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Antibacterial activities of Zinc Sulphide nanoparticles using leaf extract of Lawsonia inermis

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Abstract

In the present study, the objective was to study the synthesis and analyse the zinc sulphide nanoparticles from *Lawsonia inermis* leaf extract. The study revealed that the plant extract possessed significant phytochemicals. The nanoparticles were synthesized using the leaf extract and analysed using UV, FTIR, XRD. Different functional groups were found to be present indicating the presence of diverse compounds in the extract. The zinc sulphide nanoparticles also possessed potent antibacterial activity against many pathogenic organisms The methanol leaf extract was found to possess alkaloids, phenols and carbohydrates. The nanoparticles were subjected to antibacterial study and were found to be effective In UV analysis, the maximum absorption was at 219.24 nm. The FTIR analysis showed the presence of alkyl, alkyne, Aliphatic amine, Hydroxyl, aromatic, nitro and aldehyde functional groups. Analysis revealed that the nanoparticles were of cuboidal shape. The sizes of the nanoparticles are 13.86, 8.156 and 11.85 nm. From this study, it was evident that the plant *Lawsonia inermis* can be used to synthesize nanoparticles using green chemistry methods for various biomedical and nanoelectronics applications.

Introduction

Nanotechnology is evolving as a fast growing field as it finds application in Science and Technology to manufacture new materials at nanoscale level (Albrecht *et al.,* 2006). Due to their distinctive features such as catalytic, optical, magnetic and electrical properties, metal nanoparticles have been of great interest (Singhal *et al.,* 2010). With a decrease in the size, distribution and morphology of the particles, nanoparticles have a higher surface area to volume ratio (Awwad *et al.,* 2012). In past few years, green synthesis of metal nanoparticles has become the focus of attention in the Nano science and Nano biotechnology field. There is an increasing attention on biosynthesis of the metal nanoparticles using organisms. The reduction of metal compounds into their respective nanoparticles are the result of the anti-oxidant or reducing properties of microbial enzymes or the plant phytochemicals.

From green chemistry perspective, the three main steps that are to be evaluated in the preparation of nanoparticles are the choice of the solvent medium used for the synthesis, hydrophobicity of the capping agents used (Raveendran *et al.,* 2003). Synthesis of nanoparticles using bio-organisms is attuned with the green chemistry principles because the bio organism as well as the reducing agent and the capping agent employed in the reaction are eco-friendly (Li *et al.*, 2009).The presence of some toxic chemical species adsorbed on the surface which may haveadverse effects in medical applications often occur in chemical synthesis methods (Parashar and Srivastava, 2009).

Biosynthetic and environment friendly technology which is utilized for the synthesis of zinc oxide (Zns) NPs are believed to be nontoxic, biosafe, and biocompatible and the nanoparticles have been used as drug carriers, cosmetics, and fillings in medical materials. (Rosiand Mirkin, 2005). Nevertheless most Zns nano-particles which are used commercially have some advantages such as lower cost, white appearance over silver nano-particle (Vigneshwaran, 2006). The uses of plant extracts in the biosynthetic method have drawn attention as a simple and viable alternative to chemical and physical methods (Singh, 2011).

Nano-sized materials are used as novel antimicrobial agents. Due to increasing microbial resistances against various metal ions, various antibiotics, and the development of resistant strains in multiple ways the attention of the researchers are focused on high surface area to volume ratio (Chan and Tsai, 2008). Antibacterial activity is also observed against spores are resistant to high temperature and high pressure. In the textile industry several classes of antimicrobial agents are used, many of which are biocides (Singh *et al.,* 2012).

The Zns nanoparticles exhibit bactericidal properties due to electrostatic interaction between the nanoparticles and the cell surface and also cell damage is enhanced because of increased association of the nanoparticles. Upon prolonged contact between the bacterium cell membrane and the nanoparticles the toxic effects of Zns nanoparticles towards the pathogenic species of bacteria are enhanced Due to cytotoxic behavior of Zns nanoparticles the bacterium and fungal lipid bilayer gets ruptured resulting in the drainage of the cytoplasmic contents (Feris *et al.*, 2010).

