

Study on Plant Extract Mediated Synthesis of Silver Nanoparticles Using Combination of *Cardiospermum Halicacabum* and *Butea Monosperma* & Screening of Its Antibacterial Activity

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ABSTRACT

Objective: To synthesis silver nanoparticles from aqueous leaf extract of *Cardiospermum halicacabum* and *Butea monosperma* and analyze the phytochemical constituent and evaluate the antibacterial activity using this silver nanoparticle. Materials and methods: For the synthesis of silver nanoparticle, 1mM silver nitrate is used. Preliminary phytochemical analysis of silver nanoparticle was analyzed using standard procedures. The antibacterial activity was evaluated using agar diffusion method. Results: The phytochemical analysis of the silver nanoparticle exhibited the presence of alkaloids, Tannin, phenol, carbohydrate, terpenoid. The antibacterial activity of silver nanoparticle against *Pseudomonas*, *Bacillus cereus*, *citrobacter* and *E.coli*. The whole plant of *Cardiosperm halicacabum* and *Butea monosperma* leaf parts have prominent antimicrobial properties. Conclusion: The silver nanoparticle of *Cardiospermum halicacabum* and *Butea monosperma* were found to be a powerful antimicrobial agent and this study can be continued for their structural elucidation and pharmacological activity.

Keywords: Silver nanoparticle, *Cardiospermum halicacabum*, *Butea monosperma*, antibacterial activity.

INTRODUCTION

The nanomaterials can significantly enhance the pharmacokinetics and therapeutic index of plant drugs¹. The use of nanotechnology in medicine and more specifically drug delivery is not to spread rapidly. The nanoparticles are the particle scale ranging from 1-100nm². Nanoparticles have considerable applications in the field of medicine, electronic, mechanical, optical, chemical, food, environmental etc.,³. Nanotechnology is a field that is making a mark in research day by day and making an impact in all spheres of human life⁴. Rheumatoid Arthritis (RA) is an Auto-immune Disease that results in a chronic, systemic inflammatory disorder that causes pain, swelling, stiffness and loss of function in joints⁵. It occurs more frequently in women than in men and its prevalence depends upon age. In human, RA is the common inflammatory joint disease where skeletal complications start with focal erosion of cartilage initially followed by marginal & sub-chondral bone loss. Extended joint destruction with ankylosis and generalized bone loss are characteristics for late complications⁶. The steroidal and non-steroidal anti-inflammatory drugs are used in the treatment of the disease but they offer only temporary relief and produce severe side effects including gastrointestinal bleeding and cardiovascular toxicity. Natural has a great source of medicinal plant from ancient time and numbers of modern drugs have been isolated by choosing important and useful plants. In India the pre-historic also has medicinal importance in the Ayurveda.

In the daily life the disease can be treated various medicinal those described in veda⁷. Now a day's infections disease spread widely in the developing countries including India⁸, it can be cured by medicinal plants. One of such a medical plants for rheumatic arthritis is *Butea monosperma* and *Cardiospermum halicacabum* is an indigenous plants. *Butea monosperma* are widely used for the treatment of rheumatism, diarrhea⁹. *Cardiospermum halicacabum* family sapindaceae known as the ballon plant are widely distributed in Asia & Africa. The whole plants has been used for the several centuries in the treatment of rheumatism, stiffness of limbs, snakebite, its roots of nervous disease, laxative, as a diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific. Its leaves and stalks are used in the treatment of diarrhea, dysentery and headache and as a poultice for swellings¹⁰. A literature shows the structural elucidation, antimicrobial, antioxidant and pharmacological activity of *Cardiospermum halicacabum* and *Butea monosperma* individually. None of the studies were elucidate the synergistic effect of *Cardiospermum halicacabum* and *Butea monosperma* and its potential using silver nanoparticle.

The main objective of our study is to synthesis silver nanoparticle from aqueous extract of *Cardiospermum halicacabum* and *Butea monosperma* and to screen the phytochemical qualitatively and also evaluate the antibacterial activity.

Table 1: Phytochemical analysis of silver nanoparticles.

S.No	Phytochemical	Aqueous Solution
1.	Alkaloid	Present
2.	Flavonoid	Present
3.	Tannins	Present
4.	Phenols	Present
5.	Protein	Present
6.	Anthocyanin	Absent
7.	Terpenoids	Present
8.	Carbohydrates	Present

Table 2: Antibacterial activity of silver nanoparticles against pathogenic bacteria.

Microorganism	Zone of inhibition (in cm)		
	Contr ol	Ampicil lin	Silver nanoparticle
<i>Ecoli</i>	-	0.8	0.7
<i>Citro bacter</i>	-	1.3	0.1
<i>Streptococcus aureus</i>	-	1.2	0.9
<i>Pseudomonas</i>	-	05	0.7
<i>Citro bacteria</i>	-	1.7	0.8
<i>Bacillus cereus</i>	-	0.1	0.2
<i>Proteus vulgaris</i>	-	0.8	0.1

MATERIALS AND METHODS

Sample collection

Cardiospermum halicacabum fresh and healthy plant was collected from local markets of Virudhunagar, Tamilnadu, India. *Butea monosperma* leaves were collected from our college campus. The sample was washed thoroughly under running tap water to remove dirt and then shade dried at room temperature.

