Green Synthesis of Zinc Oxide Nanoparticle from *Senna alata* and *Euphorbia hirta* Plant Extracts: Effect of antibacterial Activity against Gram-Positive *and* Gram-Negative Pathogens

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Abstract

Synthesis of nanoparticles via biological methods directly from enzymes, plant extracts and micro-organisms has already attained the status of being an eco-friendly alternative to the conventional methods. The current study explores the green route synthesis of Zinc oxide Nanoparticles (ZnO NPs) from the green aqueous extracts of herbal plants *Senna alata* as well as *Euphorbia hirta (E.hirta)*. The invirto bioactivity studies of ZnO NPs synthesized from Senna alata (sample A) were analyzed such that antibacterial activity was performed against gram-positive and gram-negative micro-organism and compared with ZnO NPs (sample B) synthesized from E.hirta for the similar activity. Inhibition zones of Maximum 20 mm and 18 mm were obtained against *Escherichia coli* & *Pseudomonas aeruginosa* for samples A and sample B, respectively. To date, as per the available literature data, no paper has reported a high inhibition Zone value of this order. The observed patterns of X-Ray Diffraction (XRD) authenticate that the particle size of both samples is around 44 nm. The ZnO NPs were further characterized by utilizing UV-Visible spectrophotometer, Fourier transforms spectroscopic analysis (FTIR) and Scanning electron microscopy (SEM). The energy dispersive X-ray analysis (EDAX) clearly validates the elemental presence of both zinc and oxide in the prepared NPs.

Keywords : *Senna alata*, *Euphorbia hirta*, Zinc Oxide nano particles, XRD, UV-VIS, FTIR, EDAX, SEM, Antibacterial activity.

Introduction

The search for efficient methods, with which the synthesis of application-oriented nanoparticles (NPs) could be achieved, continues to be in the limelight consistently such that the synthesis of NPs via green routes persists to be a prominent research topic in recent years. Of late, the synthesis of nanoparticles (NPs) that could be obtained by undertaking eco-friendly routes has attracted researchers all around the globe because of their low cost, eco-compatibility, environmental protection, synthesis in ambient conditions, non-

toxicity, etc. In bio-nanotechnology, green synthesis is recognized as an emerging area with which the benefits of both economic as well as environment-friendly aspects could be attained so that the same is followed for the current research work. [1] The technique with which ZnO NPs are synthesized from green extracts has established itself as an effective alternative to the existing chemical and physical methods.[2]. Nontoxic reagents that involve in green extracts are not only bio-safe but also eco-friendly.[3] Zinc oxide (ZnO) is a well-known classic inorganic metal oxide that is being synthesized in nanosize by means of various methods whereby it exhibits nanostructures of various kinds.[4] These metal oxides possess good photocatalytic and photo oxidizing abilities against biological as well as chemical species [5]. In this work, we intend to undertake green Synthesis of Zinc Oxide Nano Particles from environmentally benign plant leaf extracts of *Senna alata* and *Euphorbia hirta* which have exceptional therapeutic properties [6]. Food and Drug Administration of the U.S has approved ZnO as pretty safe such that it could be considered for further possible procurement of extracts through which synthesis of nanoparticles is feasible because of its low cost, properties of UV blockage, considerable catalytic activity, large surface to volume ratio, white appearance and for their significant applications featuring in the field of medicine as well as agriculture [7,8]. Of late, ZnO nanoparticles are found to have broadly used in antibacterial activity as well as environmental remediation [9].

Senna alata is a familiar medicinal herb belonging to the *Leguminosae* family[10] which is distributed mostly in the regions of tropical and humid as found in Mexico, India and West Indies.[11] It is generally known to be candle bush, acapulo, craw-craw plant, ringworm bush, or ringworm plant. The plant is also predominantly found in the continents of Asia as well as Africa. It has several names with respect to the locality where it is found and it has an array of bioactive chemical compounds. The chemical constituents that have been reported are such as phenolics (rhein, kaempferol, chrysophanol, aloe-emodin, and glycosides), fatty acids (oleic, palmitic, and linoleic acids), steroids, and terpenoids anthraquinones (alatinone and alatonal), (sitosterol, stigmasterol, and campesterol). A Number of biological activities are displayed by such kinds of secondary metabolites that have been reported (*Oluwole Solomon Oladeji et.al., 2020*). The extract of the plant is being traditionally used over a long period in the treatment of typhoid, malaria, diabetes, asthma, and ringworms.[12] The shrub stands around 3-4 meter tall while its leaves grow up to 50-80 cm long and its inflorescence has a similar look to a yellow candle.[13] Moreover, the ingredients of the plant leaves have fungicidal properties[14].

The plant *Euphorbia hirta* is known to be a small annual herb that is pretty often noticed in open wastelands, as a weed in cultivation lands such as rice fields and wheat fields. It could also be seen along the roadsides, pathways and grasslands. The plant is known to be a common herb which is found in pan-tropic , partly subtropic areas and almost throughout the world which includes Australia, Western Australia, Northern Australia, Northern Territory, New South Wales, Queensland, Central America, Africa, Indo-Malaysia, Philippines, China and India. The plant is native to Central America *(Asha.S et.al., 2014). Euphorbia hirta* is a typical herb that possesses medicinal qualities of being antibacterial anti-inflammatory, antimalarial, galactogenic, antiasthmatic, antidiarrheal, anticancer, antioxidant, anti-fertility, antiamoebic and antifungal activities. The plant can grow up to 6 cm.[15] The leaf extract of the plant is being used in south India as ear drops and for the treatment of wounds, especially for boils. In order to prevent pathogen infection, the latex of the plant can be used as warts and cuts. To enable induced milk flow, the decoction of leaves is utilized while the leaf chewed with palm kernel could pave the way for the restoration of virility. The plant leaves are very effective in the treatment of ulcers. The leaves of the plant add to the foodstuff as they are eaten as vegetables *(Asha.S et.al., 2014).*

