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BIO-SYNTHESIZED NANO-FORMULATION OF ZINC OXIDE – *ALOE VERA* AND TO STUDY THEIR CHARACTERIZATION AND ANTIBACTERIAL ACTIVITIES AGAINST MULTIPLE PATHOGENS

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XRD, FTIR, SEM, antibacterial

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ABSTRACT: Nano-sized ZnO particles of specific morphology were synthesized using the plant leaf extracts of *Aloe vera*. In modern science Nanotechnology is an ablaze field for the researchers. Nanoparticles having a size of 1-100 nm in one dimension are used significantly concerning medical chemistry, atomic physics and all other known fields. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. The biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, eco-friendly and less time consuming. The semiconductor ZnO has gained substantial interest in the research community in part because of its large exciton binding energy 60 meV which could lead to lasing action based on exciton recombination even above room temperature. The Green synthesis was done by using the methanol of *Aloe vera* extract and zinc oxide. A fixed ratio of plant extract to metal ion was prepared and the color change was observed which proved the formation of nanoparticles. The nanoparticles were characterized by UV-vis Spectrophotometer, FTIR Analysis, XRD, and SEM.

INTRODUCTION: Nano materials have attracted tremendous interest due to their noticeable performance in electronics, optics, and photonics. Nano materials are typically classified into three groups: 0- dimensional, 1-dimensional and 2-dimensional. 0- dimensional nanostructures, referred to as quantum dots or nanoparticles with an aspect ratio near unity, have been extensively used in biological applications [10]. Nanotechnology emerges from the physical, chemical, biological and engineering sciences where new techniques are being developed to probe and maneuver single atoms and molecules for multiple applications in different field of scientific world.

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In nanotechnology, a nanoparticle is defined as a small object that behaves as a whole unit in terms of its transport and properties. The science and engineering technology of nanosystems is one of the most exigent and fastest growing sectors of nanotechnology [12].

Zinc oxide (ZnO) is a class of inorganic metal oxides available and exhibits a wide range of nanostructures. Photocatalytic and photo oxidizing ability against chemical and biological species are used to characterize these metal oxides [21].

U.S. Food and Drug Administration have recognized ZnO as safe [11]. Lower cost, UV blocking properties, high catalytic activity, large surface area, white appearance and their remarkable applications in the field of medicine and agriculture are the advantages of ZnO particles[7]. Recently, ZnO have been used extensively in environmental remediation and antibacterial activity [14].

Zinc oxide is a semiconductor with wide band gap (3.37), high excitation binding energy (60 meV) at room temperature [13] and has unique optical and as well as excellent thermal and chemical stability [5]. ZnO nanoparticles have gathered the increasing interest of the scientific and industrial community due to diverse application in solar energy conversion, sensors, catalysis, cosmetics, paints, fibers, drug-delivery antibacterial and luminescence properties.

In this work we have used environmentally benign plant leaf extracts of *Aloe vera* which have exceptional therapeutic properties [3]. As surface stabilizing agents which act as bio template for the synthesis of ZnO NPs. The structural, optical, thermal, photo catalytic and anti-bacterial properties of the ZnO NPs have been evaluated [1].

MATERIALS AND METHODS:

Extraction of the plant material: All the chemical reagents used in this experiment were of analytical grade. The *Aloe vera* leaves were collected from in and around Salem, Tamilnadu, India. The fresh plant materials were washed with running tap water and shade dried. Then Aloe-gel broth extract at different concentrations were prepared with distilled water and the volume was made up to 250 ml. The collected extracts were stored and then taken up for further investigations.

Phytochemical analysis: Preliminary phytochemicals analysis was carried out for all the *Aloe vera* extracts as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids: *Aloe vera* extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Detection of Flavonoids:

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

b) H_2SO_4 test: Extracts were treated with few drops of H_2SO_4. 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 H_2SO_4. The color 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: 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 color indicates the presence anthraquinones.

Detection of Phenols:

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

b) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow color 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 color 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.

Antimicrobial activity: 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 petriplates. 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 ºC for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Synthesis of ZnO Nano Particles: Preparation of zinc oxide NPs For the synthesis of NPs, 50 ml of Aloe gel- extract was taken and boiled at 60º–80 ºC by using a stirrer-heater. Then, 5 g of zinc nitrate was added to the solution as the temperatures reached at 60 ºC. 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 60 ºC 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.

