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Biological Synthesis And Validation Of Silver Nanoparticles From Leaves Of Lawsonia Inermis L. – An Ethnomedicinal Plant For Skin Diseases

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Abstract: Lawsonia inermis L. an ethnomedicinal plant. The ethnic groups of Kurnool district, Andhra Pradesh, India are extensively using to cure leucoderma. Biological synthesis of silver nanoparticles was carried out from leaves aqueous extract of Lawsonia inermis 10 ml root extract was mixed to 90 ml of 1 mM aqueous of Ag(NO3)2 and was heated at 60-800C for 20 min. The colour change of aqueous solution from orange to dark brown color was observed. For characterization using UV-Vis spectrophotometer and AFM. AFM, UV-Vis spectrophotometer showed the formation of silver nanoparticles with spherical shape and average size 27.66 nm. SNPs have good antimicrobial activity against different microorganisms.

Keyword: Medicinal plant; Silver nanoparticles; Atomic Force Microscope (AFM); Inhibition zone; Secondary metabolites; Anti microbial efficacy.

INTRODUCTION:

Since ancient time, people have been exploring the nature particularly plants in search of new drugs. In this connection a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic, Sidha and Unani. WHO is encouraging the traditional drugs because of its less side effects and matter of low cost and easy availability1. Everyday new inspiring information is being added to folklore medicine for the development of drugs2.

Lawsonia inermis L. belongs to family Lythraceae. A native of North Africa, South-West, Asia, India, Sri Lanka and middle East3. This plant has been described in Charaka Samhita for the treatment of epilepsy and jaundice and for dying grey hair4. The Ayurvedic pharmacopeia of India indicated the use of leaves in dysuria, bleeding disorder, prurigo and other obstinate skin diseases5. Leaves are used in vulnerary, diuretic, headache, hemicranias, lumbago, bronchitis, boil, ophthalmic, syphilitics, sores and scabies. Leaves have an orange-red dye and leaf paste or powder is widely used for decorations hands, nails and feet with patterns. It is used for alternating jaundice, skin diseases6.
The Ethnic groups (Chenchu, Sugali, Yerukala and Yanadi) of Kurnool district, Andhra Pradesh, India. Traditional healers of these ethnic groups are extensively using to treat leucodarma and ringworm infections applying that paste of Lawsonia inermis. Leucoderma is a skin disease lack of pigments due to absence of melanocytes7. Skin diseases are common occurrence among rural masses due to poor hygienic conditions.

Lawsonia inermis L. leaf powder contains a variety of important chemical compounds. Lawsone is the principle of natural dye, napthaquinone8. Tannins, Gallic acid, glucose, mannitol, fat, resin and mucilage. Leaf powder consists of antidiabetic activity, immune modulatory effect, hepatoprotective, antioxidant, antimicrobial, antiviral, antiparasitic, antifertility analgesic, anti-inflammatory, cytotoxic, anti-sickhing, enzyme inhibitory activity, memory and behavior effectiveness anticoagulant, wound healing activity and nematicidal effect6 . Leaves are used to treat ringworm infection9.

In present scenario synthesis of silver nanoparticles (SNPs) play vital role in the field of nanomedicine. Silver has good conductivity, chemical stability, catalytic and antimicrobial activity10. SNPs are non-toxic to human and most effective against microorganism at low concentrations11. By using plant materials for the synthesis of nanoscale metals was initially reported by Gradea-Torresdey et al.,12-13. Biological synthesis of SNPs from aqueous silver nitrate through a sample is eco-friendly route using.

Silver has been described as being “oligodynamic” it has ability to exert a bactericidal effects at minute concentration14. It has ability to exhibit antimicrobial properties against bacteria15 and viruses 16. Last few decades that the mode of action of silver as an antimicrobial agent has been studied with any rigor17.
In this study ethno-medical botanical studies, phytochemical screening, biological synthesis of SNPs and screening of SNPs for microbial efficacy by using leaves of Lawsonia inermis L.

MATERIALAND METHODS

Plant material

The fresh leaves of Lawsonia inermis L. was

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collected, Ahobilam hills, Kurnool District of Andhra Pradesh, India, in February 2012 and identified based on ethnobotanical data and interviews with traditional healers in parallel with the ayurvedic physician for authentication. The leaves were washed thoroughly with water, finally distilled water and air dried under shade and ground using a pestle and motor, fine powder used for further studies.

