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Biosynthesis of zinc sulphide nanoparticles using Phyllanthus emblica and their antimicrobial activities

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ABSTRACT

In the present study, the objective was to study the synthesis and analysis of zinc sulphide nanoparticles from Phyllanthus Emblica leaves and fruit extract. The study revealed that the plant extract possessed significant phytochemicals. The nanoparticles were synthesized using the leaves and fruit extract and analyzed 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 antimicrobial activity against many pathogenic organisms.

Introduction

Nanotechnology is the tool for design, production, characterization and applications of nanostructure materials. It generally deals with the structures sized between 1-100 nanometers in at least one dimension. It has been emerged as a growing and rapidly changing field and presents potential opportunities to create better materials and products. Nanostructured materials are a technically considerable object that possesses optical and electrical properties that depend impressively on the dimension and shape of the nanoparticles. This is due to confinement of the charge carriers in the constricted space of the nanocrystal[1,2]. The properties of nanoparitcles powerfully depend on their size. Their high specific surface area results in high chemical reactivity. The decrease of their size also leads to an increase of the band-gap energy that is known as quantum size effect. Recently, II-VI semiconductor nanoparticles are playing consideration in enormous fields due to their exceptional and distinctive optical and electrical properties which present a major benefit over their mass counterparts[3-4].

Amongst those ZnS is an important member in II-VI group semiconductors having a better value of band gap energy[5]. Polymers are also excellent host materials as capping agents and stabilizers as they check agglomeration and precipitation of the particles. Sulfide is a semiconductor nanomaterial processing a lot of remarkable physical properties and potentially used in mesoscopic electronic[6] biolabeling[7] and photocatalysis[8].

Metals have been used for centuries as bactericidal agents; silver, copper, gold, titanium, and zinc have fascinated particular consideration, each having different properties and spectra of activity[9]. The antibacterial, antifungal, and antiviral actions of sulfide nanoparticles have been broadly investigated in comparison with other metals[10-12].

In the present work, we report the synthesis of zinc sulphide nanoparticles by the chemical co-precipitation method. UV-Vis spectrophotometer and X-ray diffraction techniques are used to characterize the synthesized sulphide nanoparticles. Oral pathogens can cause severe break which may show the way to serious issues in human disease like blood circulation and coronary disease. Various oral foods, including toothpaste, now integrate powdered zinc salts to control the development of dental plaque. The antimicrobial activity is assessed against oral pathogens such as Streptococcus sp. Staphylococcus sp. and Candida albicans and these results confirmed that the zinc sulphide nanoparticles are exhibiting good bactericidal activity.

Zinc Sulphide (ZnS) is a simple inorganic compound known for its practical applications in photoconductors, solar cells, field effect transistors, sensors transducers, optical coatings and light emitting materials [13] It may be pointed out here that simple inorganic substances as antimicrobial agents may prove to be advantageous as they contain mineral substances essential for human consumption and may exhibit powerful action even when administered in small amounts. In view of the information on presence of Antimicrobial, nanoparticles of ZnS were prepared in our laboratory and were evaluated for the antimicrobial potentiality along with that many pathogenic organisms and the study was aimed at biosynthesizing Zinc Sulphide nanoparticles from Phyllanthus Emblica leaves and fruit extract and study their characteristics and antimicrobial activity.

Materials and Methods

Extraction of the plant material

The fresh plant materials were washed with running tap water and shade dried. The leaves and fruit of Phyllanthus Emblica were crushed to coarsely powdered by grinder.

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These coarse powders (30g) 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.

Phytochemical Screening

Preliminary phytochemical analysis was carried out for all the Phyllanthus Emblica extracts as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

Phyllanthus Emblica extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent.

Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

H2SO4 test: Extracts were treated with few drops of H2SO4. Formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H2 SO_4. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthraquinones

Borntrager's test

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

Detection of Phenols

Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of Saponins

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins**.**

Detection of Tannins

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of Oils and Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Quantitative phytochemical analysis Estimation of Alkaloids

Alkaloid determination using Harborne (1973) method. 5g of the Phyllanthus Emblica sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in methanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids

Ten grams of Phyllanthus Emblica leaf and fruit sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference (Krishnaiah et al, 2007).

Estimation of Steroids

10 ml of Phyllanthus Emblica extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at $70^0C \pm 20^0C$ for 30 minutes with occasional shaking and diluted to the mark with distilled water.

Determination of Saponin

20g of Phyllanthus Emblica leaf and fruit sample was dispersed in 200 ml of 20% methanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of normal butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage (Nahapetian and Bassiri, 1975).