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.

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.

SEM analysis: 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.

XRD Analysis: ZnO 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:

Phytochemical Qualitative Analysis: The qualitative phytochemical analysis of the *Aloe vera* leaf methanol extract was done to test for presence of various phytochemicals. The plant was found to alkaloids, flavonoids, steroids, anthroquinone, phenols, tannin and carbohydrates. Terpenoids, saponins, oils and resins were absent in *Aloe vera* extract (**Table 1).**

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *ALOE VERA* EXTRACT

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

Antimicrobial activity of *Aloe vera* (gel) extract:
The antimicrobial activity of Aloe-gel 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 *B.subtilis* at concentration of 20 µl better activity was seen against *S.aureus* and *S.typhi* than the other organisms (**Fig. 1**). The lowest inhibition activity was found to be *B.subtilis* (**Table 2**).

TABLE 2: ANALYSIS OF ANTIMICROBIAL ACTIVITY OF *ALOE VERA* (GEL) EXTRACT

S. no.	Organisms	Zone of Inhibition (mm)				
		Control	Concentration of Sample 20µl	Concentration of Sample 30µl	Concentration of Sample 40µl	Concentration of Sample 50µl
1	*S.typhi*	31mm	9mm	15mm	18mm	21mm
2	*B.subtilis*	29mm	0mm	9mm	12mm	15mm
3	*P.aeruginosa*	27mm	12mm	13mm	16mm	20mm
4	*S.aureus*	34mm	10mm	16mm	18mm	21mm
5	*E.coli*	30mm	10mm	12mm	14mm	15mm

Aloe-gel extract exerted highest activity on bacterial agents tested compared to the other extracts. The aloe-gel extract at the concentration of 100 µg/ml showed 25 mm diameter zone of inhibition against *E. coli* (**Fig. 1**). This was followed by 20, 18 and 15.5 mm zone of inhibition against *B. subtilis*, *B. cereus* and *S. aureus* [4]. De Boer *et al*., 2005 reported that the results of the study showed that the methanolic extract was more effective than aqueous extract. This may be due to the better solubility of the active components in organic solvents.

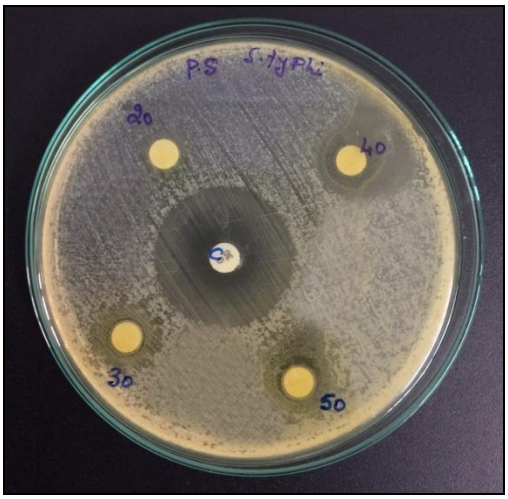

Fig.1 (a)

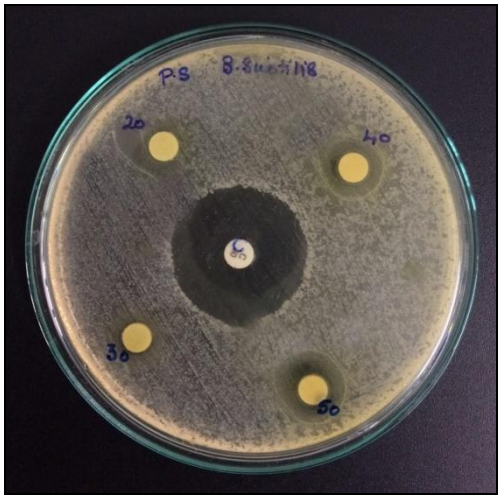

Fig.1 (b)

Fig.1 (c)

