IJPSR (2017), Volume 8, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 March, 2017; received in revised form, 02 November, 2017; accepted, 11 November, 2017; published 01 December, 2017

BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *HIBISCUS ROSA SINENSIS*: AN APPROACH TOWARDS ANIMICROBIAL ACTIVITY ON FISH PATHOGEN AEROMONAS HYDROPHILA

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Keywords:

Hibiscus rosa-sinensis, Silver nanoparticles, *Aeromonas Hydrophila*, UV- Vis Spectra, FT-IR, SEM

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ABSTRACT: *Aeromonas hydrophila* is a gram negative bacterium which is primary or secondary cause of ulcers and hemorrhagic septicaemia in fish. The treatments for this infection are only restricted to some antibiotics. So, novel materials are being searched for combating with bacterial infections and the resulting consequences. In this presence study, the green synthesized silver nanoparticles using *Hibiscus rosa sinensis* extract act as reducing agent. The silver nanoparticles have shown bactericidal effects against *A. hydrophila* isolated from infected fish *Catla catla*. The silver nanoparticles were characterized by using UV–Visible, fourier transform infra-red spectroscopy (FT-IR) and scanning electron microscopy (SEM) techniques. SEM results showed that the particles were in the size range of 5µm. FT-IR prediction indicated the presence of C–H stretch, C–H bend, N–H bend, –C≡C– stretch, H–C=O: C–H stretch, C–H stretch respectively on the AgNPs. The maximum zone of inhibition 10 mm observed by normal cured against *A. hydrophila* is. The synthesized silver nanoparticles exhibited maximum zone of inhibition 11 mm against *A. hydrophila*. As a conclusion of the study, these biologically synthesized nanoparticles were found to be highly effective against *A. hydrophila* infected fish. This study revealed that *H. sinensis* synthesized silver nanoparticles exhibit good and potent antimicrobial activity against fish pathogen.

INTRODUCTION: Aquaculture is the fastest growing food producing sector in the world, with an average annual growth rate of 8.9% and practiced in a variety of agro-climatic zones ranging from tropical to temperate area [1]. It includes farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Fish is an important component of human diet and one of the cheapest sources of quality animal protein and beneficial fat and various micro nutrients available to millions across the world.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(12).5241-46

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (12).5241-46

Moreover, during the past several decades fisheries and aquaculture are contributing to global food security, poverty alleviation, rural livelihoods, employment and income generation [2]. Fish disease is one of the major threats to the sustainable development of aquaculture causing loss of millions of dollars annually.

The bacterial infections are considered the major cause of mortality in aquaculture. Among the common fish pathogens, *Aeromonas hydrophila* is a gram negative bacterium that can grow both in aerobic and anaerobic condition and causes a variety of diseases in fish. There are contradicting views on whether the microbe is a primary cause of diseases or an opportunistic one causing diseases to hosts that are immune compromised and stressed [3]. Day by day, the resistance to existing antibiotics or

drugs is increasing for one or more reasons. This increasing incidence of antibiotic resistance among the microbial organisms necessitates an alternate therapy to curb the resistant infectious micro - organisms [4]. A new approach to prevent or combat microbial pathogens is by the use of silver nanoparticles especially synthesized with the help of natural medicinal plants. Medicinal plants are already known for many therapeutic values and have been used since ages for curing many diseases and disorders including infectious diseases.

In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. One of the plants are used is *Hibiscus rosa sinensis* is an ornamental plant traditional used as for anti microbial, demulcent, aphrodisiac, anodyne, laxative, emollient. The phytochemicals found in this are medicinally steroids, flavonoids, tannins, reducing sugar, anthocyanin pigment, carotene, thiamine, riboflavin, niacin and ascorbic acid [5].

It has various activities like antitumor, antidiarrheal, antiestrogenic, antispermatogenic, ndrogenic, antiphologistic [6], anti-fertility [7], wound Healing anticonvulsant [8]. The bacterial infection play important role in many of the pathological conditions where extracts of *H. sinensis* extracts have been used as traditional medication. Considering the therapeutic potential of this plant. Hence, by keeping the above concept in mind, in the present study, we wish to synthesis, characterization and evaluation of silver nanoparticles as a new class drug against *A. hydrophila* isolated from infected fish.

MATERIALS AND METHODS:

Collection of Experimental Plant: Fresh healthy Hibiscus rosa sinensis was collected from their natural habitat of Herbal Garden, AMET University campus, Chennai and authenticated according to the manual of Mukerjee [9].

