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Evaluation of antimicrobial activity of silver nanoparticles synthesized from *Abutilon indicum*

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Abstract
Abutilon indicum is a medicinal plant. Plants root, bark, flowers, leaves and seeds are very much used in siddha medicines. The leaves of plant *Abutilon indicum* were collected from Pallamalai hill slopes at the month of January. Phytochemical analysis shows the presence of Flavonoids, tannins, Phenolic compound, alkaloid and safonins. The green synthesis of Silver Nanoparticles using plant extract was done. The Silver nanoparticles have been studied for antimicrobial activity. It has been compared with plant extract results shows more inhibitory than extract and control for *E. coli* and *Staphylococcus aureus.*

Keywords
Abutilon indicum, antimicrobial, silver nanoparticles, phytochemicals.

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1. INTRODUCTION

Medicinal plants are used to treat disease. It is used in healthcare in tackling the disease of public health importance. The use of medicinal plants has attained a commanding role in health system in all over the world. The medicinal are not only used for the treatment of diseases but also for maintain good health. The application of Nanomaterials is an emerging field of nanotechnology. Technological and environmental challenges can be solved by Nanomaterials. Nanomaterials possess more surface area, it shows more catalytic activity (1).

The nanoparticles used for all the aforesaid purposes, the metallic nanoparticles considered as the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains. Among the all noble metal nanoparticles, silver nanoparticle are an arch product from the field of nanotechnology which has gained boundless interests because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities which can be incorporated into composite fibers, cryogenic superconducting materials, cosmetic products, food industry and electronic components (2). For biomedical applications, being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions' as an antiseptic and displays a broad biocidal effect against microorganisms through

the disruption of their unicellular membrane thus disturbing their enzymatic activities.

Abutilon indicum belonging to family Malvaceae is distributed throughout all tropical zones. A. indicum is reported to be used to treat ulcers, headaches, gonorrhea, bladder infection, inflammation, hepatic, and pulmonary disorders (3). There are several reports proved that this plant also used as demulcent, aphrodisiac, laxative, diuretic and sedative (leaves), diuretic; laxative, expectorant, and demulcent. The leaves can also be used to treat ulcers, headaches, gonorrhea, and bladder infection (4). Such plants root, bark, flowers, leaves, and seeds are very much used in Siddha medicines. The leaves are also used for pile complaints. So far not adequate characterization of its analgesic and anti-inflammatory activity has not been yet confirmed. Hence, this study shows the synthesis of AgNPs from ethanolic extraction of *A. indicum* and it is anti-inflammatory and antioxidant properties of in *in vitro*.

2. METHODOLOGY

2.1. Collection of plant sample:

The leaves of plant Abutilon Indicum were collected from Pallamalai hill slopes at the month of January.

2.2. Identification of plants:

The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, and Coimbatore by referring the deposited specimen. The voucher number of the specimen is 3709 The fresh leaf of this species was washed under running tap water, shade dried at room temperature and powdered.

2.3. Preparation of plant extract:

A. indicum leaf extract was used for the preparation of silver nanoparticles, because of the medicinal properties and easy availability of the plant in all vegetation. The leaves were washed with water to remove all type of contaminants, then washed with distilled water and air dried at room temperature. 20 gms of leaves were weighed and cut into small pieces. It was then added to a beaker containing 200ml of distilled water and boiled for 30 minutes. The extract was cooled and filtered by Whatman filter paper and the extract was stored at 4^{o}c for future use.

2.4. Qualitative phytochemicals screening:

The crude extract checked for the presence of the following secondary metabolites such as alkaloids, phenols, flavonoids, saponins, steroids, cardiac glycosides and tannins by standard procedures in hexane and methanol.

i. Cardiac Glycosides

Keller-kiliani test was performed to assess the presence of cardiac glycosides. The crude dry powder of crude extract was treated with 1 mL of $FeCl_3$ reagent (mixture of 1 volume of 5% $FeCl_3$ solution and 99 volumes of glacial acetic acid). To this solution a few drops of concentrated H_2SO_4 was added. Appearance of greenish blue color within a few minutes indicated the presence of cardiac glycosides.

ii. Steroids

Liebermann-Burchard reaction was performed to assess the presence of steroids. A chloroform solution of the crude dry powder of the extract was treated with acetic anhydride and a few drops of concentrated H_2SO_4 were added down the sides of the test tube. A blue green ring indicated the presence of terpenoids.

iii. Alkaloids

The crude extract was evaporated to dryness in a boiling water bath. The residue was dissolved in 2 N HCl. The mixture was filtered, and the filtrate was divided into 3 equal portions. One portion was treated with a few drops of Mayers reagent; one portion was treated with equal amount of Dragondroffs reagent and the other portion was treated with equal amount of Wagners reagent. The creamish precipitate, orange precipitate and brown precipitate, indicated the presence of respective alkaloids.

