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Phyto–engineered *Andrographis alata* zincoxide nanoparticles and their potentials of antibacterial and antioxidant activities under *In vitro*

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Abstract

The study assessed the anti-oxidant and anti-bacterial effect of fabricated green zincoxide nanoparticles (ZONs) of *Andrographis alata* whole plant ethanol extract. Phyto-fabricated nanoparticles (NPs) were characterized, which shows and confirms the formation of *Andrographis alata* ZONs through absorbance at 370 nm in UV-Visible spectra, shape and size were flower/star-like bunches with variable nm sizes by SEM and HR-TEM. From XRD and SAED confirms the purity of crystallite nature and crystalline phase and FTIR shows the functional groups involved in reducing as well as capping agents in the synthesis. EDAX shows the percentage of Zinc as 70.54% and Oxygen as 20.18% which shows the presence of Zinc and Oxygen in the synthesized *Andrographis alata* ZONs. Particle size distribution was measured by DLS was 22nm and PDI value was 1.000. Synthesized *Andrographis alata* ZONs exhibits good DPPH radical scavenging activity with IC50 values of 51.11µg/mL and displays good antibacterial activity against gram positive (Gm+) *Corynebacterium diphtheriea, Bacillus subtilis, Streptococcus pyogenes* and *Staphylococcus aureus*; gram negative (Gm-) bacteria *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhii* than leaf extract. Overall, the outcomes exposed that phyto-fabricated *Andrographis alata* ZONs can be splendid approach to form biocompatible and stable agents with significant potential in biological applications.

Keywords: *Andrographis alata*; ZONs; Anti-oxidant; Anti-bacterial; IC_{50} Value

1. Introduction

Nanobiotechnology, the amalgamation of nanotechnology and biotechnology to develop bio, synthetic and environment-kindly technology for the fabrication of nanomaterials. Metal oxides have notable properties like optimal chemical stability, adhesiveness and solubility. For the account of environmental aspects, metal oxide NPs synthesis is stimulated by using green chemistry methods through biological systems due to progress in metal oxide NPs usage in environmental applications [1,2]. The spectra in environmental and biological research areas plays a significant role in medicinal applications, electronic devices, contaminant detection and radiolabeling is broadened by the reaction of a ligand or salt [3].

In the fabrication of advanced nanomaterials, nature makes the ways and insights. For the synthesis of pure metal oxide NPs via biomimetic method, that biological systems can act as the 'bio-laboratory' has now been reported in the literature.

Latest reports specify that the NPs, including ZONs fabricated by the biological technique, rather than chemical and physical methods, it was harmless, biocompatible, bio-safe and had worthy inhibition against different microbes compared to chemically derived NPs. Because of their hexagonal phase, n-type semiconductor, and wurtzite structure,

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ZONs have acquired special notice. The distinctive property of ZONs for targeted drug delivery is chiefly ascribed to its semiconductor nature that induces ROS (reactive oxygen species) production and ultimately leads to cancer cell death. ZnO (zinc oxide) is generally recognized as safe (GRAS)) by the FDA [21CFR182.8991] [4]. Zinc oxide NPs can commonly use in the production of anti-septic lotion, surgical tapes, anti-itch creams, anti-bacterial Band-Aid's, anti-microbial powders, diaper powders, anti-dandruff lotion and ceramics. As well, an advanced bio sensing ability of ZONs was resulting due to the luminescence and conductivity of ZnO, boost the UV filtering property and optical absorption. Because of the unique optical, chemical and electrical properties of ZONs, they are used in various applications like metal-insulator-semiconductor diodes, light-emitting diodes, solar cells, drug delivery, photocatalytic degradation, and personal care products for instance sunscreens and cosmetics, which make them the most viable, time-efficient and economically feasible approach as the chemicals are fewer perilous to the ecosystem.

Phyto-fabrication of ZONs by using different plant extracts were assessed to develop antioxidant, anti-diabetic, anti-cancer, anti-fungal and antibacterial potentials of NPs.