Preparation of Extraction: The coarse powder plant material was extracted with ethanol by using soxhlet apparatus. The solvent were removed under reduced pressure to get semisolid mass. The phytochemical screening of the extract was followed by the standard method [10].

Preparation of 1 mm Silver Nitrate Aqueous Solution: The synthesis of silver nanoparticle was performed by Vijayaraj *et al*., [11]. 5 mL of the ethanolic leaf extract of *H. sinensis* was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50 mL of 1 mM $AgNO_3$ solution was added drop wise with constant stirring of 120 rpm at 50 - 60 °C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 5 hours was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the ethanolic extract of *H. sinensis* to generate extremely stable silver nanoparticles.

Characterization Techniques:

UV- Vis Spectra Analysis: The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV - Vis spectrum of the PerkinElmer spectrophotometer at a resolution of 1 nm (from 300 - 600 nm) in 2 mL quartz cuvette with 1 cm path length.

SEM Analysis: The Morphological characterization of the samples was done using JEOL Jsm- 6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater. After that the material was subjected to analysis.

FT-IR Analysis: The characterization of functional groups of samples were investigated by FT IR analysis (Shimadzu) and the spectra was scanned in the range of 4000 cm^{-1} to 400 cm^{-1} range at a resolution of 4 cm^{-1}. The sample was prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc.

Antibacterial Assay: Antibacterial activity of test sample was investigated against *A. hydrophila* bacteria were isolates from infected fish, collected from Kanathur fish landing centre. The *A. hydrophila* was isolated from infected fish by the method of Carnahan *et al*., [12] The isolated microorganism was cultured on nutrient agar at 37ºC for 24 hrs. The antibacterial activity was carried out by agar well diffusion method using different concentration 20 µg/mL, 50 µg/mL, 100

µg/mL, and 200 µg/mL. Four wells of 5 mm size each made into the agar plates with the help of sterile cork borer. The wells were loaded with 50 µL of crude flower extract and silver nanoparticle synthesized extract. All the plates were incubated at 37 °C for 24 hours. After incubation, the plates were observed for clear inhibition zone around the well. Indicated the presence of antibacterial activity of tested samples. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well. Compare the effects with Tetracycline as a reference antibacterial drug. The experiments were performed in triplicates and the optical density values are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION: Herbal medicines are popular remedies for a number of diseases and used by a vast majority of the world's population. Since pre historic times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Further, most of our marketed medicines are distillations, combinations, reproductions or variations of substances which are abundantly found in nature. Our forefathers recommended some of the substances, which are abundantly found in nature long before their pharmacological actions were demonstrated and understood by scientific validations [13]. The medicinally active plant compounds are usually their secondary metabolites like terpenoids, quinones, flavonoids, tannins *etc*. Natural drugs have been a part of the evolution of human, healthcare for thousands of years.

Nowadays nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered [14].

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, *etc*. which have been found in vitro to have medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds [15].

Phytochemicals from medicinal plants serve as lead compounds in antimicrobial discovery [16]. Literature reports revealed that natural materials areas sources of new antibacterial agents. In the presence study phytochemical analysis was performed based on standard method. **Table 1** represent phytochemical content such as alkaloids, terpenoids, flavonoids, Phenol, Tannins, phytoserol, Carbohydrates and saponins are present in the test sample.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *H. SINENSIS*

S. no.	Phytochemical compounds	Results
1	Alkaloids	+
2	Flavonoids	+
3	Steroids	+
4	Saponins	+
5	Tannins	+
6	Terpenoids	+

The use of nanotechnology to synthesize compounds with improved anti microbial properties is an area of current research by many scientists. In our study, we report the non toxic, practical and environmentally benevolent approach for the synthesis of silver nanoparticles using the ethanolic extract of *H. sinensis* plant with potent anti microbial activity. The production of cytokines are key events in the regulation of an microbial response and recent attention has been focused on the effect of the synthesized nanoparticles as selective cytokine inhibitory agents [17].

Biological synthesis of nanoparticles, the aqueous metal ion precursors from metal salts is reduced and as a result a colour change occurs in the reaction mixture. This is the first qualitative indication that nanoparticles are being formed. One interesting property of colloidal particles in solution, due to their size and shape, is their ability to be seen when a laser beam passes through the colloidal solution. This effect is known as the Tyndall effect and is a simple and straight forward technique that can be used to detect the presence of nanoparticles in solution [18].

The change of colour indicates the formation of silver nanoparticles (SNPs) [19]. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol.