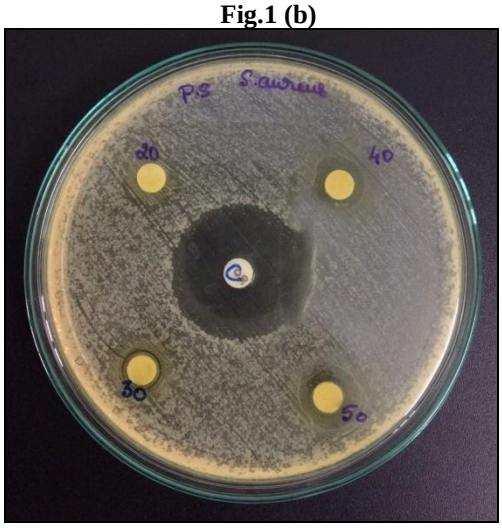

Fig.1 (d)

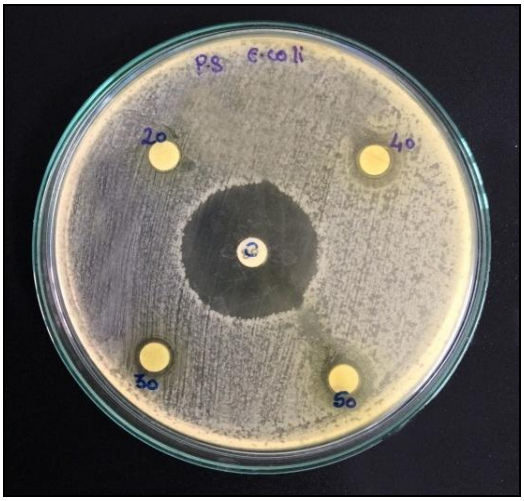

Fig.1 (e)

FIG. 1: ANTIBACTERIAL ACTIVITY OF ZnO NANOPARTICLES SYNTHESIZED USING 50ml OF *ALOE VERA-GEL* EXTRACT AGAINST MULTIPLE PATHOGENS AT DIFFERENT CONCENTRATIONS 20µl, 30µl, 40µl AND 50µl

UV analysis of *Aloe vera* (gel) extract: The UV analysis of the methanol extract synthesized zinc oxide nanoparticles of *Aloe vera* gel extract have the maximum absorption peak it was obtained at 436 nm wavelength (**Fig. 2**).

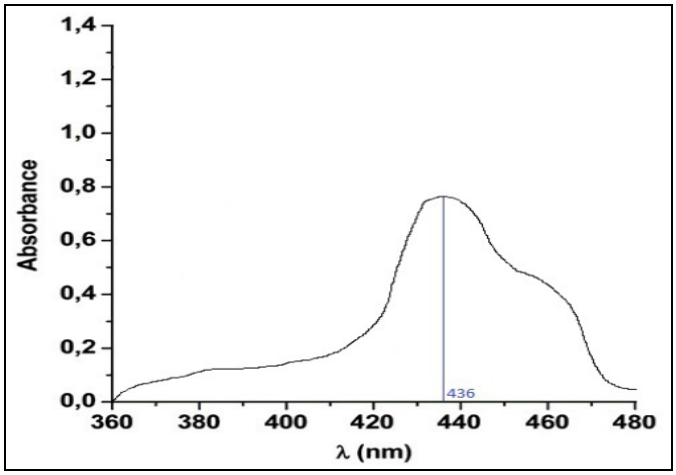

FIG. 2: UV SPECTRUM OF ZnO NANOPARTICLES SYNTHESISED USING 50ml OF ALOE-GEL EXTRACT

FTIR analysis of *Aloe vera* (gel) extract: The appearance of peaks at 540.91 cm-1shows that the aliphatic iodo compounds, C-I stretch. Thioesters, CH_3-S-(C-S stretch) appeared at 631.58cm^{-1}. Aromatic phosphate (P-O-C stretches) at 1240.07cm^{-1}. Secondary amine, NH bend appeared 1541.82 cm^{-1}. Alkenyl C=C stretch (C=C) is 1636.68. Methoxy, methyl ether O-CH_3, (CH) stretch at 2851.44cm^{-1}. Methyl C-H asym. / Sym. Bend at 2964.17cm^{-1}. Hydroxy group, H- bonded OH stretch (O-H) (**Fig. 3**).