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, 20ml of leaf and fruit extract was taken and boiled at 60^0C - 80^0C by using a stirrer-heater. Then, 14.4 g (80 ml) of zinc sulphate, DI water solution was added to the solution as the temperatures reached at 60^0C. This mixture was then boiled until it converted to a deep green colored suspension. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 200^0C for 2 h. A light white colored 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

1.X-Ray Diffraction (XRD) analysis

The XRD results as shown in Fig 1, it is clear that pure ZnS nanoparticles were obtained in powder form. The broadened peaks in the XRD pattern indicated the formation of ZnS nanocrystals with small crystallites. The three diffraction peaks at 2θ values of 28.978^0, 47.62^0, 56.65^0 corresponding to the (111), (220) and (311) diffraction planes, respectively of the spherical nanocrystalline structure of ZnS were observed.

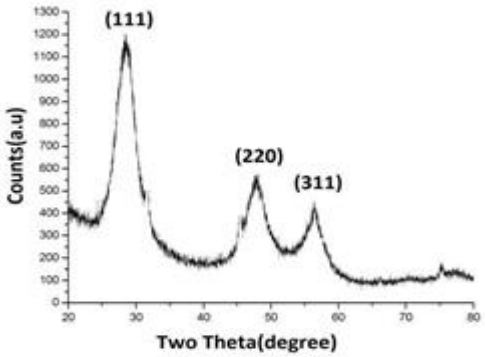

Fig 1:XRD Pattern of Phyllanthus Emblica leaves and fruit Extract ZnS nanoparticles.

The average crystallite size (D) was calculated from the full-width at half-maximum (FWHM) of the most intense peak of the (111) plane of ZnS nanoparticles using the Debye-Scherrer formula for spherical particles [Eq. (1)].

$$D = 0.89\lambda/(\beta \cos \theta) \quad (1)$$

Where λ is the wavelength (Cu Kα), β is the full width at the half-maximum of the ZnS nanoparticles and θ is the diffraction angle. From this equation the average particle size was estimated to be 4.49 nm

2.SEM analysis

The SEM Image of the ZnS NPs as shown in Fig 2,the powder which clearly shown the size of particles to be less than 100 nm as expected ,the prepared NPs were found to be in cluster form. The surface morphology of the ZnS NPs is spherical in shape. in some place various sizes of the particles are observed, that is particle size randomly distributed.

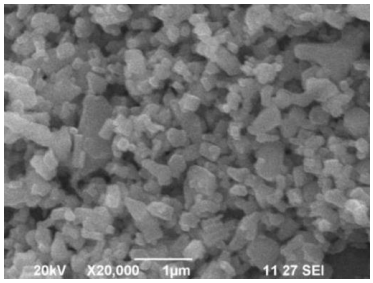

Fig 2.SEM Analysis of Phyllanthus Emblica leaves and fruit Extract ZnS nanoparticles

3.UV-Vis spectra analysis

In order to confirm the optical property and substitution of ZnS NPs as shown in Fig 3 the power were characterized by UV-Vis transmission spectra analysis. The UV-Vis transmission spectra analysis of ZnS NPs powder are highly transparent in the visible region and a sharp fall in transmission is observed below 200-300nm region. It is found that the absorption edge shifts toward longer wavelength with doping of ZnS.

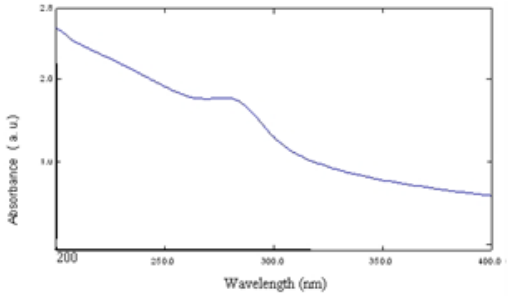

Fig 3.UV-Vis spectra Analysis of Phyllanthus Emblica leaves and fruit Extract ZnS nanoparticles.

4. Fourier transforms infrared (FTIR) studies

Fig: 4 Represents the IR spectra of ZnS nanoparticles in the range 4000-400 cm-1 for ZnS nanoparticle the peaks at 1123.67, 682.4, and 570.85 cm-1 are because of the vibrational stretching of ZnS. One peak at 735.96 cm-1 is for the assigned of C=O bond. In addition there is only one peak at 1599.7 cm-1 as assigned of bending vibration of O-H bond. Low number of peak for bond is indicating the absorption of water on the surface of ZnS nanoparticles.

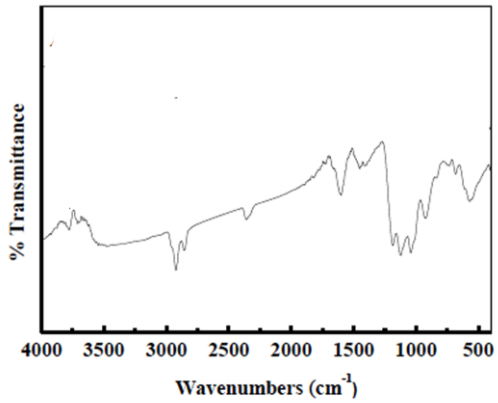

Fig 4.FTIR Studies of Phyllanthus Emblica leaves and fruit Extract ZnS nanoparticles.