Materials and Methods

Collection of plants

A random collection of the fresh samples of *Euphorbia hirta, and Senna alata* were undertaken from the yercaud hills, Tamil Nadu, the southern state of India. Running tap water was used to wash the sample materials that were air-dried. Thereafter, the respective samples were homogenized into fine powders so as to be stored in airtight bottles. Then, the bottles were stored in a refrigerator so as to be taken up for further investigations.

Fig 1: Senna alata *Fig 2: Euphorbia hirta*

Extraction of the Plant Material

Crude extracts of both *Euphorbia hirta, and Senna alata* were achieved by employing the method of Soxhlet extraction. Powdered samples of 20 gm each were packed uniformly into two different thimbles and the extraction was enabled by 250 ml of Methanol solvent separately. The extraction process was in progress for 24 h until the solvents in siphon tubes of extractors became colourless.

Phytochemical Analysis

The standard methods demonstrated by Brain and Turner, 1975 and Evans, 1996 were followed for the preliminary phytochemical analysis of all the prepared methanol extracts.

Detection of alkaloids

Dilute hydrochloric acid was used to dissolve the respective extracts such that the filtration process was followed. The obtained filtrates were utilized for the possible test of the presence of alkaloids so that the following tests were undertaken.

a) **Mayer's test**: On treating the respective filtrates with Mayer's reagent, yellow cream precipitates were formed such that the presence of alkaloids could be confirmed.
b) **Wagner's test**: On treating the respective filtrates with Wagner's reagent, brown/ reddish brown precipitates were formed which confirmed the presence of alkaloids.

Detection of Flavonoids

a) **Lead acetate test**: On treating the respective extracts with a few drops of lead acetate solution, yellow precipitates were formed which confirmed the presence of flavonoids.
b) **H_2SO_4 test**: The presence of flavonoids was identified as a few drops of H_2SO_4 were treated with the extracts such that the resultant mixture turned orange.

Detection of Steroids

Liebermann- Burchard test: On adding 2 ml of acetic anhydride with 0.5g of the respective extracts along with 2ml of H_2SO_4, the resultant colour changed from either violet to blue or violet to green depending on the sample which could designate the presence of steroids.

Detection of Terpenoids

Salkowski's test: On mixing carefully 2ml of chloroform and concentrated H_2SO_4 (3ml) onto the extract of the respective plant samples measuring 0.2g, a layer was found to have formed. The appearance of reddish-brown coloration on the inner face confirmed that terpenoids were present.

Detection of Anthraquinones

Borntrager's test: The extracts of both the plants measuring 0.2g were boiled with 10% HCl in a water bath for about a few minutes. Thereafter, the filtrates were allowed to cool such that the resultant filtrates were added with $CHCl_3$ measuring equal volume. Then, a few drops of 10% NH_3 were added to the respective mixtures and heated. The appearance of the pink colour indicated the formation of anthraquinones.

Detection of Phenols

a) **Ferric chloride test**: Both the plant extracts had to be treated with 5% of ferric chloride solution measuring up to a few drops. The appearance of bluish-black could confirm that phenol was present.

b) **Lead acetate test**: The extracts formed yellow precipitate after treating with a few drops of lead acetate solution so that the presence of phenol was confirmed.

Detection of Saponins

Froth test: 5ml of distilled water was shaken with 0.2g of each of the extracts, respectively such that frothing (formation of creamy small bubbles with stable persistence) was formed which predicted that saponins were contained in both the extracts.

Detection of Tannins

Ferric chloride test: The extract of little amount was mixed with a small quantity of water and heated making use of a water bath such that the resultant mixture was filtered. Thereafter, 0.1% ferric chloride was poured into the filtrate so that a dark green formation was seen with which the presence of tannins could be confirmed. This test was performed for both extracts.

Detection of Carbohydrates

Fehling's test: The extracts of both the plants measuring 0.2 gm were boiled in a water bath separately with 0.2 ml each of Fehling solutions A and B such that the presence of sugar was indicated by the obtained red precipitate.

Detection of Oils and Resins

Spot test: The respective test solutions of both the extracts were applied on two different filter papers whereon a transparent nature appeared on the respective filter papers such that the presence of oils and resins could be witnessed.

Green Synthesis of Zinc Oxide Nanoparticles

ZnO NPs were obtained by following biogenic synthesis which was accomplished according to the method of Elumalai *et al*., 2015 with the implementation of a few modifications. The *Euphorbia hirta* and *Senna alata* extracts (about 25 mL) were heated separately (60–80 °C) utilizing a magnetic stirrer. As soon as the temperature of the respected extracts attained 60 °C, Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) measuring 2.5 g was mixed in the respective extracts and allowed for settlement about 1 h such that a white precipitate was formed for both the cases. Then, the resulting pastes were collected in two ceramic crucibles and treated in air-heated furnaces at 60 °C for about 2 h, respectively. A set of light white powder was obtained which were carefully collected. Both the materials were powdered into fine particles utilizing a mortar and pestle so that the final products of synthesized ZnO nanoparticles were obtained and the respective products were named as sample1 (*Senna alata*), sample 2 (*Euphorbia hirta*), respectively for further discussions. Samples 1 and 2 were used for further characterizations.

