BIO SYNTHESIS AND ANTHELMINTIC ACTIVITY OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *SARACA INDICA* LEAVES

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ABSTRACT

Present study was conducted to know the anthelmintic activity of colloidal solution of silver nanoparticles using *Saraca indica* leaves extract. The bio-synthesized nanoparticles were characterized using UV-visible (UV-vis) spectrophotometer, transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infra-red (FTIR) spectrometry. The nanoparticles were found to be spherical in shape and up to 50 nm in size.

It was found that anthelmintic activity of colloidal solution of silver nanoparticles was more than the aqueous extract. Overall the anthelmintic activity revealed the concentration-dependent nature of the aqueous extracts and colloidal solution of silver nanoparticles. Different concentration of aqueous extracts and colloidal solution of silver nanoparticles were tested against adult Indian earthworms (*Pheretima posthuma*) as test worms. The bioassay involved determination of the time of paralysis and time of death control. Piperazine citrate (10mg/ml) was used as a standard reference drug. Normal saline was used as a control.

Key words: *Saraca indica*, silver nanoparticles, aqueous extract, *Pheretima posthuma*, anthelmintic activity.

INTRODUCTION

Nanoparticles have shown exceptional electronic, catalytic, optical, magnetic and other physical and chemical properties that are quite different from the bulk one [1]. Silver nanoparticles have proven useful in antibacterial clothing, burn ointments and as coating for medical devices because of their mutation-resistant antimicrobial activity [2]. To fulfill the growing need of environmental friendly nanoparticles, researchers are using microorganisms for the synthesis of various metal nanoparticles [3-5]. Plant leaf extract of *onion* [6], *syzygium cumini* [7], *basil* [8] and *banana peel* [9] had been used for the synthesis of gold and silver nanoparticles, which lead to formation of pure metallic nanoparticles of silver and gold and can be used directly. The chemical methods are extremely expensive and use toxic chemicals which may pose potential environmental and biological risks. *Saraca Indica* (Family Caesalpinaceae) plant had been used for medicinal purposes long before reported history. In *Mahākāvya,* or Indian epic poetry, the ashoka tree is mentioned in the Ramayana in reference to the Ashoka Vatika (garden of Ashoka trees) where Hanuman first meets Sita. It is medium sized and evergreen tree up to 9 m in height with orange yellow coloured flowers and arranged in dense corymbs. It occurs throughout India up to an altitude of 750 m in central and eastern Himalayas (10). Useful parts of the plant are barks, leaves, flowers and seed. The plant is also effective in dyspepsia, fever, burning sensation, colic, ulcer, menorrhagia, leucorrhoea, pimples, etc. It has already been mentioned that barks and leaves of the plant possess anthelmintic activity (11,12). Extract of *Saraca indica* leaves has been reported to possess potent anthelmintic, analgesic, anti-microbial activity, CNS depressant, antiulcer, anti-inflammatory, larvicidal, anti-diabetic, shigellocidal, uterinetonic activity (13). Moreover, traditional system of medicine reports the efficacy of several natural products eliminating helminths. Considering its importance, the present communication deals with the evaluation of anthelmintic activity of aqueous leaves extract of *Saraca indica* and silver nanoparticles using *Saraca indica leaves*.

MATERIALS AND METHODS

2.1. Preparation of aqueous extract and Synthesis of silver nanoparticles

Freshly collected *Saraca indica* leaves were washed with deionized water and 100 ml double distilled water was added to the flask containing 5 g finely chopped leaves and heat it for 20 minutes at 60^0C. Then the raw extract obtained was filtered in hot condition with 11-micron mesh to remove fibrous impurities. The resultant clear extract was used for the synthesis of silver nanoparticles. For

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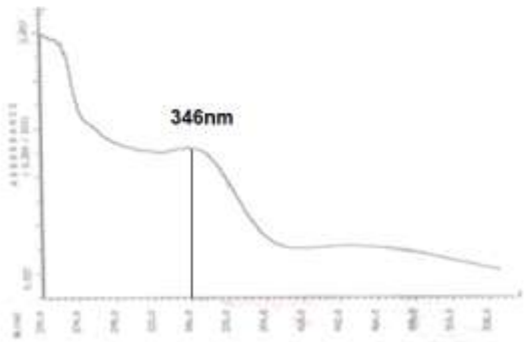
reduction of Ag^+ ions, 10 ml aqueous *Saraca indica* leaves extract was added to 50 ml of 10^{-3} M aqueous $AgNO_3$ solution. Optical density was measured by diluting suitable aliquots of the sample to a final volume of 2 ml using deionized water and subjected to UV-vis spectroscopy measurements. The synthesized nanoparticles were centrifuged at 8000 rpm for 10 min and subsequently re-dispersed in deionized water twice to get rid of any unbound biological molecules.