Table 1. Qualitative phytochemical analysis of Phyllanthus Emblica leaves and fruit Extract ZnS

Phytochemicals	Observations	Sample A
Alkaloids Mayer's test Wagner's test	Cream colour Reddish brown solution/ precipitate	 + +
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange colour precipitate	+ +
Steroids Liebermann-Burchard test	Violet to blue or Green colour formation	-
Terpenoids Salkowski test	Reddish brown precipitate	-
Anthroquinone Borntrager's test	Pink colour	-
Phenols Ferric chloride test Lead acetate test	Deep blue to Black colour formation White precipitate	+ +
Saponin	Stable persistent	-
Tannin	Brownish green / Blue black	-
Carbohydrates	Yellow / brownish / blue / green colour	+
Oil and Resin	Filter paper test	-

5.Antimicrobial activity of Phyllanthus Emblica leaves and fruit f Extract ZnS

The qualitative phytochemical analysis of the leaf methanol extract of Phyllanthus Emblica leaf extract ZnS was done to test for presence of various phytochemicals. The plant was found to alkaloids, Flavonoids ,phenols and carbohydrates. steroids, Terpenoids, anthroquinone, saponins, tannins, oils and resins were absent in Phyllanthus Emblica extract. The antimicrobial activity of Phyllanthus Emblica leaf extract ZnS methanol leaf extract was studied at concentrations of 30, 40, 50 and 60µl against the organisms, E.coli, B.subtilis, S.aureus, M.smegmatis, c.albicans and f.oxysporum. There was no activity against M.smegmatis, c.albicans and f.oxysporum at concentration of 30µl. Only E.coli, B.subtilis, S.aureus were inhibited at concentration 30µl. At concentration 40 µl, 50 µl and 60 µl, highest inhibition was found against B.subtilis, S.aureus followed by E.coli, f.oxysporum.

Table 2. Analysis Antimicrobial activity of Phyllanthus Emblica leaves and fruit extract ZnS.

S.No.	Name of Organism	Concentration of Samples			
		30 µl	40 µl	50 µl	60 µl
1.	E.coli	14±0.5	16±0.5	24±0.5	26±0.5
2.	B.subtilis	14±0.5	18±0	25±1	27±2
3.	S.aureus	12±0	17±0	25±0	27±.12
4.	M.smegmatis	00	15±0.5	21±0.5	24±1
5.	c.albicans	00	16±1	20±0.5	24±1
6.	f.oxysporum	00	15±0	23±0	25±0

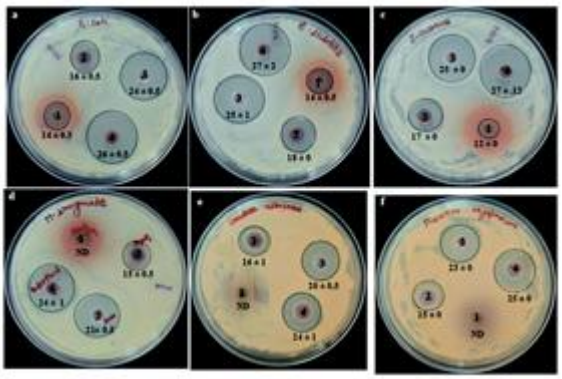

Fig 5 . Antimicrobial activity of ZnS Nanoparticles using 20ml of Phyllanthus Emblica leaves and fruit extract

Note: (1) 30 µl, (2) 40 µl, (3) 50 µl, (4) 60 µl concentrations of 20 ml Phyllanthus Emblica extract ZNS

Conclusion

Thus after this entire study it can be conclude that Phyllanthus Emblica extract ZnS NPs have maximum Anti bacterial activity against B.subtilis, S.aureus followed by E.coli. However in case of f.oxysporum and M.smegmatis have maximum Antifungal activity. In the present study, the ZnS nanoparticles were synthesized using Phyllanthus Emblica methanol leaf and fruit extract have Antimicrobial activity against the entire test organism. The methanol leaf extract was found to possess alkaloids, Flavonoids, phenols and carbohydrates. The nanoparticles were analysed using UV, FTIR, SEM and XRD. In UV analysis, the maximum absorption was at 200-300 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 spherical in shape, The sizes of the nanoparticles are less than 100 nm. From this study, it was evident that the plant Phyllanthus Emblica can be used to synthesize ZnS nanoparticles using green chemistry methods for various applications.

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