Results and Discussion

Phytochemical Qualitative analysis:

The phytochemical analysis of qualitative nature for sample1 and sample 2 was enabled so as to identify the constituents present in the form of phytochemicals.

Table 1: Qualitative phytochemical analysis of *Senna alata* and *Euphorbia hirta*

Phytochemicals	Observations	Methanol extract of *Senna alata*	Methanol extract of *Euphorbia hirta*
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	++	++
Arthroquinone 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	++	++
Oils & Resins	Filter paper method	--	--

++ indicates Present and -- indicates absent

Structural characterizations were carried out for the samples with the help of XRD technique.

XRD analysis

The XRD patterns of sample1 and sample 2 are shown in Fig 4 and Fig. 5, respectively. The reflected characteristic (h k l) peaks obtained for 2θ values represent the respective lattice planes (100), (002) and (101) which agree well with the standard JCPDS card 036 – 1451 to be hexagonal wurtzite ZnO. The maximum intensity could be observed for the (002) plane in comparison with the (100) and (101) planes. This leads to the conclusion that growth has taken place in the c-axis in the Hexagonal wurtzite crystal structure.

From the obtained XRD data, the crystalline size could be established for the prepared Zinc oxide NPs using Debye Scherrer's formula. $D = K\lambda/\beta \cos\theta$ A°, Where, λ = 1.5418 A° that is the constant wavelength used for the X-ray radiation. θ is known to be the Bragg diffraction angle while β is referred to full width at half maximum (FWHM) for the intensity of the obtained diffraction pattern measured in radian. The procured crystallite sizes of Samples 1 and 2 are 44.4 nm and 44.6 nm, respectively.

Table 2: Grain sizes of ZnO NPs **with different plant extracts**

Samples	**2θ(°)**	**FWHM (°)**	**Grain size (nm)**
ZnO sample 1 (*Senna alata)*	34.47	0.1968	44.4
ZnO sample 2 (*Euphorbia hirta)*	34.46	0.1968	44.6

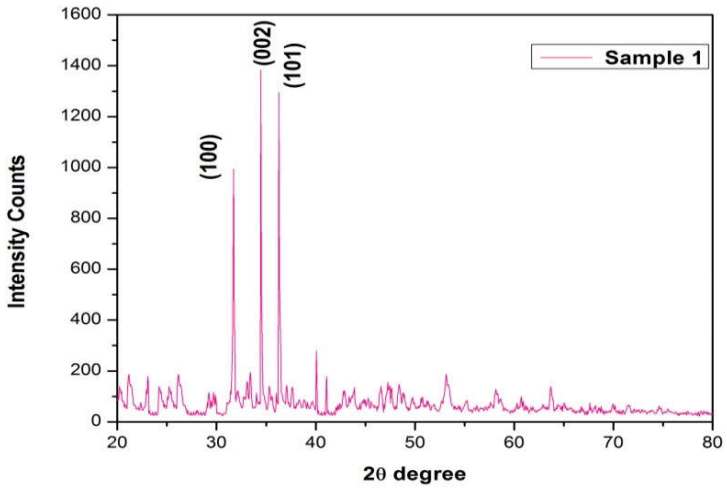


Fig 4: X- ray diffraction patterns of sample 1 (Green synthesized ZnO NPs ***using Senna alata leaf extract)***

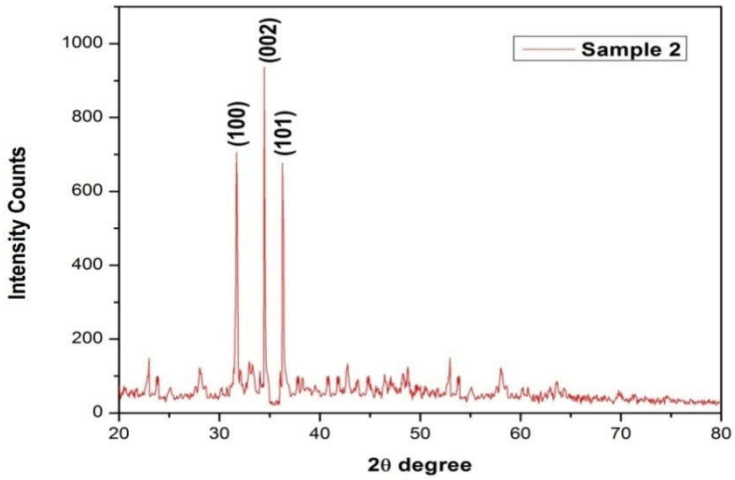


Fig 5: X- ray diffraction patterns of sample 2 (Green synthesized ZnO NPs ***using Euphorbia hirta leaf extract)***

SEM Analysis of Green Synthesized ZnO Nanoparticles

Scanning electron microscopic (SEM) analysis could be achieved by utilizing a Hitachi S-4500 SEM machine.