Figure 1: UV vis- spectra of biosynthesized silver nanoparticles



2.2 Earthworms

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil, washed with normal saline to remove the external matter and were used for anthelmintic activity. Adult Indian earthworms *Pheretima posthuma* of 3-4 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (14).

2.3 Anthelmintic activity

Prepared *Saraca indica* leaves extract and silver nanoparticles were used for anthelmintic study. Solution of standard anthelmintic 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 number in each group, were released into each petridish. Observations were made for the time taken for paralysis and death of individual worms. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^0C. The anthelmintic activity was evaluated by adopting the standard method (15).

RESULTS AND DISCUSSION

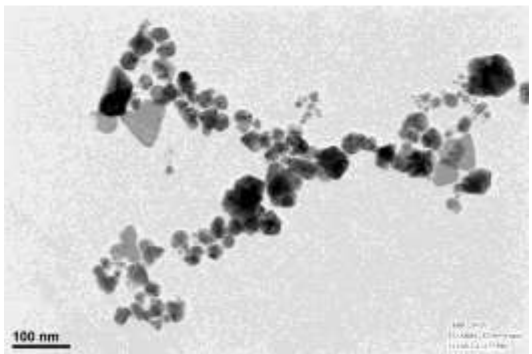
3.1. UV-vis spectral analysis

Formation and stability of silver nanoparticles in aqueous colloidal solution are confirmed using UV–vis spectral analysis. The tinge of brownish black colour was remarkable with the first appearance after 10 minutes of addition of leaves extract to aqueous silver nitrate solution due to the formation of silver nanoparticles. Thereafter intensity increases with the reaction time thus confirming the formation of silver nanoparticles. After of 24 h the intensity of brownish black colour did not increase confirming that that the reaction has come to an end. Characteristic surface plasmon absorption bands are observed at 346 nm for brownish black coloured silver nanoparticles synthesized from 10^{-3} M $AgNO_3$ solution using aqueous extract of Saraca indica leaves.

3.2. TEM analysis

TEM image of silver colloidal solution synthesized by treating from10^{-3}M $AgNO_3$ solution using Saraca indica leaves extract is shown in Fig. 2. It illustrates that the particles are predominantly spherical in shape with diameter ranging from 5 to 50 nm.

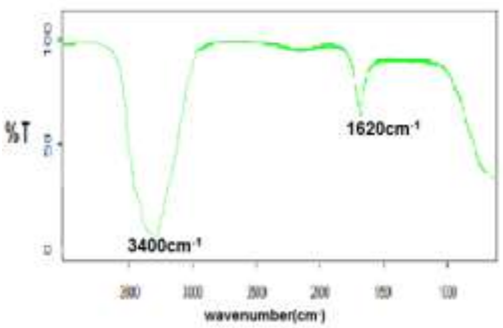
Figure 2: TEM image of biosynthesized silver nanoparticles



3.3 FTIR analysis

The FTIR spectra of silver nanoparticles are shown in Fig. 3. Some pronounced absorbance bands were observed at around 3400 cm-1 (OH stretching in alcohols and phenolic compounds) and 1620cm^{-1} (amide I arising due to carbonyl stretch in proteins), suggest the presence of proteins on the surface of Ag-core particles, and plant proteins in the NPs shell. As plant molecules get adsorbed onto the Ag NPs surface, the amide groups intends to form stronger bonds with Ag atoms, which will break most of the H-bonds between the N–H groups and lead the narrowing and blue-shifts of the amide bond. These results confirm the presence of possible proteins acting as reducing and stabilizing agents. These results indicated that the carbonyl group of proteins gets

Figure-3: FTIR of biosynthesized silver nanoparticles



adsorbed strongly to metals, indicating that proteins could have also formed a layer along with the bio-organics, securing nanoparticles. These IR spectroscopic studies confirmed that carbonyl group of amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nanoparticles and acting as capping agent to prevent agglomeration and providing stability to the medium (16).