Preparation of extract

25 g of plant powder was taken into 250 ml conical flask and added 100 ml of sterile distilled water and boiled for 10 min at 1000C on water bath. Then plant material extracts were collected in separate conical flask by standard filtration method and stored in refrigerator for further use.

Phytochemical screening

10 ml leaf extract was used for preliminary phytochemical screening. The qualitative analysis of secondary metabolites was carried out by using the methods for flavonoids18, steroids, alkaloids and phenols19, triterpenoids and glycosides20, tannins, anthraquinons, leucoanthocyanins and emodins21, saponins22, reducing sugars and anthocyanins23.

Preparation of 1 mM Silver nitrate solution

1 molar silver nitrate stock solution was prepared by 1.7 g of Ag(NO3)2 was dissolved in 10 ml distilled water. 1 mM solution was prepared by 1 ml of 1 M solution was made up to 100 ml with 99 ml of distilled water. This solution was stored in amber colored bottle for further use.

Synthesis of silver nanoparticles

SNPs were synthesized by using leaf extract of Lawsonia inermis. The reduction of pure Ag2+ ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5th h after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV-Vis Spectrophotometer. The size and shape of SNPs were confirmed with AFM.

UV-Vis spectra analysis

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3 hrs. after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was carried out by using UV-Vis spectrophotometer (Systronics type 118).

AFM analysis

The silver nanoparticles extracted by the above protocol were visualized with an Atomic Force Microscope (AFM). A thin film of the sample was prepared on a glass slide by dropping 100 l of the sample on the slide and was allowed to dry for 5 min, the slide were then scanned with the AFM.

Microorganisms

Pure cultures of Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi species of bacteria and Paecilomyces varioti, Pencillium rubrum and Aspergilus flavus species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India.

Antimicrobial activity

The antimicrobial activities of SNPs were carried out with paper disc diffusion method using nutrient agar medium and potato dextrose agar medium for bacterial and fungal cultures respectively. Zones of inhibition for control as a plant extract, SNPs of Lawsonia inermis, silver nitrate, were measured after 24 h and 7 days and compared with standard drugs Gentamycin and Nystatin for bacterial and fungal growth respectively. The experiments were repeated thrice and mean values of inhibition zone diameter were measured.

RESULTS AND DISCUSSION

The ethnic groups (Chenchu, Sugali, Yerukala and Yanadi) of Kurnool District, Andhra Pradesh, India. Traditional healers of these groups have staunch confidence to treat leucoderma skin disease. The leaves are soaked in coconut oil for a week along with the flowers of Thespesia populnea L. and the oil infusion is used to treat leucoderma. This ethnomedicinal information was cross checked with Ayurvedic physicians, Sri Venkateswara Ayurvedic college, Tirupati, Andhra Pradesh, India for authentication. In this regard Lawsonia inermis L leaves are extensively used in Ayurvedic medicines to treat leucoderma. The secondary metabolites screening of Lawsonia inermis L. showed that the leaf extract is rich in alkaloids, anthraquinones, coumarins, fat, flavonoids, glycosides, phenols, steroids, tannins and lacking of anthocyanins, emodins, lignins, leucoanthocyanins, reducing sugars, saponins and triterpenoids (Table-1). Flavonoids, phenolic compounds are medically used as antistomatic, diarrhoea and anti-inflammatory, anti cancer and anti oxidative.

Table-1: Secondary metabolites of leaf extract of Lawsonia inermis L.

S. No.	Secondary metabolites	leaf extract
1.	Alkaloids	+
2.	Anthocyanins	-
3.	Anthraquinones	+
4.	Coumarins	+
5.	Emodins	-
6.	Fatty acids	+
7.	Flavonoids	++
8.	Glycosides	+
9.	Lignins	-
10.	Leuco anthocyanins	-
11.	Phenols	+
12.	Reducing sugars	-
13.	Saponins	-
14.	Steroids	+
15.	Tannins	++
16.	Triterpenoids	-

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Note: '+' indicates presence, '++' indicates presence of more amounts, '-' indicates absence

In the present study SNPs were synthesized by using leaf aqueous extract of Lawsonia inermis L rapidly with in 20 min was able to be followed by color change. The fresh suspension of Lawsonia was orange in color. However, after adding of Ag (No3)2 the sample turned to dark brown color. The colour change in aqueous solution is due to the surface- Plasmon resonance (SPR) phenomina. The reason could be that the quantitative variation in the formation of SNPs (or) availability of H+ ions to reduce the silver. It is well known that SNPs exhibit dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles24. The appearance of dark-brown color in the reaction vessels indicates the formation of SNPs25.