iv. Flavonoids

In a test tube containing 0.5 mL of crude extract, 5-10 drops of diluted HCl and small piece of zinc or magnesium were added, and the solution was boiled for few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.

v. Phenols

The extract is dissolved in 5 mL of distilled water. To these few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds.

vi. Tannins

The crude extract was treated with alcoholic $FeCl_3$ reagent. A bluish black colour, which disappears on addition of a little dilute H_2SO_4 was followed by the formation of yellowish-brown precipitate.

vii. Saponins

The presence of saponins was determined by Frothing test. The crude dry powder of fungal extract was vigorously shaken with distilled water and was allowed

to stand for 10 min. No froth indicates absence of saponins, and stable froth more than 1.5 cm indicated the presence of saponins.

2.5. Synthesis of silver nanoparticles from plant extract:

Silver nitrate GR used as such (purchased from Merck, India) 100 ml, 1mM solution of silver nitrate was prepared in an Erlenmeyer flask. Then 1, 2, 3, 4 and 5 mL of plant extract was added separately to 10 ml of silver nitrate solution keeping its concentration at 1mM. Silver nanoparticles were also synthesized by varying concentration of $AgNO_3$ (1mM - 5mM) keeping extract concentration constant (1 mL). This setup was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature. Reduction of Agþ to Ag0 was confirmed by the colour change of solution from colourless to brown. Its formation was also confirmed by using UV Visible spectroscopy.

2.6. Determination of antibacterial effect for synthesized silver nanoparticles:

The antibacterial assay was done on human pathogenic *Escherichia coli* and *Staphylococcus aureus* by using standard disc diffusion method. Mac Conkey broth (HiMedia) medium was used to subculture bacteria and were incubated at 37 °C for24 h (4). Fresh overnight cultures were taken and spread on the Mac Conkey agar plates to cultivate bacteria. Sterile paper discs of 5 mm diameter saturated with plant extract, silver nanoparticle and double distilled water (as control) were placed in each plate and incubated again at 37°C for 24h and the antibacterial activity was measured based on the inhibition zone around the disc impregnated with plant extract and synthesized silver nanoparticle.

3. RESULT

3.1. Screening of qualitative phytochemical analysis:

The plant extract exhibited presence for anthraquinones, phenols and glycosides for hexane extract whereas flavonoids, saponins and steroids ere present in both the extracts.

Table 1: Qualitative phytochemical analysis for *A. indicum*

TEST	RESULT	
	Hexane extract	Methanol extract
Anthraquinones	+	-
Alkaloids	-	+
Flavonoids	+	+
Saponins	+	+
Tannins	-	-
Phenolic compounds	+	-
Glycosides	+	-
Terpenoids	-	-
Steroids	+	+

'+' denotes presence; '-' denotes absence.

3.2. Determination of antibacterial activity:

The plant extract and those mediated silver nanoparticles were immediately tested for respective antimicrobial activities towards both gram positive (*S. aureus*) and gram negative (*E. coli*) bacterial strains showing the zones of inhibition. Based on the zone of inhibition produced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against *E. coli* and *S. aureus*. On the other hand, control and plant extract alone did not exhibit any antibacterial activity. The silver nanoparticles showed efficient antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms (4).

Table 2: Anti-bacterial activity of plant extract and AgNPs

S. No	Micro organism	Control(mm)	Plant extract (mm)	Silver nanoparticle(mm)
1	*E. Coli*	Nil	15	17
2	*S. aureus*	Nil	13	15

4. DISCUSSION

Infection diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants. The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and wellbeing. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment. In this study, silver nanoparticles synthesized from plant extract and it showed more antimicrobial activity when compare with plant extract (4). It shows that nanoparticles chelated with plant compounds shows increased surface activity.

CONCLUSION

A simple one green synthesis of stable silver Nanoparticles using *A. indicum* leaf extract at room temperature was

reported in this study. Synthesis was found to be efficient in terms of reaction time as well as stability of the synthesize nanoparticles which exclude external stabilizers/reducing agents. It proves to be an eco-friendly, rapid green approach for the synthesis providing a cost effective and an efficient way for the synthesis of silver nanoparticles. Therefore, this reaction pathway satisfies all the conditions of a 100% green chemical process. The synthesized silver nanoparticles showed efficient antimicrobial activities against both E. coli and S.aureus. Benefits of using plant extract for synthesis I that it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer products. This eco-friendly method could be a competitive alternative to the conventional physical/chemical methods\used for synthesis of silver nanoparticle and thus has a potential to use in biomedical applications and will play an important role in opto-electronics and medical devices in near future.

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