Lawsonia inermis (L) Wall is an annual herb commonly found in the Western Ghats distributed in the Southern moist mixed forests, grown among rocks, also known as Karpuravalli and Padukurkka. The stem is bluntly 4-angled, often tinged with red Leaves are simple, opposite, broadly ovate, obtuse, crenate, base subcordate or rounded, some- what fleshy, usually pubescent. Flowers are pale purple, in dense cylindric spikes. Seeds are small, suborbicular, compressed, and brown (Nambiar *et al.,* 1985; Jayaweera, 1981). Traditionally the plant has been used as hepatoprotective agent, stimulant, anti-ulcer, anti-inflammatory (Sirsi and Rao, 1956; Ravikumar and Santhosh, 2008; Grover *et al.,* 2001). The present study was aimed at synthesizing zinc sulphide nanoparticles from *Lawsonia inermis* leaf extract and to study their characteristics and antibacterial activity.

Materials and Methods

Extraction of the plant material

The fresh plant materials were washed with running tap water and shade dried. The leaves of *Lawsonia inermis* were crushed to coarsely powdered by grinder. These coarse powders (25g) were then subjected to successive extraction in 250ml of each solvent (methanol) by using Soxhlet apparatus. The collected extracts were stored and then taken up for further investigations

Antibacterial activity Preparation of inoculums

Stock cultures were maintained at 4^0C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37^0C and 25^0C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^0C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Synthesis of zinc sulphide nanoparticles

Preparation of zinc sulphide NPs For the synthesis of NPs, 50ml of plant leaves extract was taken and boiled at 60^0C - 80^0C by using a stirrer-heater. Then, 5 g of zinc sulphate powder was added to the solution as the temperatures reached at 60^0C. This mixture was then boiled until it converted to a deep yellow coloured suspension. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400^0C for 2 h. A light white coloured powder was obtained and this powder was carefully collected and sent for different

characterizations. The material was powered using a mortar and pestle so, that got a fine powder, which is easy for further characterizations.

Ultra- Violet Spectroscopy

The UV spectrum provides a useful means of detecting conjugated unsaturated chromophores within a molecule such as polyenes, α, β-unsaturated ketones and aromatic compounds. This can be particularly helpful in the identification of chromophores and flavones. The UV spectrum may be caused by the summation of chromophores from different parts of a polyfunctional molecule, and this should be considered in the light of deduction drawn from other spectroscopic methods and chemical degradation.

FTIR Spectroscopy

Infrared light from suitable source passes through a scanning Michelson inferometer and Fourier Transformation gives a plot of intensity versus frequency. When a powdered plant sample is placed in the beam, it absorbs particular frequencies, so that their intensities are reduced in the inferogram and the ensuing Fourier transform is the infrared absorption spectrum of the sample.

Scanning Electron Microscope

Scanning electron microscopic (SEM) analysis was performed using the Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by simply dropping a very small amount of the sample on the grid, with excess solution being removed using blotting paper. The film on the SEM grid was then allowed to dry by putting the grids under a mercury lamp for 5 min.

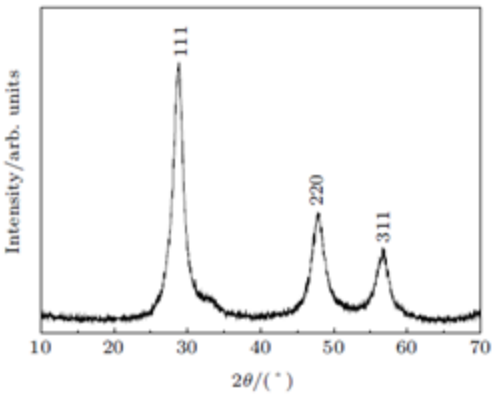
X-Ray Diffraction

ZnS nanoparticles were examined by X-ray diffractometer. The powdered metal was sticked in the cubes of XRD and then the result was taken in the XRD equipment.

