+ +	- -
Saponin	Stable persistant	+	-
Tannin	Brownish green / Blue black	+	-
Carbohydrates	Yellow / brownish / blue / green color	+	+
Oils & Resins	Filter paper method	-	+

Table 2: Antibacterial activity of *Mollugo pentaphylla*

S.No.	Organisms	Control	C 30	C 60	S 30	S 60
1	*S.aureus*	25	13	17	14	19
2	*P.aeruginosa*	29	11	21	16	27
3	*E.coli*	27	10	23	11	25
4	*B.subtilis*	26	11	19	10	16
5	*S.typhi*	25	10	18	12	20



S.aureus ***P.aeruginosa*** ***E.coli***



B.subtilis ***S.typhi***

Figure 1: Antibacterial activity in *Mollugo pentaphylla*

UV, FTIR and SEM described the silver nanoparticles from the ethanol extract of *Mollugo pentaphylla*. A light beam from a visible and/or UV light source is separated by a prism or diffraction grating into its segment wavelengths. In addition, each monochromatic (single wavelength) beam is separated by a half-mirrored system into two equivalent intensity beams. One light, the specimen beam, moves through a small crystal-clear (cuvette) container containing a solution of the compound being analyzed in a transparent solvent. The other tube, the reference, passes through the same cuvette that contains only the solvent. Such light beams ' intensities are then measured and evaluated by electronic detectors. The spectrometer scans all the component wavelengths in the manner defined automatically over a short period of time. The measured area of ultraviolet (UV) is typically between 200 and 400 nm, and the visible component is between 400 and 800 nm.

3.4 Characterization of Synthesis of Silver Nanoparticles

The silver nanoparticles ' UV analysis was done. At 424.76 nm (Figure 2), the peak absorbance was seen. FTIR measurements are performed to identify potential *Mollugo pentaphylla* extract biomolecules responsible for nanoparticles formation and stabilization. Infrared Absorption Bands of 3338.41 cm^{-1} in Intermolecular hydrogen bond (Strong), 2976.09 cm^{-1} in Acids (Medium), 2928.13 cm^{-1} in Acids (Medium), 1867.34 cm^{-1} in Aromatic methane (Weak), 1571.18 cm^{-1} in Secondary amines (Weak), 1282.17cm^{-1} in Secondary amines (Strong), 1153.48 cm^{-1} Aromatic ketones (Strong) and 789.11 cm^{-1} in Aromatic methane (Strong) (Figure 3). The silver nanoparticles synthesized from *Mollugo pentaphylla* were characterized by Scanning Electron Microscopy (SEM). SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan patterns. SEM technique was employed to visualize the size and shape of silver nanoparticles (Figure 4).

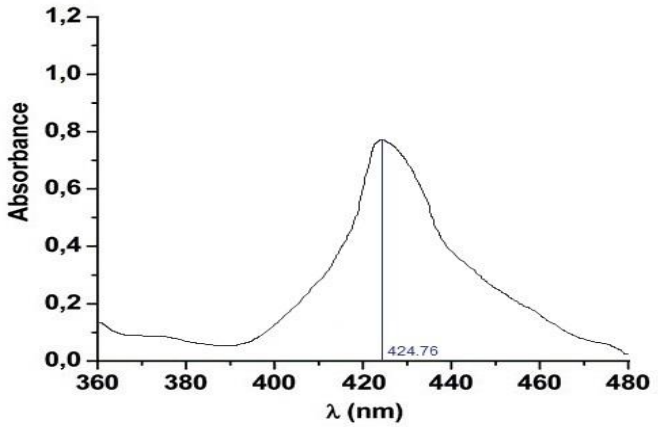


Figure 2: UV analysis of Synthesis of Silver from *Mollugo pentaphylla*

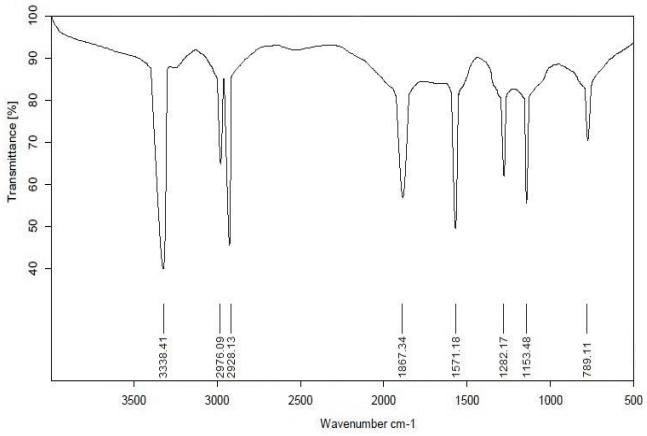


Figure 3: FTIR analysis of Synthesis of nanoparticles Silver Nanoparticles in *Mollugo pentaphylla*

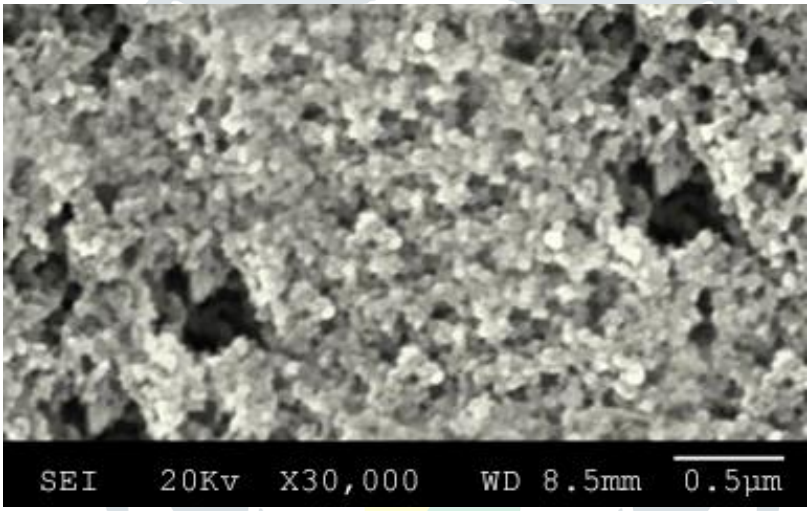


Figure 4: SEM analysis of Synthesis of Silver Nanoparticles in *Mollugo pentaphylla*

4. Conclusion

Mollugo pentaphylla is historically a very valuable medicinal herb. There is a lot of ayurvedic preparation with *Mollugo pentaphylla* such as, Anti-asthma kada, sirisa twak kvatha, vasadikwath etc. Phytochemical research has shown that *Mollugo pentaphylla* contains therapeutically useful alkaloids, hormones, tannins, and phenols. *Mollugo pentaphylla* ethanol extract synthesized the silver nanoparticles and characterized by UV, FTIR and SEM. There was substantial antibacterial activity in the extracts. It can be concluded that in various activities, *Mollugo pentaphylla* appears to be promising crop. The crop can therefore be further studied on different isolated pure compounds pharmacologically. The silver nanoparticles have potential applications in the biomedical field from a technical point of view, and this has many advantages such as cost-effectiveness, accessibility for medical and pharmaceutical applications, as well as large-scale commercial production.

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