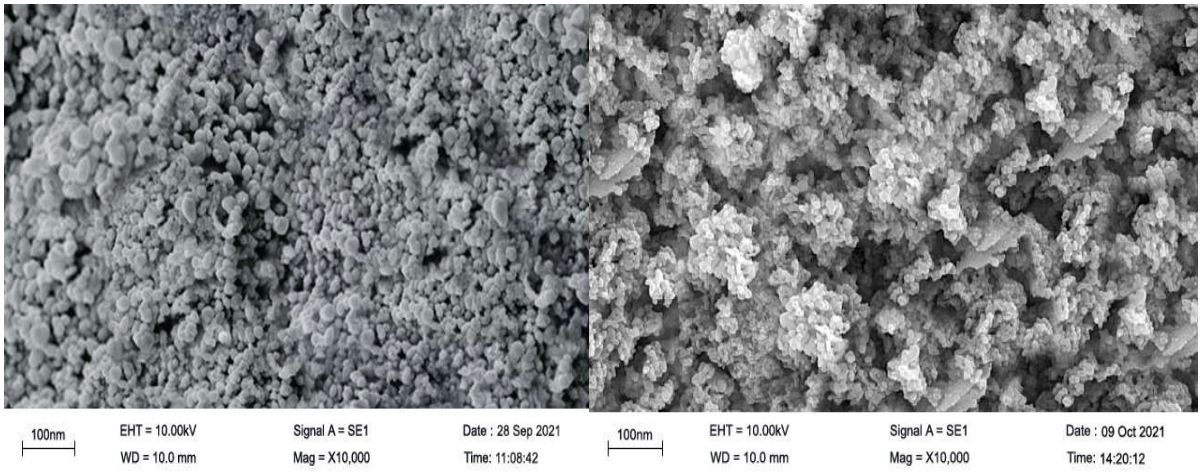


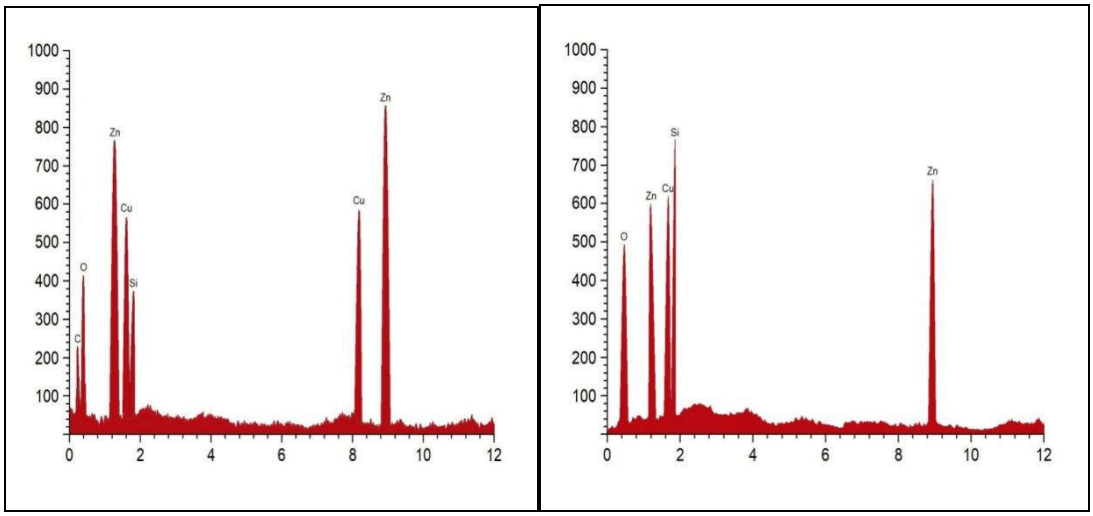
Fig 6(a) ***Fig 6(b)***

Fig. 6(a), (b) : SEM images of sample 1 and 2 (Green synthesized ZnO NPs ***from the leaf extracts of Senna alata and Euphorbia hirta)***

Figure 6(a) and 6(b) exhibit the SEM images of sample 1 and sample 2. These images depict the surface texture and porosity. Nano-clusters in the form of nano-spheres are present. It is clear that ZnO NPs exhibit rough surfaces with heterogeneous porous nature which designates a good possibility for adsorption. Hence, ZnO NPs could be considered for antibacterial activity. Similar particle size is observed from SEM, as it is reflected in the result of XRD such that the result is in good agreement with the earlier report by *Elilarassi et al*.

EDAX Analysis of Green synthesized ZnO Nanoparticles

The EDAX spectra of the respective Green route synthesized ZnO NPs are displayed in fig.7. The amount of Zn and O obtained by EDAX analysis for sample 1 is 42.15% and 14.2%, respectively. For sample 2, the amounts of Zn and O are 49.51% and 8.16 %, respectively. The percentage of Zn, O, and Cu elements available in the respective samples are shown in Table 3.



(a) *(b)*

Fig. 7(a) and (b): EDAX Analysis of sample 1 and 2 (Green synthesized ZnO NPs ***using a) Senna alata and b) Euphorbia hirta leaf extract)***

Elements	*Intensity*	*% Weight*
C	0.16	4.34
O	0.25	8.16
Zn	1.23	23.72
Cu	1.56	15.21
Si	1.65	7.01
Cu	8.13	15.77
Zn	8.97	25.79

(a)

Elements	*Intensity*	*% Weight*
0	0.25	14.27
Zn	1.23	20.69
Cu	1.56	19.98
Si	1.65	23.60
Zn	8.97	21.46

(b)

Table 3(a) and (b): EDAX Table of sample 1 and 2 (Green synthesized ZnO NPs ***using a) Senna alata and b) Euphorbia hirta leaf extracts)***

FTIR analysis

i) FTIR spectrum of Green Synthesized ZnO NPs from *Senna alata* leaf extract

FTIR analysis:

The chemical composition available in the synthesized Zinc oxide NPs was analyzed by making use of a FTIR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer).