Results and Discussion

XRD analysis of *Lawsonia inermis* extract

The crystalline nature of the prepared nanosize ZnS powder is evident from the x-ray diffraction pattern The most significant feature within the observed pattern, at 2θ=29.6264 , is assigned to the (111) reflection of the cubic zinc blende structure of ZnS (JCPDS No 5-566) .Two



other prominent features are observed at 2θ = 48.7355 and 57.5339 ,which belong to (220) and (311) reflections. The Nanosized by applying Debye- scherrer formula

Fig 5. ZRD images of ZnS Nanoparticles

$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad \text{.....(1)}$$

Where D is the mean particle size, λ is the wavelength of incident X-ray (1.5406 ˚A), θ is the degree of the diffraction peak , and β is the full width at half maximum (FWHM)of the XRD peakappearing at the diffraction angle θ. The broadening of the absorption spectrum could be due to the quantum confinement of the nanoparticles. The mean calculated crystallite average size of the ZnS nanoparticles is 4.6 nm

Antimicrobial activity of *Lawsonia inermis*

The antimicrobial activity of *Lawsonia inermis* methanol leaf extract was studied at concentrations of 20, 30, 40 and 50µl against the organisms *S.typhi, S.aureus, B.subtilis, E.coli* and *P.aeruginosa.* There was no activity against any organisms at concentration of 20µl. Only *S.aureus, E.coli* and *P.aeruginosa* were inhibited at concentration 30µl. At concentration 40 µl and 50 µl, highest inhibition was found against *P.aeruginosa* and *B.subtilis* followed by *S.typhi.*

S.No.	Name of Organism	Control	Concentration of Sample A		
			30 µl	40 µl	50 µl
1.	*S.typhi*	22	03	5	9
2.	*S.aureus*	23	06	8	11
3.	*B.subtilis*	21	04	9	12
4.	*E.coli*	22	08	08	10
5.	*P.aeruginosa*	24	07	09	10

Table 1. and Fig 1:
Analysis of Antimicrobial activity of *Lawsonia inermis*

FTIR analysis of *Lawsonia inermis* extract

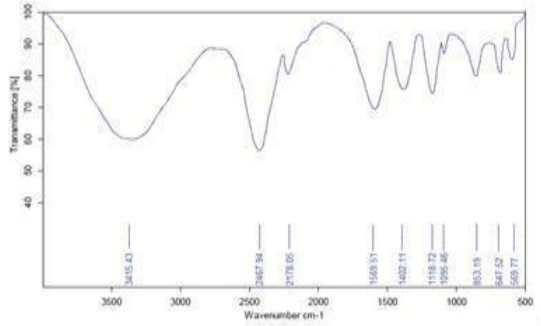


Fig 3. FTIR- spectra of ZnS Nanoparticles synthesised using 50ml of *Lawsonia inermis* leaf extract

The appearance of peaks at 569.77 cm^{-1}ascribed to Presence of Halogen atom combined with alkyl group. The C-H bending in alkyne group (strong stretching) appeared at 647.52 cm^{-1}. The intense band at 853.19cm^{-1} and 1095.46 cm^{-1}relates to the =CH-H Stretching and Aliphatic amine (C-N) respectively. The Hydroxyl group stretching (-OH) gives the band at 1118.72cm^{-1}. the strong absorptive peaks at 1402.11$cm^{-1}$and 1569.51 cm^{-1} are attributable to C-C Stretching (in ring) aromatic(-NO) nitro group asymmetric stretching (medium) respectively. The peaks at 2178.05 cm^{-1}indicates presence of triple bond stretching in alkyne compounds. The peaks at 2467.94 $cm^{-1}$and 3405.43 $cm^{-1}$are attributed to C-H medium stretching in aldehyde (HC=O) and N-H stretching (medium).

Conclusion

In the present study, the ZnS nanoparticles were synthesized using *Lawsonia inermis* methanol leaf extract. The methanol leaf extract was found to possess alkaloids, phenols and carbohydrates. The nanoparticles were subjected to antibacterial study and were found to be effective. The nanoparticles were analysed using UV, FTIR, SEM and XRD. In UV analysis, the maximum absorption was at 219.24 nm. The FTIR analysis showed the presence of alkyl, alkyne, Aliphatic amine, Hydroxyl, aromatic, nitro and aldehyde functional groups. SEM analysis revealed that the nanoparticles were of cuboidal shape. The sizes of the nanoparticles are 3.99, 4.47 and 5.32 nm. From this study, it was evident that the plant *Lawsonia inermis* can be used to synthesize nanoparticles using green chemistry methods for various applications.

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