The colour change in aqueous solution is due to the surface- Plasmon resonance (SPR) phenomina (Fig-1). The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of SNPs synthesized from the leaf extract Lawsonia inermis L. have the characteristic absorbance peaks at 230 and 380nm respectively (Fig-1). This illustrated the presence of homogenous distribution of hydrosol SNPs after 20 min of stirring26. Silver nanoparticles have free electrons, which give rise to an SPR absorption bonds27, due to the combined vibration of electrons of metal nanoparticles in resonance with the light waves28-29. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles.

The size and shape of SNPs was detected by using AFM (Atomic force microscope). Size of SNPs was 27.66 nm, spherical in shape (Fig-1). The result obtained in this study is interesting because it can serve as a foundation in terms of identification of potential forest plants for synthesizing SNPs.

The leaf extract of Lawsonia inermis L. SNPs showed highest percentage of bacterial inhibition zones both gram-positive (Staphylococcus and Bacillus) and gram-negative (Salmonella and E.coli) (Graph-1). The results were compared to that of standard antibacterial antibiotic Gentamycin. The antifungal activity was studied and the results were compared to that of antifungal antibiotic Nystatin. The result showed that Pencillium rubrum has sensitive followed by Aspergillus flavus and Paecilomyces varioti. The maximum toxicity was observed in SNPs of Lawsonia use that Ag(No3)2. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction. The extract showed broad fungi toxic spectrum when tested against 13 ringworm fungi30. The SNPs are also reported to be nontoxic to human and most effective against bacteria, viruses and other eukaryotic micro-organisms at very low concentrations and without any side effects31. SNPs synthesized from Shorea tumbuggaia and Boswellia ovalifoliolata32-33 and Thespesia populnea34, Curcuma longa exhibit the antibacterial activity35.

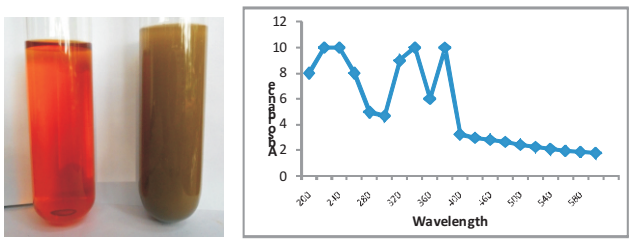

Fig-1: The color change of leaf extract of Lawsonia inermis (1) blank extract without silver nitrate (2) leaf extract with 1 mM silver nitrate; b) UV-Vis spectroscopy of synthesized of silver nanoparticles, (c, e & f) AFM of Topography of SNPs (d) Three dimensional structure of SNPs

Table-2: Antimicrobial activity of SNPs isolated from leaf extract of Lawsonia inermis

S. No.	Micro-oragansims	$Ag(NO_3)_2$	Plant extract control	SNPs	Standard : Gentamycin / Nystatin
	Bacterial species				
1.	*Staphylococcus aureus*	6.4±0.28	6.4±0.29	10.4±0.24	10.4±0.44
2.	*Salmonella typhi*	6.0±0.24	5.4±0.20	6.4±0.16	10.7±0.32
3.	*E.coli*	6.8±0.40	5.9±0.26	10.6±0.27	11.6±0.86
4.	*Bacillus*	6.2±0.24	8.3±0.23	9.5±0.24	15.5±0.24
	Fungal species				
5.	*Paecilomyces varioti*	7.4±0.37	6.6±0.33	8.1±0.35	13±0.83
6.	*Pencillium rubrum*	7.5±0.68	5.77±0.66	**11.8±0.20**	18±1.7
7.	*Aspergillus flavus*	6.4±0.50	5.3±0.74	8.0±0.33	13.7±0.68