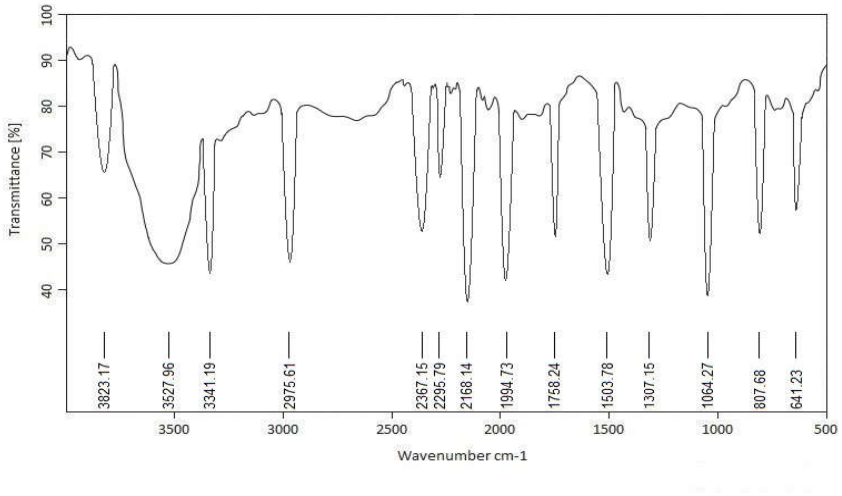


Fig.8: FTIR Analysis of sample 1 (Green synthesized ZnO NPs ***using Senna alata leaf extract)***

S.No	Frequency Range (cm^{-1})	Functional Group
1	3823.17	Primary alcohol (Variable)
2	3527.96	Primary amines (Weak to medium)
3	3341.19	Primary amines (Weak to medium)
4	2975.61	Vinyl terminal (Medium)
5	2367.15	Alkanes (Weak)
6	2295.79	Alkanes (Weak)
7	2168.14	Aromatic methane (Weak)
8	1994.73	Aromatic methane (Weak)
9	1758.24	Aromatic methane (Weak)
10	1503.78	Aromatic methane (Weak)
11	1307.15	Aromatic esters (very strong)
12	1064.27	Aliphatic esters (Very strong)
13	807.68	Meta disubstituted Amines (Very strong)
14	641.23	Meta disubstituted Amines (Medium to strong)

Table 4: FTIR analysis of sample 1

Fig.8 displays the FTIR spectrum of Green synthesized ZnO NPs arising out of *Senna alata* leaf extract. The characteristic peaks obtained at 3823 cm $^{-1}$ and 3527 cm^{-1} are assigned to O-H stretching while the peaks at 1994 cm $^{-1}$ and17581 cm^{-1} are ascribed to O-H bending

vibration. The characteristic peaks at 641 cm^{-1} and 1064 cm^{-1} indicate the formation stretching mode of the Zn-O bond. The occurrence of the above-mentioned functional groups proves the formation of ZnO.

FTIR analysis

ii) FTIR spectrum of Green Synthesized ZnO NPs using *Euphorbia leaf* extract

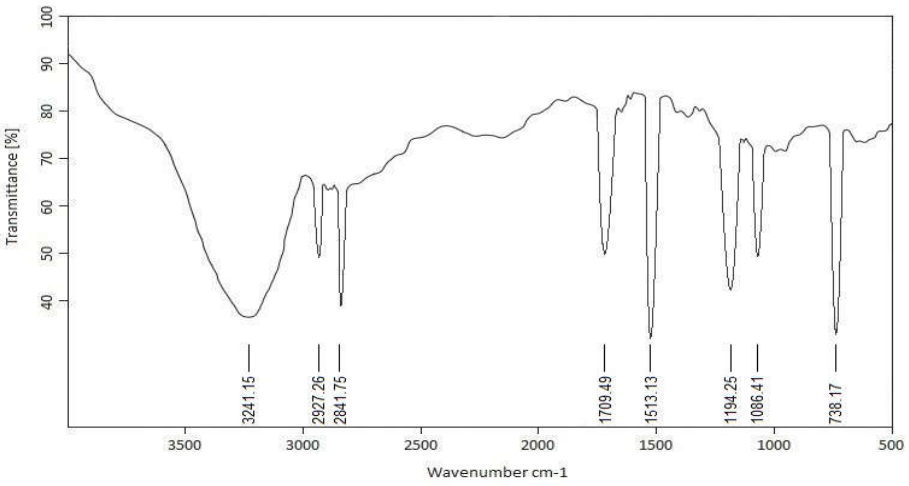


Fig. 9: FTIR Analysis of sample 2 (Green synthesized ZnO NPs ***using Euphorbia hirta leaf extract)***

S.No	Frequency Range (cm^{-1})	Functional Group
1	3241.15	Intra-molecular hydrogen bonded OH (Strong)
2	2927.26	Alkanes (Medium)
3	2841.75	Methoxy (Medium)
4	1709.49	Aliphatic Acids (Very strong)
5	1513.13	Aromatic C-C (Medium to Strong)
6	1194.25	Aromatic esters (Very strong)
7	1086.41	Aliphatic esters (Very strong)
8	738.17	Ortho disubstituted Amines (Very strong)

Table 5: FTIR analysis of sample 2

Fig.9 displays the recorded FTIR-spectrum of Green synthesized ZnO NPs from Euphorbia hirta leaf extract. The characteristic peaks are observed at 3241 cm^{-1}, 2927 cm^{-1}, 2841 cm^{-1}, 1709 cm^{-1}, 1513 cm^{-1} and 738 cm^{-1}. The peaks at 3241 cm^{-1} and 1709 cm^{-1} are assigned to O-H stretching and O-H bending vibrations, respectively. The characteristic peaks at 738 cm^{-1} and 1086 cm^{-1} indicate the formation stretching mode of Zn-O bond. The

formation of the ZnO is authenticated by the availability of various chemical functional groups.