Andrographis alata. Nees (Acanthaceae) is a rare and erect herb dispersed widely in Southern India [5]. It is an endemic medicinal plant and reported to have the highest concentration of neoandrographolide with an effective antioxidant, hepatoprotective, antimalarial and anti-inflammatory properties [6,7]. *Andrographis alata* has also been reported to contain a number of unusual flavones and flavone glycosides [8,9].

Current work is aimed to fabricate the environ-friendly *Andrographis alata* plant mediated ZONs and its characterization using UV-Vis spectra, XRD, SEM, EDAX, HR-TEM, SEAD, FTIR and DLS. In this phyto-synthesis of ZONs, *Andrographis alata* whole plant extract acts as a capping and stabilizing agents that have owe pharmacological activity. In this research antioxidant and antibacterial activities of *Andrographis alata* mediated ZONs will be studied.

2. Materials and methods

2.1. Materials

Zinc acetate dihydrate [Zn(O2CCH3)2(H2O)2], DPPH(1,1-diphenyl2-picrylhydrazil), Methanol, were procured from Emplura L-Ascorbic acid, Muller-Hilton Agar, Nurient Broth were purchased from Hi-media, Agar-Agar from Fisher Scientifics, Microorganisms of Gm+ Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis and Corynebacterium diptheriea and four Gm- bacteria Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Salmonella typhii all are procured from Department of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh.

2.2. Plant collection

The whole plant of the *Andrographis alata* was collected from Thalakona near Tirupati, Andhra Pradesh and identified by Prof. C. Varadarajulu Naidu, Department of Biotechnology, Dravidian University, Kuppam.

2.3. Preparation of plant extraction

Collected whole plant was washed thoroughly with running tap water followed by Type-1 water and allowed it to air-dry then to shade-dry. The dried plant material was blend to fine powder. 5gm of whole plant dry-powder was taken into 250 mL round-bottom flask and 100 mL of ethanol was added to it and kept on magnetic stirrer for 2 hours at 70 ^{0}C. After this, the plant extract was permitted to cool at room temperature then filtered with vacuum filter by What-man filter paper No.1 and the filtrate was stored for further experiments.

2.4. Green-fabrication of zinc oxide nanoparticles (ZONS)

0.2M Zinc acetate dihydrate [Zn(O2CCH3)2(H2O)2] was dissolved in 50mL type-1 water in a 250mL round bottle flask and kept on magnetic stirrer for one hour at 70 ^{0}C [10]. To this, 25mL of plant extract was added and allowed it to stirr for complete mixing of the added solutions for 2-3 hours and for maintaining pH-12, 3M sodiumhydroxide solution was added drop wise. The color of the solution was changed from white to pale green confirmed the synthesis of ZONs. From the reaction solution the precipitate was separated by centrifugation at 10,000 rpm at 30 ^{0}C for 20 min and pellet was collected. The filtrate was discarded and then pellet was washed type-1 water for 3 times and then with ethanol for 2 times, after that pellet was collected into watch glass and dried it in hot-air oven at 80 ^{0}C for 2 hr., then preserved it in air-tight bottle for further studies.

2.5. Morphological and structural characterization of ZONs

Characterization of phyto-synthesized ZONs of *Andrographis alata* was done by various analytical techniques like UV-Vis, FTIR, XRD, SEM, EDAX, DLS, HR-TEM, and SAED to determine the quality and quantity of synthesized ZONs.

UV-Vis spectrophotometer: The absorption spectrum of *Andrographis alata* ZONs was recorded on Shimadzu 1800 UV-Vis spectrophotometer using 200-800nm.

FTIR spectroscopy: For the functional groups of *Andrographis alata* identification FTIR spectra was attained on FTIR spectrophotometer (Bruker-Tenson 37) in the range of 4000-400cm^{-1}, using *Andrographis alata* ZONs powder.

X-ray diffraction: Phase purity and average size of *Andrographis alata* ZONs was analyzed using Powder-XRD diffractometer (Rigaku-Miniflex600, Japan) working at kV detector voltage, 15mA current and a nickel filter (2θ range of 20^0 - 90^0).

SEM: Images of *Andrographis alata* ZONs were obtained by scanning electron microscope (JEOL JSM-IT500) operating with 15-18kV proximal current and 15eV acceleration voltage to know the surface morphology. The sample was placed on a carbon-coated tape and coat with gold sputtering for 30 minutes and then used for imaging.