The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. In the present findings the UV-Vis spectrum of silver nanoparticles synthesized from *H. sinensis* have strong absorbance peaks at 437 nm **Fig. 1**. The observed peak at 437 nm is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field [20].

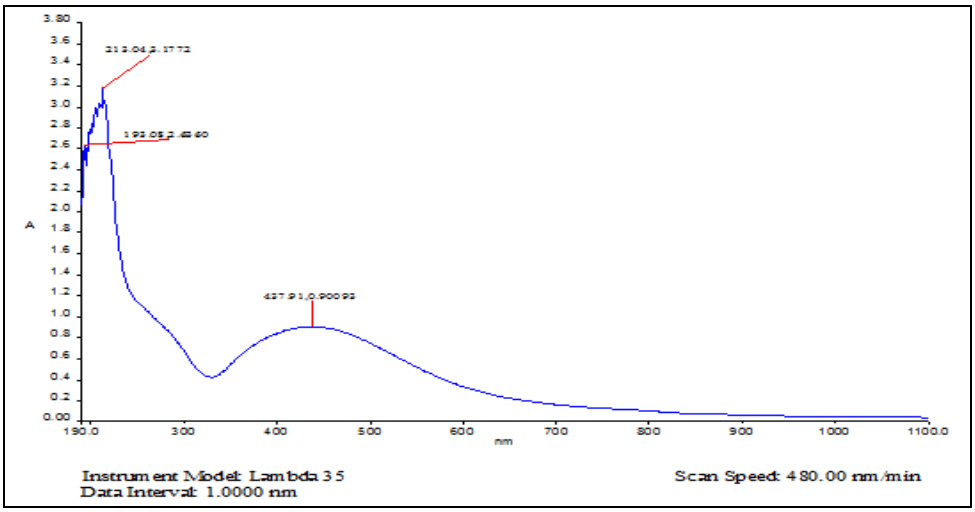

FIG. 1: UV-VIS SPECTROSCOPY OF *H. SINENSIS*

According to Schaffer *et al.*, [21] the silver nanoparticles are cubical, rectangular, triangular and spherical in shape with uniform distribution. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilizes the nanoparticles. The morphological features of synthesis silver nanoparicles were studied by SEM analysis **Fig. 2**.

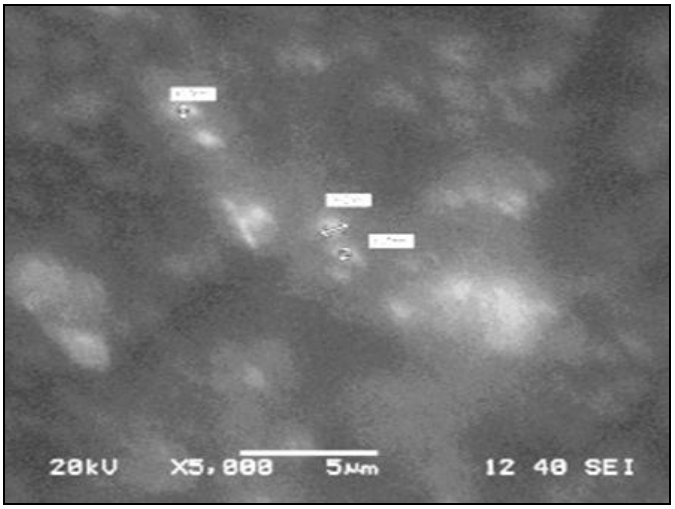

FIG. 2: SEM ANALYSIS OF *H. SINENSIS* SILVER NANOPARTICALES

SEM analysis of *H. sinensis* capability to synthesize silver nanoparticles are most of the particles are spherical in shape. The size of the silver nanoparticles was found to be 5 - 50 nm, with an average size 15 nm. The particle size, size of distribution and shape of silver nano particles are the important parameters that govern the properties and hence it has wide applications in medicinal fields.

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules [22]. FTIR gives the information about functional groups present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the bio fabrication of silver nanoparticles mediated by the plant extracts. **Fig. 3** shows the FTIR spectrum of *H. sinensis* mediated synthesized AgNPs, the silver nitrate salt and dried *H. sinensis*. In $AgNO_3$ peaks were observed at 653.87 cm^{-1}, 1411.89 cm^{-1}, 1647.21 cm^{-1}, 2048.40 cm^{-1}, 2522.89 cm^{-1}, 2835.36 cm^{-1} which are associated C–H stretch, C–H bend, N–H bend, –C≡C– stretch, H–C=O: C–H stretch, C–H stretch respectively. It may be inferred that these biomolecules are responsible for capping and efficient stabilization of synthesized nanoparticles.