UV-VISIBLE SPECTROSCOPY

Utilizing UV-Visible spectroscopy, it is possible to deal with the implication of the measured intensity as well as the wavelength of the observed absorption of UV region and visible region of EM radiation by the sample. Wherever absorption takes place, it could be observed with the formation of a peak such that a graph is plotted against the wavelength and intensity of absorbed radiation. The required confirmation of biosynthesized ZnO-NPs on their absorption was enabled utilizing a UV-Visible double beam spectrophotometer.

UV-Visible Absorbance Spectra

Fig.10 depicts the UV-Visible spectra of green synthesized ZnO NPs wherein an absorption peak appears at 321nm for *Senna alata* plant extract (Sample 1) and at 231 nm for *Euphorbia hirta* plant extract (Sample 2). The absorption region of the biosynthesized ZnO NPs covers entirely the UV-Visible and near IR region. Photoionisation occurring in semiconductor atoms lead to the occurrence of excitation for the valence electrons moving to the conduction band such that the required energy has to be either greater or equal to the value of the bandgap. Photoionisation occurring in impurity atoms lead to the transition of electrons which move either from the donor energy level to the conduction band or from the valence band to the acceptor level. Therefore, it is possible to use the UV region for better analysis of antimicrobial activity. The values of optical band gap values have been measured by Tauc plot method (Direct method). The obtained optical band gap value for *Senna alata* is 2.90eV and for *Euphorbia hirta* is 3.00 eV.

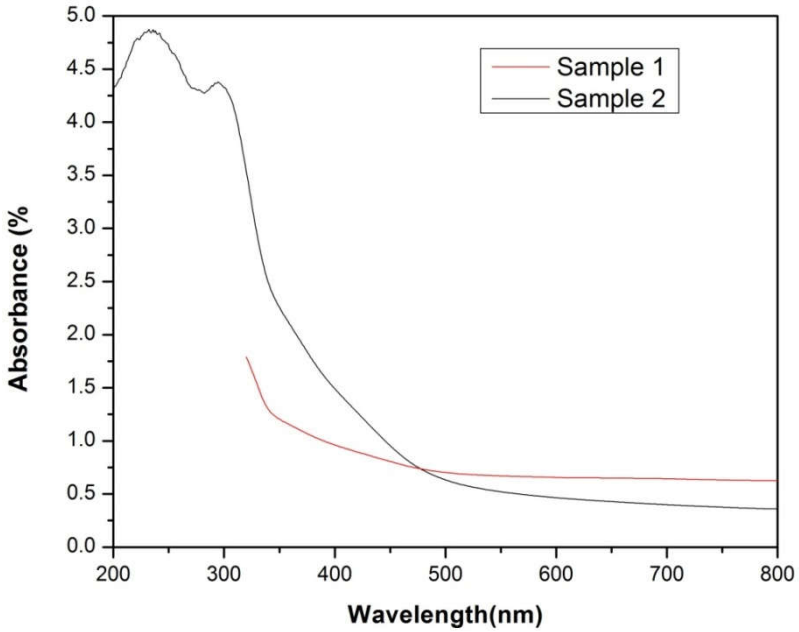
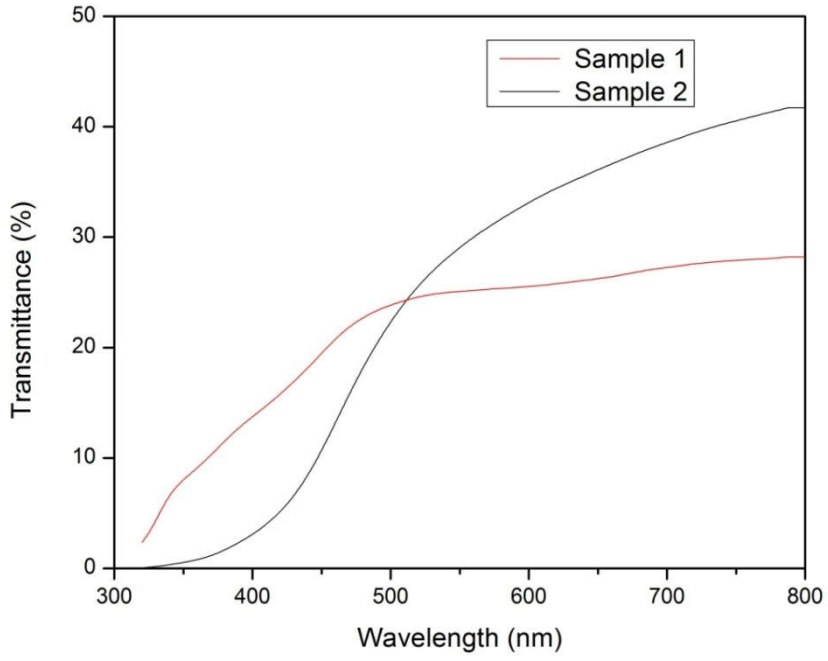


Fig. 10: UV-Vis Absorbance spectra of samples 1 and 2 (Green Synthesized ZnO NPs ***using Euphorbia hirta and Senna alata leaf extracts)***