EDAX: Elemental composition data of bio-fabricated ZONs was found by elemental dispersive X-ray analysis which is connected with SEM.

DLS: Synthesized ZONs particle size distribution was determined by dynamic light scattering analyzer (Malvern, ZETASIZER, Nano series Nano-S90) instrument at 2 0^0C. *Andrographis alata* ZONs sample was dispersed in type-1 water (1mg/ 1mL) was taken into DLS cuvette for analysis.

HR-TEM: By using high resolution transmission electron microscopy (FEI Tecnai F20 S-Twin), images were attained operating at 200kV to measure particle size and structural characterization of synthesized ZONs of *Andrographis alata*.

SAED: Selective area electron diffraction was used to know the determination of growth directions of synthesized ZONs of *Andrographis alata*.

2.6. Antibacterial activity

Andrographis alata ethanol extract and green ZONs antibacterial potential was investigated by using agar disc diffusion method [11]. For attaining active organisms, all bacterial strains were subcultured in a nutrient broth and incubated at 37^0C overnight. From this 100µL was taken and dispensed in autoclaved nutrient broth and adjusted to Mcferland standard (1× 106 cfu/mL). Petriplates are filled with 20mL of sterilized Mueller Hinton Agar media (MHA). From this 100µL was taken and dispersed on media. Sterilized discs containing 50 and 100µL of test samples extract and ZONs were impregnated on the MHA media and incubated for overnight at 37^0C. inhibition zones by bacterial growth around the discs were measured by measuring scale.

2.7. Determination of minimum inhibitory concentration (MIC)

The MIC was evaluated by micro broth dilution technique [12] for extract and ZONs. All bacterial strains were streaked on nutrient agar plates. After incubation for overnight, 2-3 colonies were picked and dispensed in nutrient broth and kept in rota-incubator for overnight at 37^0C. From this, 100µL were suspended in 10mL sterilized nutrient broth to obtain Mcfarland standard. Stock solutions of extract and ZONs (10mg/mL) were prepared in sterile type-1 water. In the flat-bottomed 96-well microplate, first column was sterility control, twelveth column was taken as growth control and second to eleventh were taken with different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9 and 1.95µg/mL) of *Andrographis alata* ZONs, 100µL of autoclaved nutrient broth and 5µL of bacterial inocula were added and incubated. Turbidity was observed before and after incubation at 630nm absorbance.

2.8. Determination of minimum bactericidal concentration (MBC)

50µL of bacterial suspension from MIC plate was taken and inoculated on sterile MHA media and incubated for 24 hours. Concentrations at no visible colonies were taken as MBC.

2.9. *In-vitro* antioxidant assay

Andrographis alata ethanolic extract and ZONs antioxidant potency is evaluated by standard spectrophotometric methods such as DPPH and ABTS radical scavenging assays. The radical scavenging percentage was attained by below formula:

Radical scavenging percent = OD Control – OD Sample / OD Control × 100

Using concentrations Vs percentage inhibition curves, IC_{50} were calculated.

2.10. DPPH radical scavenging assay

Various concentrations 20, 40, 60, 80 and 100µg/mL of plant extract, ZONs and standard ascorbic acid were prepared in methanol. Fresh stock solution of DPPH (125mM) was prepared by dissolving 2.45mg in methanol and kept in dark conditions at room temperature. Different concentrations of test samples and standard were taken as 10µL and 290µL of DPPH solution were filled in 96-well plate and incubated for 30 minutes. After, absorbance was noted at 517nm wavelength using microplate reader (BIORAD iMark) 25^0C [13]. methanol alone and reaction mixture without test sample/ascorbic acid were taken as blank and control respectively.

2.11. Statistical analysis

All investigations were performed trice (n=3). The one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of control and treatments. The data were shown as mean ± SD and $p < 0.05$ accepted as the minimum level of significance.

3. Results and discussion

3.1. Visual observation

The color change of biogenic nanoparticles was the preliminary test that is from dark green to pale-green. Surface Plasmon Resonance (SPR) excitation is the cause of color change i.e. an interacting electromagnetic field induce the collective oscillation of free conduction electrons. It represents the formation of ethanol extracted green synthesized ZONs from the whole plant of *Andrographis alata*.

