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Antihelmenthic And Antimicrobial Activity Of Green Synthesized Silver Nanoparticles From Illicium Verum Hook.F. Fruit

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Abstract: *Illicium verum Hooker fillius or commonly called star anise is a member of Magnoliaceae. The synthesis of metal nanoparticles is a growing area for research due to its potentiality in the application and development of advanced technologies. In general, nanoparticles are synthesized by using chemical methods which are not eco-friendly. Here, we have used a fast, convenient and environment-friendly method is green synthesis of silver nanoparticles. The objective of the present study is to synthesize silver nanoparticles by reducing Ag+ ions present in the aqueous solution of silver nitrate with the help of fruit extract of Illicium verum and to study the antimicrobial, antihelmenthic activity of green synthesized silver nanoparticles. The antibacterial activity of these nanoparticles was studied against Bacillus subtilis, S. cerevesiae. and Escherichia coli. Silver nanoparticles showed inhibition of growth suggesting antibacterial property and the time intervals between paralysis and death of the earthworms shows the anthelmenthic activity of the synthesized silver nanoparticles.*
Keywords: *Antihelmenthi, Bacillus subtilis, Environment friendly, Green synthesized nanoparticles, Silver nanoparticles*

I. Introduction

Nanoparticles have properties which are based on the characteristics such as size and shape. Nanoparticles are being viewed as fundamental blocks of nanotechnology. The most important and Distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. The most effectively studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Nanotechnology deals with the production and stabilization of various types of nanoparticles [1]. In order to obtain nanoparticles in large quantities within a short period, physical and chemical procedures are used .(Ag NPs) have rapidly increased due to their unusual optical, chemical, electronic, photo-electrochemical, catalytic, magnetic, antibacterial, and biological labeling properties [2] chemically synthesized Silver nanoparticles were used in broad range of applications like biomedical [3], drug delivery [4], food industries [5], agriculture [6], textile industries [7], water treatment [8] as an antioxidant [9], antimicrobial [10], anti-cancer [11], cosmetics [12], ointments [13], and larvicides [14]. Nanoparticle synthesis is usually carried out by various physical and chemical methods using various hazardous and toxic chemicals. The green synthetic approaches for AgNPs are an alternative source to conventional method and currently several groups of researchers are concentrating on biomimetic approaches such as plant or plant leaf extracts, nuts, microorganisms and yeast to synthesize the metal nanoparticles which is a "green chemical or phytochemical" approach. Biologically synthesized silver nanoparticles (Ag-NPs) have wide range of applications because of their remarkable physical and chemical properties. The use of green chemistry is an interesting synthetic procedure for nanoproducts, which can be applied potentially in different fields of medicinal [19, 20], biolabelling [21], microelectronics [22], information storage [23] optoelectronic devices [24] and in the catalysis of chemical reactions [18]. At present, several groups of researchers concentrating on biomimetic approaches such as plant or plant leaf extracts, nuts, microorganisms and yeast to synthesize the metal nanoparticles called as "green chemical or phytochemical" approach [25-28]. Illicium verum is an evergreen medium sized tree belonging to family Magnoliaceae and is the native of China. The Fruits of the illicium consist star like appearance and fruit is the main valued product of the plant hence it called as star anise the fruits and seeds are valued as a spice used in cooking. The fragrant wood is used for construction and furniture. Star anise is mainly grown for the essential oil that is extracted from seeds and fruit. The oil of star anise is used worldwide in medicine. It is used as stimulant, eupeptic, carminative, mildly expectorant and diuretic. It is found to be useful in flatulence, spasmodic pains and dysentery. It relieves colic and is a common ingredient of cough lozenges and cattle sprays. The oil is employed as an applicant in rheumatism, as an antiseptic, useful against fevers, scabies, body lice, bedbugs, and highly useful in constipation and insomnia activities as well. So far, there have been no reports on the synthesis of nanoparticles by using fruit extract. The current work is on the extracellular synthesis of AgNPs at room temperature using the aqueous extract of the fruit of Illicium verum as a simple, low cost and reproducible method and to evaluate the antibacterial and antihelmenthic activity of these nanoparticles.

II. Materials and Methods

2.1 Preparation of Illicium verum extracts

The fruits of Illicium verum were collected and were thoroughly washed thrice with double distilled water, sliced into pieces, shade dried and then crushed into powder using a grinder (Joya, 16-002). An intense brown colour Illicium verum extract was obtained after mixing 10 g of the fruit powder in 200 mL double distilled water and then boiling it for 10 min on a hot plate. The residue was separated by filtration through Whatman No. 1 filter paper. The filtrate obtained was stored in a refrigerator for further use.