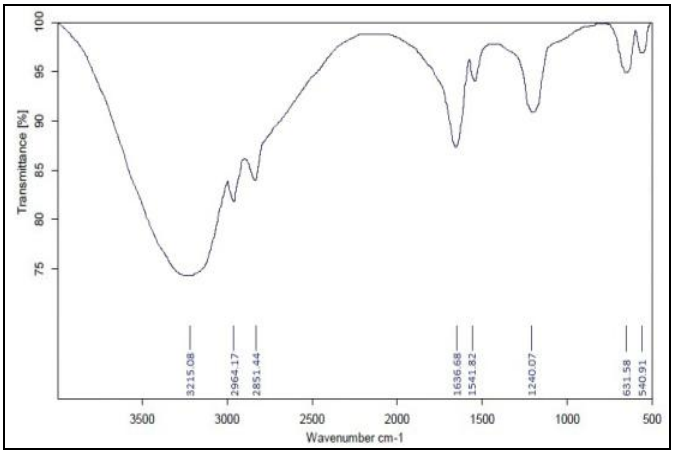

FIG. 3: FTIR- SPECTRA OF ZnO NANOPARTICLES SYNTHESIZED USING 50ml OF *ALOE VERA* GEL EXTRACT

SEM analysis of *Aloe vera* (gel) extract: The SEM analysis of the zinc oxide nanoparticles synthesized from *Aloe vera* extract revealed that their shapes were nearly spherical and also hexagonal particles. The nanoparticles were found to form aggregates and their average sizes were less than 5µm. The surface of nanoparticles were rough (**Fig. 4**).

XRD analysis of *Aloe vera* (gel) extract: XRD patterns of ZnO synthesized for *Aloe vera* leaf extract are shown in the **Fig. 5.** The diffraction peaks at 2θ=39.62, 46.49, 64.71, 74.64 corresponding to (111), (200), (220) and (311) planes respectively were observed and compared with the standard powder diffraction card of JCPDS No. 36-1451.

According to the XRD data, the mean crystalline sizes (D) of the Zinc oxide nanoparticles calculated using Debye Scherrer's formula. The obtained sizes are 10.35 nm, 11.66 nm, 18.75 nm and 30.16 nm.

D = Kλ/β Cos θ Å, Where, λ=1.5418 Å is the wavelength of the X-ray radiation used. The term θ is the Bragg diffraction angle and β is the full width at half its maximum intensity of diffraction pattern (FWHM) in radian.