3.2. UV-visible spectra analysis

The phyto-synthesized ZONs bio reduction was monitored with an UV-Vis spectrophotometer. In accordance to surface plasmon resonance (SPR) effect, at a certain wavelength range conducting electrons start oscillate. At 370nm, spectra revealed a characteristic absorbance [14] in Figure 1. This can be attributable to intrinsic bandgap absorption of ZnO and electron transitions from the valence band to the conduction band. The bandgap (Eg) of bulk ZnO material is Eg =3.45148eV. The bandgap was calculated from the absorption spectra using the below equation [15]:

$$\alpha h\nu = D(h\nu - E_g)^n$$

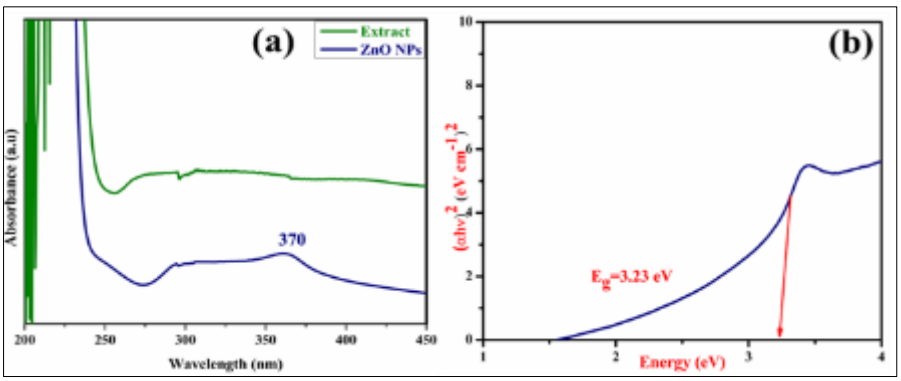


Figure 1 UV-Vis spectra of *Andrographis alata* (a) ethanol extract and synthesized ZONs and (b) Tauc plot

Where α is absorption coefficient, h is incident photon energy, D is energy independent constant and Eg is the bandgap energy of the material. The transition data provides the best linear in the region for n =2.

The corresponding bandgap obtained was 3.23eV, this also reported in previous reports [16]. The bandgap is increased by decreasing of nanoparticle size. Biological properties like antibacterial, substrate binding, cleavage activities and antioxidant are correlated with the bandgap energy [17].

3.3. Scanning electron microscopy (SEM)

SEM study exhibits the shape, dispersal and size of the bioreducted nanoparticles. Figure 2 shows the SEM images shows the morphology of *Andrographis alata* ZONs are flower /star-like bunches and nanometre scaled rod shape. SEM was seen in various magnification ranges like 1µm - 500nm.