3.4 XRD analysis

Fig. 4 shows the XRD patterns of silver nanoparticles synthesized from the aqueous leaves extract of Saraca indica. A number of Bragg reflections with 2θ values of 38.03°, 46.18°, 63.43°and 77.18 correspond to the (111), (200), (220) and (311) sets of lattice planes are observed which may be indexed as the band for face centered cubic structures of silver. The XRD pattern thus clearly illustrates that the silver nanoparticles synthesized by the present green method are crystalline in nature.

3.5 Anthelmintic activity analysis

Silver nanoparticles using *Saraca indica* leaves showed better anthelmintic activity when

Table-1:Anthelmintic activity of aqueous extract of Saraca Indica leaves and silver nanoparticles using Saraca indica leaves

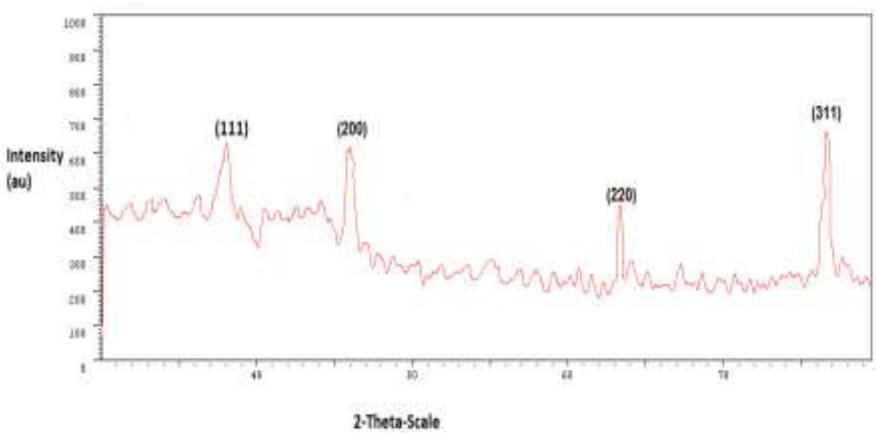
Drug tested	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Normal Saline	-	-	-
Perazine Citrate (standard)	10	12	22
Aqueous extract	50	10	20
	25	22	36
	10	38	68
Nano silver particles	0.1	4	19
	0.05	18	42
	0.025	27	65

compared with the aqueous extract of *Saraca indica* leaves. Nanoparticles show least time to cause paralysis and death of worms followed by aqueous extract. The anthelmintic activities of normal saline, standard drug, different concentrations of aqueous extract and silver nanoparticles are shown in Table 1. Overall the anthelmintic activity revealed the concentration-dependent nature of the extracts and silver nanoparticles. It was found that colloidal solution of silver nanoparticles using *Saraca indica* leaves possessed more anthelmintic activity than aqueous extract of *Saraca indica* leaves. From this study it may be concluded that, in addition to products of plants, silver nanoparticles using *Saraca indica* leaves have more anthelmintic activity.

CONCLUSIONS

The present study demonstrated the bio-reductive synthesis of nano sized silver particles

Figure4: XRD of biosynthesized silver nanoparticles



using *Saraca indica* leaves extract. *Saraca indica* proteins act as reductant as well as a capping material to protect the nanoparticle surfaces and prevent the particles from aggregation. It is a simple and efficient route of spherical shaped, fcc structured nanoparticles, with diameter range of 5 to 50 nm. Biogenic synthesis is also favoured due to its lower toxicity to environment denoting its merit and prompt for preference in various industrial and medical applications. It clearly indicates that the silver nanoparticles using *Saraca indica* leaves extract are more potent as compared to aqueous extract of *Saraca indica* leaves because silver nanoparticles took less time to cause paralysis and death of the earthworms.

ACKNOWLEDGEMENTS

We are grateful to Dr Sunita Rattan, Dr A.L.Verma, Dr B. Shukla and Dr A.K Chauhan of Amity University, Noida, UP, India for their encouragement and providing facilities for the fulfilment of this study.

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