Preparation of Leaf extract

About each 15g of *Cardiospermum halicacabum* and *Butea monosperma* sample were weighted separately and transferred into 500ml beaker containing 300 ml of distilled water and boiled for 20 minutes. The extracts were then filtered thrice through Whatmann No.1 filter paper to remove particulate matter and to get clear solution and stored in dark place used for the further analysis.

Silver nanoparticle synthesis

1mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. Sample and silver nitrate was added in 1:2 ratio kept at dark place at 72 hours inhibition time. The leaf extract was added for reduction of silver ions into silver nanoparticle. In the mean time, color change of the mixture from green color to dark black.

Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the silver nanoparticle¹¹.

Test for alkaloids- Wagner's Test

A fraction of extract was treated with Wagners test reagent (1.27g of iodine and 2g of potassium iodide in

100ml of water) and observed for the formation of reddish brown colour.

Flavonoids- Sodium hydroxide Test

A small amount extract was treated with aqueous sodium hydroxide and hydrochloric acid and observed for the formation of yellow orange color

Test for Tannins- Braymers Test

Few ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish color solution.

Test for Phenols- Ferric Chloride Test

The fraction of extract treated with 5% ferric chloride and observed of deep blue or black color.

Test for Protein- Ninhydrin Test (Aqueous)

The few ml of extract was treated with aqueous ninhydrin and observed the formation of purple color it indicate the presence of protein.

Test for anthocyanin - sodium hydroxide test

A small amount of extract was treated with sodium hydroxide and observed for the formation of blue green color.

Antibacterial activity

The agar well diffusion method was employed for determination of the antibacterial activity of silver nanoparticle. Six different suspension culture *Escherichia coli*, *streptococcus aureus*, *Pseudomonas aerogeinosa*, *citrobacter*, *Bacillus cereus* and *micrococcus* was spread on nutrient agar medium by spread plate technique. The plates were incubated at 37°C for 24hours. Water served as control. Ampicillin is used as standard. The diameter for the zone of inhibition was measured in centimeter (cm).

RESULTS AND DISUCSSION

The formation of silver nanoparticles was carried by aqueous extract of *Cardiospermum halicacabum* and *Butea monosperma*. The extract was incubated for reduction of silver ions to silver nanoparticles as there was rapid reaction. The appearance of a brown color indicates the formation of colloidal silver nanoparticles. Excitation of Plasmon vibration may be cause of this color change¹². Intensity of color increased result from high nanoparticles formation. Phytochemical screening suggests that silver nanoparticles of aqueous extract of *Cardiospermum halicacabum* and *Butea monosperma* contain various constituents are tabulated in table I. The preliminary phytochemical analysis showed the presence of alkaloid, flavonoid, tannins, phenols, protein, terpenoids, and carbohydrates. The presence of wide range of phytochemical constituent indicates that the plant could be used in a multiple ways may be beneficiary to the population¹³. An important part of natural products from plants, biomolecules and secondary metabolites usually exhibits some kind of biological activities. They are widely used in the human therapy, veterinary, agriculture, scientific research and in countless other areas¹⁴. The usefulness of plant materials medicinally is due to the presences of bioactive constituents such as alkaloid, flavonoid, tannins, phenols, protein, terpenoids, and carbohydrates compounds¹⁵. The presence of flavonoids and tannins in all the plants is likely to be

responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers¹⁶. Anthocyanins exhibit important antioxidant and anti-inflammatory actions as well as chemotherapeutic effects¹⁷. Results of antibacterial activity of the nanoparticles on the pathogenic bacteria showed in table 2. It was obvious from the results, the extract showed significant activity against *Pseudomonas* and *Streptococcus aureus* compare to positive control. *Bacillus cereus* and *Proteus vulgaris* organism has minimum inhibitory effect against this extract. Phytochemical constituents such as alkaloid, flavonoid, tannins, phenols, protein, terpenoids, and carbohydrates are secondary metabolites of medicinal plants that serve as defense mechanism against many microorganisms and insects¹⁸.

Based on earlier reports, secondary metabolites found in plants are phenols and terpenoids which represent the main antimicrobial agent. Similarly, aromatic compounds such as phenolic acids, alkaloids and flavonoids have also been identified as antimicrobial agents¹⁹. Flavonoids show anti allergic, anti-inflammatory, anti-microbial and anti-cancer activity²⁰. Our study report also coincides with Siddiqui S, et al¹⁹.

CONCLUSION

A phytochemical screening carried out with silver nanoparticle extract has revealed the presence of alkaloid, flavonoid, tannins, phenols, protein, terpenoids, and carbohydrates compounds which are responsible for antimicrobial activity. The results of this study have shown a significant antimicrobial activity against the tested organisms. This extract could serve as potential sources of new antimicrobial agents.

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