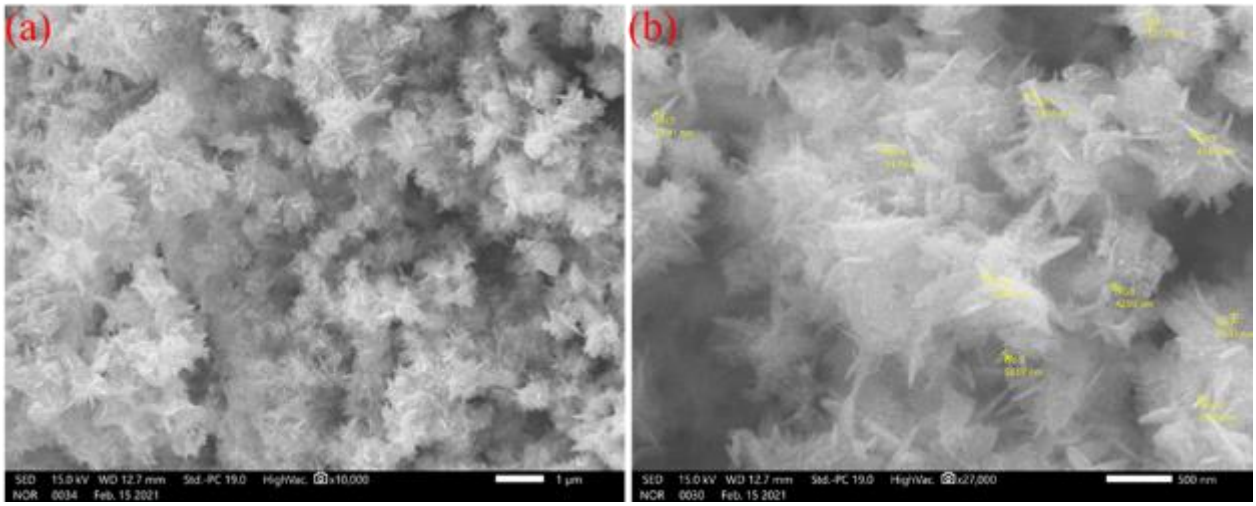
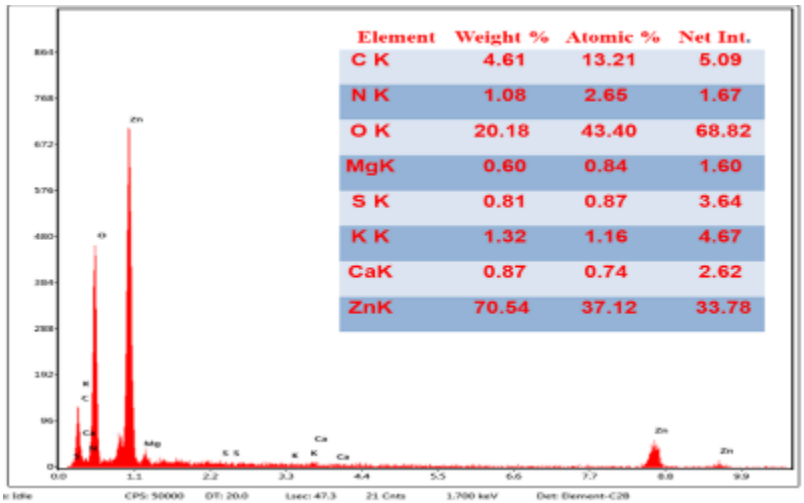


Figure 2 Scanning Electron Microscope (SEM) images of synthesized ZnO NPs of *Andrographis alata* ethanol extract; (a)1µm, (b)500nm.

These images revealed the size ranges between 41.06 to 79.05nm and some are in above 100nm and even micron size. The nanoparticles could be exhibited in the form of agglomeration. The cause for the agglomeration is due to high surface energy of ZONs and also viably because of densification that of thin space between nanoparticles.

3.4. Energy dispersive x-ray analysis (EDAX)



Element	Weight %	Atomic %	Net Int.
C K	4.61	13.21	5.09
N K	1.08	2.65	1.67
O K	20.18	43.40	68.82
MgK	0.60	0.84	1.60
S K	0.81	0.87	3.64
K K	1.32	1.16	4.67
CaK	0.87	0.74	2.62
ZnK	70.54	37.12	33.78

Figure 3 Energy Dispersive X-ray (EDAX) spectra of ZONs from *Andrographis alata* ethanol extract

Elemental configuration and purity of green synthesized ZONs of *Andrographis alata* was endorsed by the EDAX, that equipped with SEM instrument. This spectra analysis unveils strong signals pertinent to zinc and oxygen with percent weights 70.54 and 20.18% respectively displays in the Figure 3. This divulges clears that no impurity peaks were found other than elemental peaks of zinc and oxygen. This configuration evidently e exemplified that plant extract reduce the Zn salt and yields in uncontaminated ZONs. And weak signals at C, N, K, Mg and Ca might due to secondary metabolites such as carbohydrates, gums, tanins, musilases, terpenoids and saponins etc. EDAX spectrum evidently portrays the presence of extreme active role of stabilizing and capping agents in the *Andrographis alata* plant ethanol extract [18,19].