UV-Vis Transmittance Spectra



Analysis

Fig.11: UV-Vis Transmittance spectra of sample 1 and 2

Fig.11 depicts UV-Vis Transmittance spectra of Green Synthesized ZnO nanoparticles of samples1 and 2. The optical transmittance of the ZnO NPs is observed to be 21 % for *Senna alata* and 41 % for *Euphorbia hirta.*

The bandgap values of Green Synthesized ZnO NPs using Senna *alata* and *Euphorbia hirta* leaf extracts are given in table 4.2. From the observed results, it is obvious that the samples1, 2 exhibit smaller bandgap and larger grain size and hence could result in enhanced antibacterial activity. The illumination of ZnO by UV light can also generate reactive species as superoxide radicals or hydroxyl radicals in an aqueous environment so that it could be used to degrade bacterial cell walls. The UV results suggest that Green Synthesized ZnO NPs could serve as promising potential candidates for antibacterial activity.

Table 6: Grain size and Band gap values of prepared ZnO Samples

Samples	Grain size (nm)	Band Gap (eV)
ZnO Sample 1 *(Senna alata)*	44.4	2.90
ZnO Sample 2 *(Euphorbia hirta)*	44.6	3.00

Substances derived from nature as the extracts of plants have been already established as prospective sunscreen resources due to their ability to potential absorption of ultraviolet rays both in the UV A and B region so that they possess antioxidant activity.[18] The available evidence emphasizes that DNA-damaging could induce the accumulation of UV light-absorbing flavonoids as well as other phenolics in the available dermal tissue of a plant body that possesses outstanding antioxidant as well as photo-protective properties. Therefore, the experimentally obtained extracts could be made use of as an effective as well as comparatively safer ingredient in sunscreen lotions.[19]

Antibacterial Activity

Screening of Antibacterial Activity

Five bacterial strains known to be *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Pseudomonas aeruginosa* were utilized for the entire investigational process. The required bacterial cultures for the analysis were performed making use of Microbial Type Culture Collection (MTCC), Institute of Microbial

Technology, Chandigarh, India. The screening procedure had been carried out well before the young bacterial broth cultures were prepared.

Preparation of inoculums

Stock cultures that were kept on slopes of nutrient agar were maintained at a constant temperature of 4^{o}C. Active cultures required for the experiment could be obtained by transferring a loopful of cells of both directly from the stock cultures to two test tubes of Muller-Hinton broth (MHB) for the growth of bacteria. Both the test tubes of samples 1 and 2 that were to be incubated were kept without any agitation at about 24 h at 37^{o}C and 25^{o}C, respectively. Fresh Muller-Hinton broth was used to dilute such that the required optical density ranging up to 2.0 X 10^{6} colony cultures forming units (CFU/ml) of bacteria were formed.

Antimicrobial susceptibility test

In order to perform the antimicrobial activity, the disc diffusion method (Bauer *et al.,* 1966) was carried out. The screening process of *Invitro* antimicrobial activity was enabled by utilizing Muller Hinton Agar (MHA) which was procured from Himedia (Mumbai). The preparation of MHA plates could be achieved by just pouring the molten media measuring 15 ml into the sterile petri plates. Thereafter, the respective plates were kept alone so as to solidify for a time period of 5 minutes such that a suspension of 0.1% inoculums was swabbed uniformly. Then, the drying process of inoculums was performed for about 5 minutes. The concentration of the respective extracts was found to be 40 mg/disc each which were loaded on two 6 mm sterile discs, respectively. The loaded discs were kept on the surface of the medium wherein the required samples of extracts were given the time to diffuse at about 5 minutes. Thereafter, the plates were maintained at 37^{o}C for 24 h in order to ensure incubation. During the end process of incubation, inhibition zones were formed around the respective discs such that the range of the respective inhibition zones was measured making use of a transparent ruler in millimetre.

i) Antibacterial Activity of ZnO NPs **synthesized from *Senna alata* leaf extract**

The antibacterial activity of the synthesized ZnO NPs from *Senna alata* was subjected towards various gram-positive and gram-negative bacteria that were screened by disc diffusion method and the resultant samples are portrayed in Figure 12. The results arrived at

out of the antibacterial activity of the synthesized ZnO NPs obtained from methanol extract of *Senna alata* show comparatively better effect as it comes against P.aeruginosa, E.coli, S.aureus and *B.subtilis*. As reflected from table 7, the observed maximum zone of inhibition is located for P.aeruginosa (20 mm), E.coli (20 mm) while the other zones of inhibition are for S.aureus (19 mm), *B.subtilis* (18 mm) and S.typhi (17 mm) in 60 µl concentration. The antibacterial activity obtained in vitro reveals that the green synthesized extract has considerable activity against all the microorganisms that have been tested.