2.2 Synthesis of silver nanoparticles from Illicium verum fruit extract

Synthesis of AgNPs Silver nitrate (AgNO3) (BDH, Poole, England) of analytical reagent The aqueous solution of 1mM silver nitrate (AgNO3) was prepared and used as a precursor for the synthesis of AgNPs. Silver nanoparticles were prepared by adding 5 mL of the extract to 50 mL of aqueous silver nitrate solution (0.1 M) at room temperature. The mixture was hand shaken and kept in the laboratory at room temperature. The experiment was continuously observed and the color change indicates the formation of silver nanoparticles.


Fig-1 fruit and fruit powder

Fig-2 Green Synthesized silver nanoparticles

2.3 Antimicrobial activity

2.3.1Antimicrobial activity of AgNPs synthesised from Illicium verum aqueous fruit extract.

2.3.1.1Test microorganisms

Bacterial strains such as, Staphylococcus aureus, Escherichia coli and Bacillus subtilis were used in the present study. All the tested strains were obtained from the Department of Microbiology, Acharya Nagarjuna University, Guntur. These test cultures were grown in nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient agar slants at 4ºC.

2.3.1.2Agar-well diffusion method

The assay was conducted by agar well diffusion method. The bacterial strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards (108 CFU/ml). 1 ml of test strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations (10 µl,20 µl,, 30 µl, 40 µl) of AgNPs solution were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37^{o}C. After incubation for 48h, the plates were observed for zones of inhibition. The diameter of zone of inhibition was measured and expressed in millimetres. $AgNo_3$ solution and plant aqueous extract was used as negative control. The experiments were conducted in triplicates.

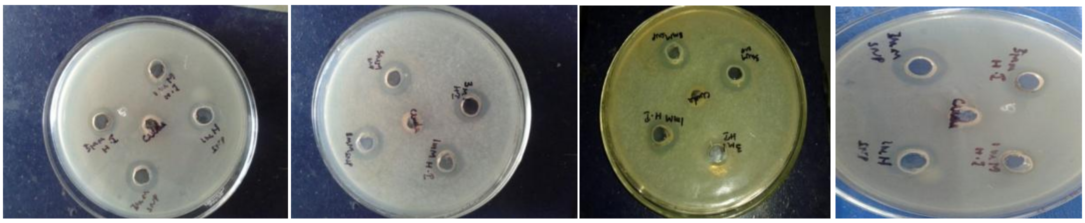


Fig:3 Anti Microbial activity of green synthesized silver nanoparticles

2.4 Anthelmintic activity

The **Illicium verum** fruit extract with suspended silver nanoparticles were checked for its antihelminthic property. Solution of standard antihelminthic drug (piperazine citrate, 10 mg/ml) was also prepared in distilled water. Normal saline is used as a control. Eight groups of approximately equal size of earthworms, consisting of six in each group, were released into the petridish. Time period taken for paralysis and death of individual worms was observed and was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^0C. The antihelmintic activity was evaluated by adopting the standard method and it was observed that the fruit extract with suspended silver nanoparticles showed better anthelmintic activity when compared with the aqueous extract of **Illicium verum** fruit. The time taken to cause paralysis and death of worm's aqueous extract. The antihelmintic activities of normal saline, standard drug, different concentrations of aqueous extract and silver nanoparticles are shown. Overall the antihelminthic activity revealed the concentration dependent nature of the extracts and silver nanoparticles. It was found that colloidal solution of silver nanoparticles using **Illicium verum** possessed more antihelmintic activity than aqueous extract of **Illicium verum** fruit. From this study it may be concluded that, in addition to products of plants, silver nanoparticles using **Illicium verum** fruit have more antihelmintic activity.

Live stage **Paralytic Stage** **Death Stage**

Fig: Antihelmenthic activity of green synthesized silver nanoparticles

III. Results

1.1 Synthesis of Ag nanoparticles using Illicium verum fruit extract (Green synthesis)

For the synthesis of silver nanoparticles, 5 ml of leaf extract was added to 50 ml of 1mM AgNO3 solution. The solution turned colourless to brown within 10 minutes. Ag nanoparticles exhibit light brown colour in aqueous solution due to excitation of surface plasmon resonance On mixing the extract with aqueous solution of the Ag ion complex, a change in the colour from light brown to dark brown was observed. It was due to the reduction of Ag+ which indicates the formation of Ag nanoparticles. A visible colour change in 10 min indicates the formation of silver nanoparticles which was confirmed by UV-visible analysis. The further change of colour to dark orange-brown is because of the increased concentration of the silver nitrate solution employed and there was no significant change in colour after 30 minutes which is an evidence for the completion of reduction reaction.