3.5. Powder x-ray diffraction (XRD)

The phyto-mediated ZONs crystallographic structure, purity, chemical composition and physical properties were confirmed by the powder XRD analysis. Figure 4 shows the *Andrographis alata* ZONs XRD spectrum. This spectrum represents the 2θ angles at 31.75^0, 34.42^0, 36.23^0, 47.53^0, 56.56^0, 62.86^0 67.99^0 and 69.11^0 corresponding to planes (100), (002), (101), (102), (110), (103) (112) and (201) confirms the hexagonal (wurtzite) crystal structure which in synchronizing with the JCPDS card no: 01-089-1397. Synthesized *Andrographis alata* ZONs crystalline structure and high purity was specified by the narrow and sharp peaks. The typical peak of ZONs is detected at plane (101) which is compared with other XRD peaks. This signifying the *Andrographis alata* mediated ZONs are free of impurities [20,21].

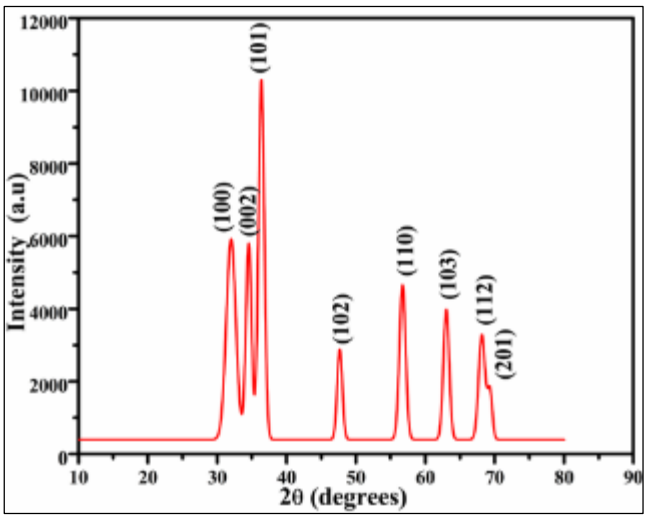


Figure 4 X-ray Diffraction pattern of ZONs synthesized from *Andrographis alata* ethanol extract

Table 1 Parameter calculation for average size calculation for *Andrographis alata* synthesized nanoparticle

Peak position	hkl	FWHM	Crystallitesize (D nm)	D average nm
31.75	100	2.436	3.13	7.85
34.42	002	0.877	8.65	
36.23	101	0.912	8.27	
47.53	102	0.695	10.45	
56.56	110	0.903	7.74	
62.86	103	0.846	8.01	
67.99	112	0.863	7.62	
69.11	201	0.732	8.93	

The particles size of the synthesized *Andrographis alata* ZONs was calculated by using Debye-Scherrer's equation [22]:

$$D = K\lambda / \beta\cos\theta$$

Where D is the crystallite size, K is a shape factor (Scherrer constant 0.9), λ is the X-ray wavelength of x-ray (wavelength of Cu Kα radiation is 1.5406 Å), β is the full-line width at half-maximum of the diffraction peak, and θ is the Bragg's angle. The average particle size of ZONs is 7.85nm. When the value of FWHM is increased the particle size decreased.

3.6. High resolution transmission electron microscopy (HR-TEM)

HR-TEM images reveals the morphological shape and size of the biosynthesized *Andrographis alata* ZONs was looks like flower and rod shapes are exhibited ranges from 2µm to 100nm represented in the Figure 5. The size of the ZONs are dissimilar with variable sizes in nm. These ZONs represents agglomeration, that is archetypal with bio-synthesis of zinc oxide nanoparticles. The agglomeration was due to high surface area and they strongly stick to each other and also might be possible due to densification resulting in very minor or no space between particles [23,24].The images corresponds the occurrence of phytochemical capping that might be dispensed to bio-organic compounds.