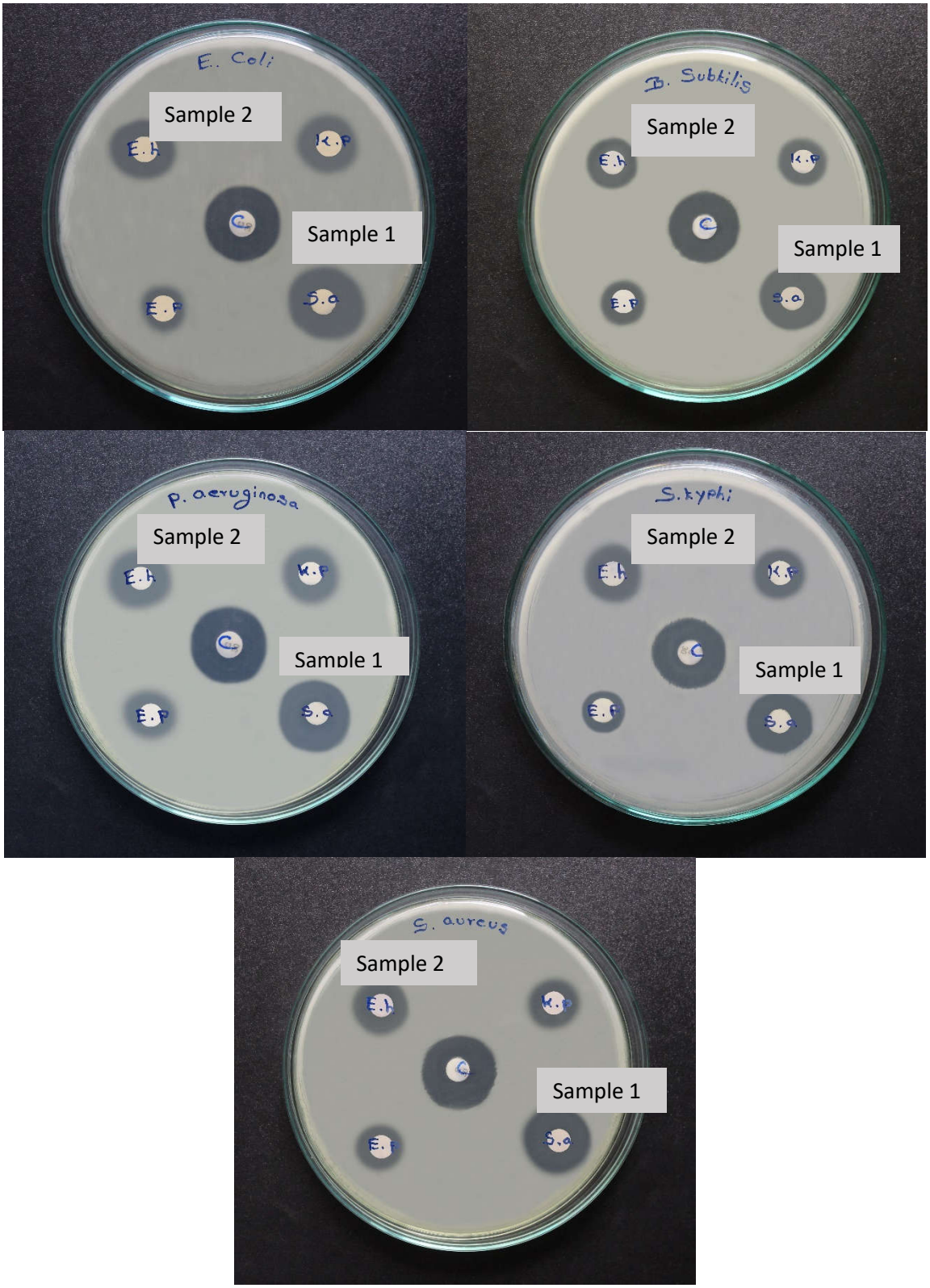


Fig. 12 : Antibacterial Activity ZnO NPs ***sample 1and 2***

S.No	Organisms	Control	Zone of Inhibition (mm)	
			Senna alata (ZnO Sample 1)	***Euphorbia hirta (ZnO Sample 2)***
1	*E.coli*	19 mm	20 mm	17 mm
2	*S.typhi*	18 mm	17 mm	15 mm
3	*P.aeruginosa*	21 mm	20 mm	18 mm
4	*B.subtilis*	20 mm	18 mm	14 mm
5	*S.aureus*	22 mm	19 mm	17 mm

Table 7: Antibacterial Activity in ZnO NPs **of *sample 1and 2***

ii) Antibacterial Activity in ZnO NPs

The ZnO NPs that have been synthesized from *Euphorbia hirta* were put under antibacterial activity for a range of gram-positive and gram-negative bacteria which were under test by undergoing disc diffusion method which is presented in Fig.12. The results of antibacterial activity obtained for the synthesized ZnO NPs from methanol extract of *Euphorbia hirta* show fairly good impact against P.aeruginosa, E.coli, S.aureus and S.typhi. As highlighted in table 7, the observed maximum for the zone of inhibition appears to be for *P.aeruginosa* (18 mm) while the other zones of inhibition are observed for E.coli (17 mm), S.aureus (17 mm), S.typhi (15 mm) and B.subtilis (14 mm) in 60 µl concentration.

Conclusion

ZnO NPs were synthesized by using methanol extract of two different plants *Senna alata and Euphorbia hirta* which were accomplished by utilizing the Soxhlet extraction method. The structural and morphological properties of the prepared ZnO samples could be characterized by using XRD and SEM. The elemental composition and functional groups were analyzed by EDAX and FTIR, respectively. The optical studies were carried out by implementing UV-Vis spectroscopy. SEM pictures reveal the adsorption nature of the synthesized NPs. The antibacterial studies of green synthesized ZnO NPs were carried out against *Escherichia coli* and *Pseudomonas aeruginosa.* The bio-synthesized ZnO NPs show better antibacterial properties against human pathogens. From the overall obtained experimental data, it could be generalized that ZnO NPs synthesized using *Senna alata and Euphorbia hirta* plant extracts have been proved to be one of the prominent antibacterial agents for both Gram-positive and Gram-negative bacteria.

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