Note: standard error of mean

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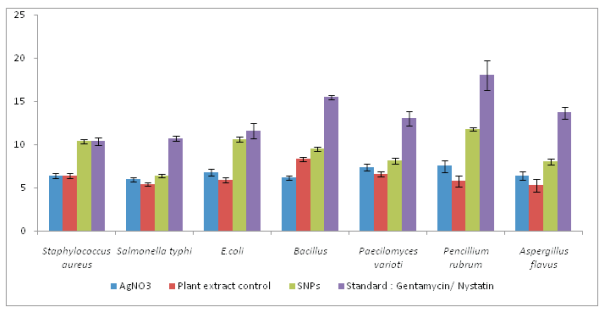

Graph-1: Antimicrobial activity of SNPs isolated from leaf extract of Lawsonia inermis

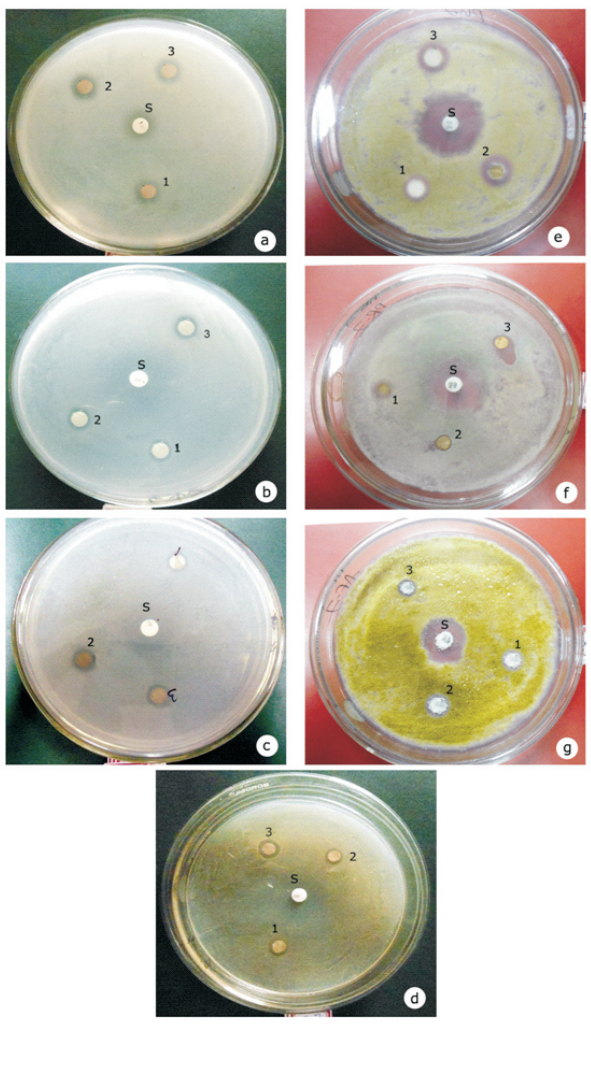

Fig-2: Antimicrobial activity of Leaves aqueous extract of Lawsonia inermis

a) Staphylococcus aureus, b) Salmonella typhi, c) E.coli, d) Bacillus, e) Paecilomyces varioti, f) Pencillium rubrum and g) Aspergillus flavus. 1) Ag(No3)2, 2) Plant extract control, 3) SNPs and S) Standard(Gentamycin/Nystanin)

The results showed that the SNPs of leaf extract of Lawsonia inermis L. could be used as an eco-friendly antimicrobial agent in the control of bacterial and fungal diseases.

CONCLUSION

The present study included ethnic groups of traditional healers are extensively using Lawsonia inermis to treat lecucoderma. Phytochemical screening is a good source for bio active principles for pharmaceutical industries. The size of SNPs was 26.66 nm and spherical in shape. SNPs were characterized by UV-Spectra and AFM analysis. SNPs have good antimicrobial activity against different microorganisms. Green synthesis of SNPs is pollutant free and eco-friendly.Hence SNPs of Lawsonia inermis L has great potential to prepare nano based biomedicines.

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Disclosure

The authors report no conflict interest in this work.

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