Antibacterial activities of zinc sulphide nanoparticles using leaf extract of Lawsoniainermisand to study their characterization

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Abstract

In the present study, the objective was to study the synthesis and analyse the zinc sulphidenanoparticles from Lawsoniainermis 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, SEM and 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. SEM 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 Lawsoniainermis can be used to synthesize nanoparticles using green chemistry methods for various biomedical and nanoelectronics applications.

Keywords: Antimicrobial Acticity, Nanoparticles,.Lawsoniainermis.

Introduction

Nanotechnology is evolving as a fast growing field as it finds application in Science and Technology to manufacture new materials at the 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 (Singhalet al., 2010). With a decrease in the size, distribution and morphology of the particles, nanoparticles have a higher surface area to volume ratio (Awwadet al., 2012). In past few years, green synthesis of metal nanoparticles has become the focus of attention in the Nanoscience 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 is the result of the anti-oxidant or reducing properties of microbial enzymes or the plant phytochemicals.

From a 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, environmentally safe reducing agent and non-toxic material for the stabilization of the nanoparticles. Till date, most of the synthetic methods reported depend heavily on organic solvents. This is mainly because of the hydrophobicity of the capping agents used (Raveendranet 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 have adverse 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 non-toxic, biosafe, and biocompatible and the nanoparticles have been used as drug carriers, cosmetics, and fillings in medical materials (Rosiand Mirkin, 2005). Nevertheless most ZnSnano-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 that 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 (Feriset al., 2010).

Lawsoniainermis (L) Wall is an annual herbcommonly found in the Western Ghats distributed in the Southern most 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 (Nambiaret 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 Lawsoniainermis leaf extract and to study their characteristics andantibacterial 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 Lawsoniainermis 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

For the synthesis of Zinc sulphideNPs, 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 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

Antimicrobial activity of Lawsoniainermis

The antimicrobial activity of Lawsoniainermis 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 anyorganisms at concentration of 20µl. Only S.aureus, E.coli and P.aeruginosawere inhibited at concentration 30µl. Atconcentration 40 µl and 50 µl, highest inhibition was found against P.aeruginosa and B.subtilis followed by S.typhi.

Table 1 and Fig. 1: Analysis of Antimicrobial activity of Lawsoniainermis

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

UV analysis of Lawsoniainermis extract

The UV analysis of the methanol extract synthesized zinc oxide nanoparticles of Lawsoniainermis leaf. The maximum absorption peak was obtained at 219.24 nm wavelength.

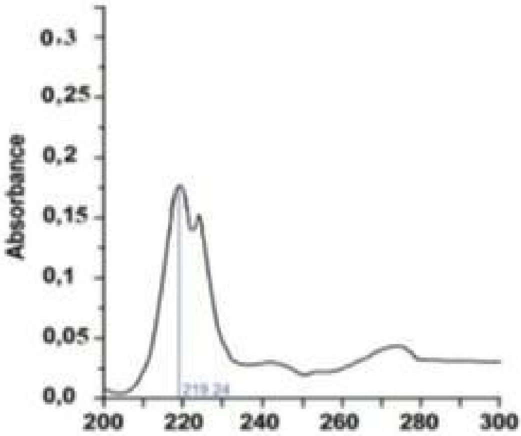

Fig 2. UV spectrum of ZnS Nanoparticles synthesised using 50ml of Lawsoniainermis leaf extract.

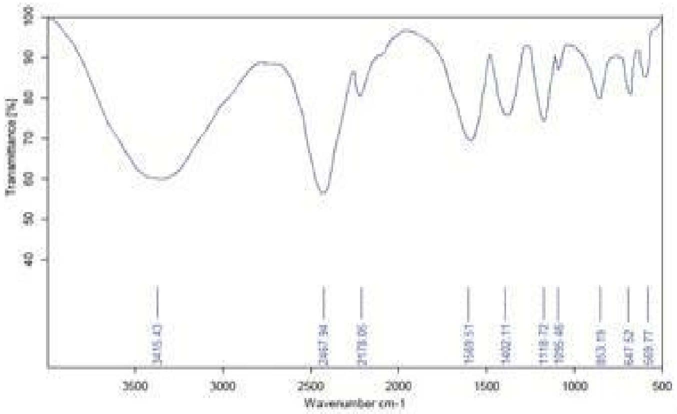

Fig 3. FTIR- spectra of ZnS Nanoparticles synthesised using 50ml of Lawsoniainermis leaf extract

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}indicate the stretching frequency of triple bond in alkyne compounds. The peaks at 2467.94 $cm^{-1}$and 3405.43 $cm^{-1}$are attributed to C–H medium stretching in (medium aldehyde (HC=O) and N–H stretching).

SEM analysis of Lawsoniainermis extract

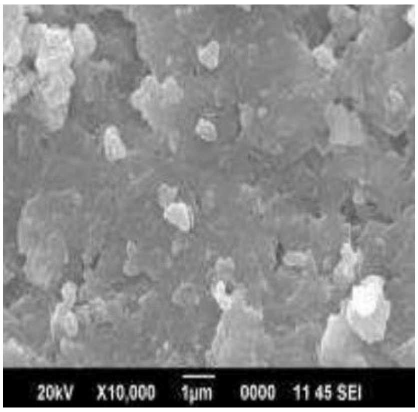

Fig 4. SEM images of ZnS Nanoparticles synthesised using 50ml of Lawsoniabinermis leaf extract.

XRD analysis of Lawsoniainermis extract

The crystalline nature of the prepared nanosizeZnS 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 size is calculated by applying Debye- Scherrer formula.

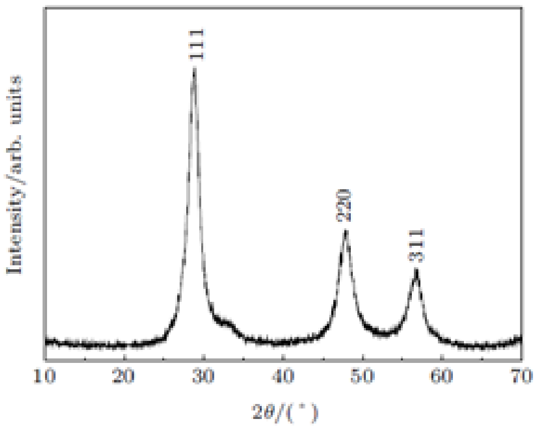

Fig 5. XRD images of ZnS Nanoparticles

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

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

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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