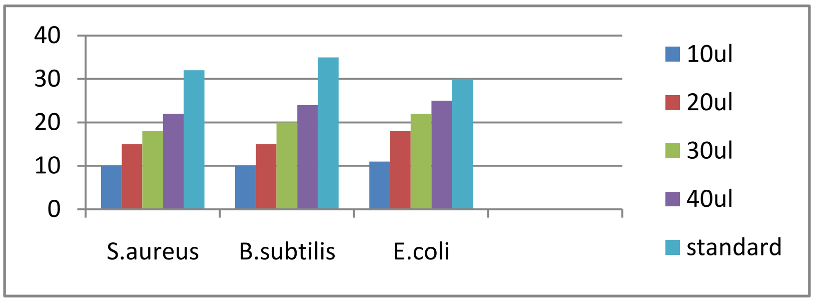
3.2 Antimicrobial activity of AgNPs of Illicium verum fruit extract

The Ag NPs of **I. verum** fruit at 40 µl/well showed maximum antibacterial activity against E. Coli, (25.00 mm), followed by Bacillus subtilis (24.00mm) and minimum of 22.00mm activity against Staphylococcus aureus. Similarly the antimicrobial activity was directly proportional to the concentration of AgNPs. Two negative controls i.e., Plant aqueous extract and $AgNO_3$ solution did not show any activity against any tested strains. Streptomycin used as standards against bacteria showed the inhibition zones of 35.00mm.

Table-1 Silver nanoparticles using Illicium verum fruit showd antimicrobial activity

Bacterial strains	10 µl	20 µl	30 µl	40 µl	standard
S. aureus	10.00	15.00	18.00	22.00	32.00
B. subtilis	10.00	15.00	20.00	24.00	35.00
E. coli	11.00	18.00	22.00	25.00	30.00

S. aureus= Staphylococcus aureus, B. subtilis= Bacillus subtilis, E. coli =Escherichia coli. S=Streptomycin



3.3 Antihelminthic activity analysis

Silver nanoparticles using Illicium verum fruit showed better anthelmintic activity when compared with the aqueous extract of Illicium verum fruits. Nanoparticles show less time to cause paralysis and death of worms followed by aqueous extract. The data obtained on the antihelminthic activities of normal saline, standard drug, different concentrations of aqueous extract and silver nanoparticles overall revealed the concentration dependent nature of the extract and silver nanoparticles in bringing out this bioactivity. It was found that the colloidal suspension of silver nanoparticles prepared in Illicium verum fruit extract showed more antihelminthic activity than the aqueous extract of Illicium verum fruits. From this study it may be concluded that, the phytochemical components along with the silver nanoparticles have more anthelmintic activity.

Table-2 Silver nanoparticles using Illicium verum fruit showd anthelmintic activity

Drug tested	Concentration(mg/ml)	Paralysis time (min)	Death time (min)
Normal Saline	-	-	-
Perazine Citrate (Standard)	10	12	22
Aqueous extract	60 30 15	8 20 30 19	15 25 50 30
Green synthesized silver particles	0.1 0.05 0.025 0.058	4 16 13 20	10 30 40 26.6

Significant dose dependent effects observed for most of the plants in current experiments indicate the fact the increasing the dose of the plant extracts increases the proportion of the chemical ingredient with pharmaceutical value in the crude plant extract.

IV. Conclusion

The treatment of nematode infections using conventional anthelmintic drugs is cost effective which will consequently lead to the rising costs of livestock management. There is research evidence reporting that some antihelminthic drugs cause common side effects are **dizziness,** drowsiness, **headache,** sweating, dryness of the mouth and eyes, loss of appetite, **diarrhea, nausea,** vomiting and **birth defects** or **miscarriage** in animal studies. More serious side effects, such as **fever,** chills, confusion, extreme weakness, **hallucinations,** severe diarrhea, nausea or vomiting, skin **rashes, low back pain,** dark urine, blurred vision, seizures, and **jaundice** have been reported. Antihelminthic drugs may interact with each other or with other drugs and a given antihelminthic drug with another medication may increase the risk of side effects from either drug . Hence the identification of novel promising anthelmintic plant extracts such as Illicium verum extract may contribute for the development of phytotherapic products that could be more cost effective, safer, and more accessible and provide a lower risk of resistance than the conventional therapeutic drugs currently employed. Further attention has to be carried out for isolation and characterization of the active components to establish an effective drug resource scientifically.

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