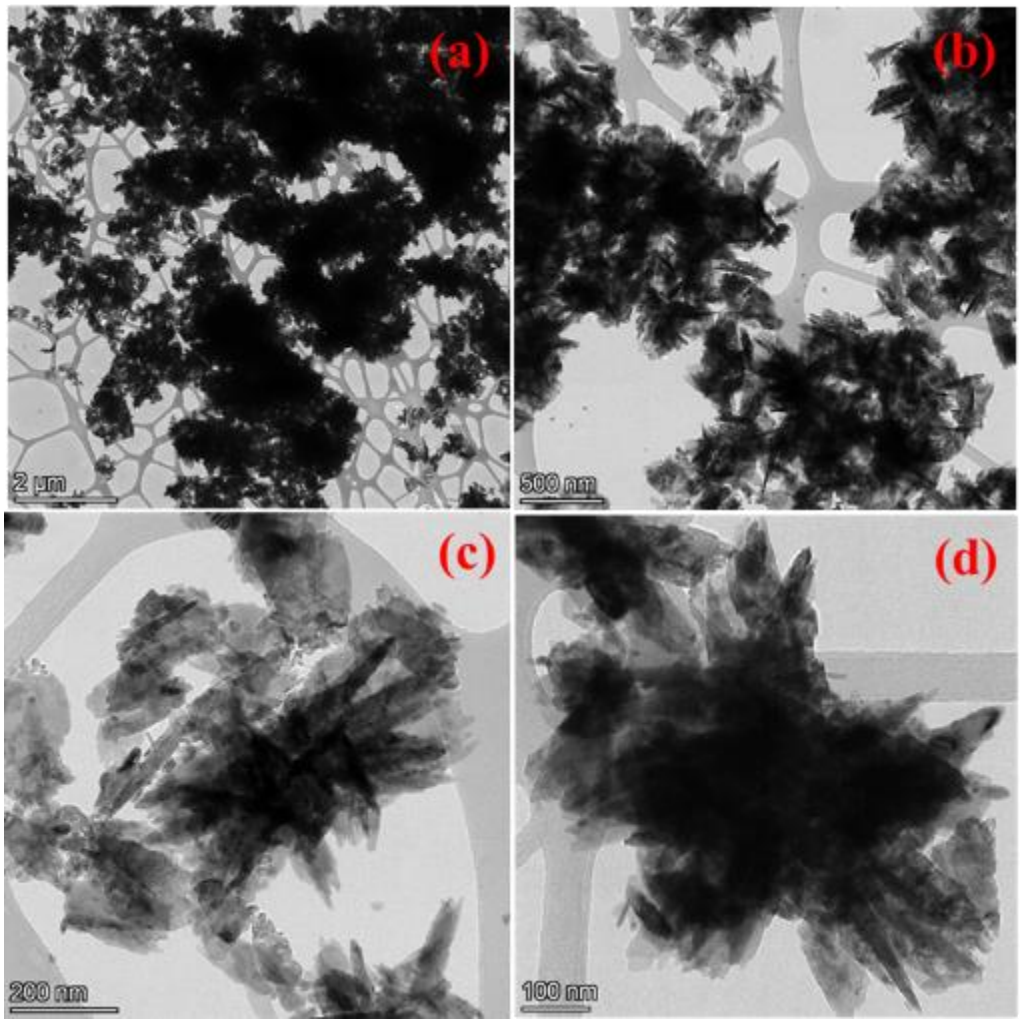


Figure 5 High Resolution Transmission Electron Microscope (HR-TEM) analysis of synthesized ZONs of *Andrographis alata* ethanol extact: (a) 2µm (b) 500nm (c) 200nm and (d) 100nm

3.7. Selective area electron diffraction (SAED)

Selective area electron diffraction (SAED) image of *Andrographis alata* ZONs shows single crystalline and the poly crystalline lattice planes which represents the obtained from the XRD. The single dotted lines represent the single crystals and the ring pattern represents polycrystalline nature are shown in Figure 6. The image shows the agglomerations of the *Andrographis alata* ZONs grains are non-porous, and these have a substantial affinity to one other. So, asymmetrical clusters are formed during NP formation processes [25].