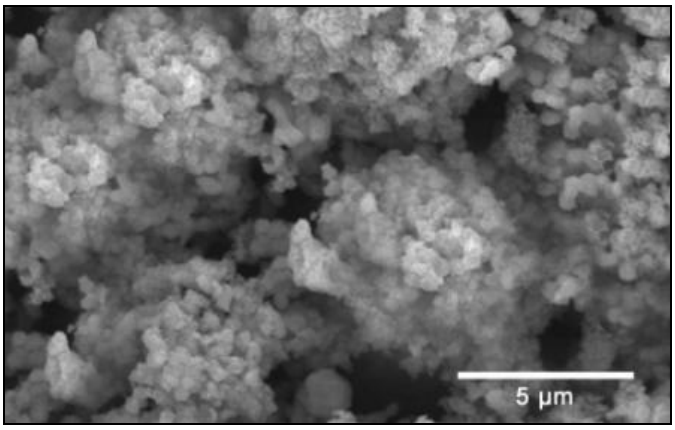

FIG. 4: SEM IMAGES OF ZnO NANOPARTICLES SYNTHESIZED USING 50ml OF *ALOE VERA* GEL EXTRACT

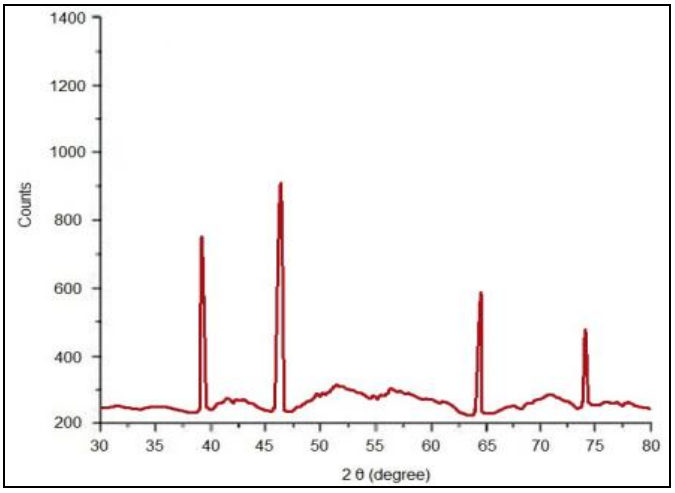

FIG. 5: XRD SPECTRUM OF ZnO NANOPARTICLES SYNTHESIZED USING 50ML OF *ALOE VERA* GEL EXTRACT

DISCUSSION: Marjorie, 1999 reported that the screening process Tannins, Saponins, Alkaloids, Flavonoids and Glycosides gave positive results while steroids and phlobatannins gave negative results.

Ejoba Raphael, 2012 reported that the *A. vera* leaves contains alkaloids, tannins, flavonoids, carbohydrates and terpenoids while the neem plant contain alkaloids, steroids, flavonoids, carbohydrates, glycosides and terpenoids. These compounds may be responsible for their medicinal uses.

The Aloe-gel extract exerted highest activity on bacterial agents tested compared to the other extracts. The aloe-gel extract at the concentration of 100µg/ml showed 25 mm diameter zone of inhibition against *E. coli*. This was followed by 20, 18 and 15.5 mm zone of inhibition against *B. subtilis, B. cereus* and *S. aureu* [4].

De Boer *et al*., 2005 reported that the results of the study showed that the methanolic extract was more effective than aqueous extract. This may be due to the better solubility of the active components in organic solvents.

The gram +ve bacteria having thick layers of peptidoglycons when compare to the gram –ve bacteria and the penetration of SNPs through cell membrane is easy. The exact mechanism of antibacterial activity is not known but some of the scientists state that SNPs may attach to the surface of the cell membrane and disturb its permeability and cause structural changes on cell membrane leads to cell death [16]. Few studies have showed that silver nanoparticles may kill fungal spores by destructing the membrane integrity [17]. There is a possibility of SNPs may also penetrate inside the bacteria and fungi causing damage by interacting with electron phosphorous and sulphur containing compounds such as DNA and proteins resulting in cell death [18].

In the present study the synthesized SNPs from *A. digitata* shows spherical shaped with diameter ranging between 5 nm to 30 nm confirmed by XRD, AFM, SEM and TEM shows potential and effective against different resistant micro-organisms. Smaller particles have larger surface area available for interaction and will give more bactericidal effect than the larger particles [21]. The same type of results were found in *Euphorbia hirta* leaf mediated synthesis of SNPs having the size between 40-50 nm shows highest zone of inhibition on *Bacillus* and *Staphylococcous*. *Acalypha indica* leaf mediated synthesis of SNPs having diameter range between 20-30 nm had excellent antimicrobial activity against water borne

pathogens *E. coli, V. cholera* and *Cochlospermum religiosum* leaf synthesized SNPs with 40 nm act as a potential antimicrobial agent [20].

CONCLUSION: Use of plant extract to synthesize nanoparticles is a promising alternative to chemical based synthesis of nanoparticles. In the present study, Zinc oxide nanoparticles were synthesized using *Aloe vera* extract as reducing agent. The synthesized ZnO nanoparticles were studied for their antimicrobial activity and it was found that the ZnO nanoparticles better activity was seen against *S.aureus* and *S.typhi* than the other organisms. The synthesized ZnO nanoparticles were characterized using UV, FTIR, SEM and XRD. The characteristic UV peak was obtained at 436 nm. The FTIR analysis revealed the presence of aliphatic iodo compounds, Thioesters, Aromatic phosphate, Secondary amine, Alkenyl C=C stretch, Methoxy, methyl ether, Hydroxy group etc. The SEM images showed that the particles were mostly hexagonal and some were spherical. The average size was found to be below 5 µm. XRD analysis confirmed the hexagonal zinc oxide structure for ZnO has an average particle size of 10.35 nm. Future research can focus on a deeper understanding of the mechanism of ZnO synthesis as this will help in maintaining a strict control over the particle size and shape.

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