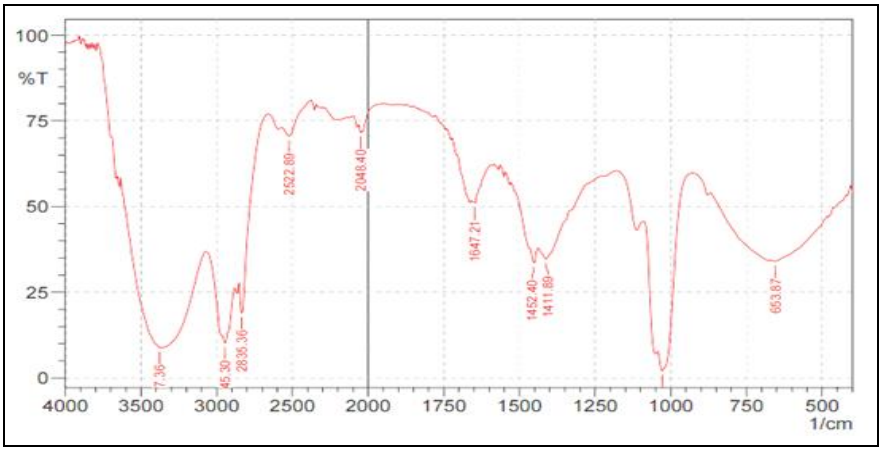

FIG. 3: FTIR ANALYSIS OF *H. SINENSIS*

TABLE 2: CHARACTERISTIC FTIR ABSORPTION OF *H. SINENSIS*

Frequency, cm^{-1}	Bond	Functional Group
653.87	C–H stretch	Alkanes
1411.89	C–H bend	Alkanes
1647.21	N–H bend	1° amines
2048.40	–C≡C– stretch	alkynes
2522.89	H–C=O: C–H stretch	aldehydes
2835.36	C–H stretch	Alkanes

According to Rai *et al*., [23] the silver nanoparticles is that they are known to have an antibacterial effect. However, the AgNPs formed during the *Aloe vera* hydrothermal method, AgNPs and *Aloe vera*, need to have bioactive functions. It is especially important to understand the functional effects on microorganisms in order to develop novel antibacterial agents. The silver ions from silver nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death [24]. The antibacterial activities of normal and silver nanoparticle synthesized *H. sinensis* extract were carried out by agar well diffusion method.

The maximum zone of inhibition by normal flower extract against *A. hydrophila* is 9 mm. The synthesized silver nanoparticles exhibited maximum zone of inhibition against *A. hydrophila* is 18 mm. Similar report was reported that the biological synthesis of silver nanoparticles from *Dracocephalum moldavica* seed extract showed excellent antimicrobial activities against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, Staphylococcus epidermidis* and *Bacillus subtilis* [25]. The silver nanoparticles shows maximum zone of inhibition against bacterial fish pathogen. These results indicated that silver nanoparticles have good antibacterial activity against *A. hydrophila*.

TABLE 3: ANTIMICROBIAL ACTIVITY OF *H. SINENSIS* SYNTHESIZED AgNPs AGAINST *A. HYDROPHILA*

Concentrations (µg/mL)	20	50	100	200
Control	0	0	0	0
Crude	16 ± 2.5	14 ± 2.5	10 ± 1.85	0
AgNPs + *H. sinensis*	14 ± 1.3	11 ± 1.7	0	0

CONCLUSION: The confirmatory reports of our studies indicate herbal medicine in fish health management and curing of disease proves to be prompt alternate. It is conclude from the obtained results, that herbal treatment for *A. hydrophila* infections of fish by *H. sinensis* are highly effective. Improvement in herbal drugs can be tried in different plants and combination. This can also be tried in other fishes so that it will be a big boom to aqua culturists. Further studies can be carried out on valuable herbal resources and standardization of dosage.

ACKNOWLEDGEMENT: Authors are thankful to the AMET University management for Facilities provided.

CONFLICT OF INTEREST: Nil.

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How to cite this article:
Vijayaraj R and Kumaran NS: Biosynthesis of silver nanoparticles from *Hibiscus rosa sinensis*: An approach towards animicrobial activity on fish pathogen aeromonas hydrophila. Int J Pharm Sci Res 2017; 8(12): 5241-46.doi: 10.13040/IJPSR.0975-8232.8(12).5241-46.