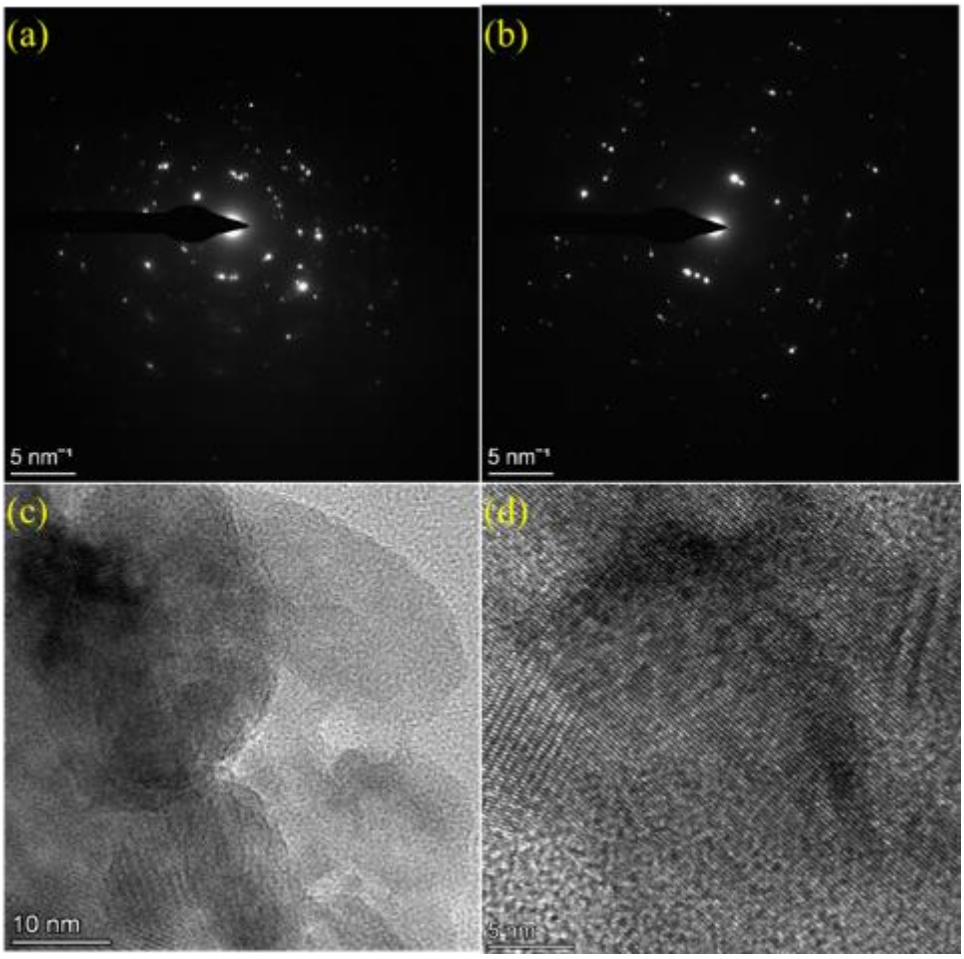


Figure 6 SAED of *Andrographis alata* ZONs: (a) polycrystalline, (b) single crystalline, and planes on crystalline grains (c) in 10nm and (d) in 5nm

3.8. Fourier transform infrared spectroscopy (FTIR)

The reduction of zincacetate dihydrate into zincoxide NPs were due to secondary metabolites present in the *Andrographis alata*. In accordance of the phenolic compounds, flavonoids, terpenoids and alkynes FTIR exhibits the formation and control of size of ZONs.

Synthesized ZONs composition and functional groups formation is declares by the FTIR spectra. Particularly Andrographolides are the major bioactive compounds responsible for the efficient capping and stabilizing on synthesized ZONs. Several peaks, were exhibited at 3425,2999,2904,2344,1972,1649,1428,1302,1009,946 and 694cm^{-1} from *Andrographis alata* ethanol extract and 3274, 2341, 1553,1381, 1077, 1022, 856, and 517cm^{-1} from *Andrographis alata* ZONs. The broad bands seems at 3425cm^{-1}, 2341cm^{-1} and1649cm^{-1} in *Andrographis alata* ethanol extract represents the presence of hydroxyl group, —N—H of amide in proteins, —OH stretch respectively and along with these other peaks are interfering the role of functional groups of ethanol extracted *Andrographis alata* in the bioreduction and stabilization of ZONs[26-29]. Small peaks in *Andrographis alata* NPs at 1553cm^{-1} have been assigned amide I, peak at 1381cm^{-1} denoting the C=C stretching vibration of aromatic ring[30], stretching vibration at 1022, 856cm^{-1} attributed to the presence of C—N stretching amine group [31,27]. Large peak at 517cm^{-1} refers the formation of NPs of zincoxide.

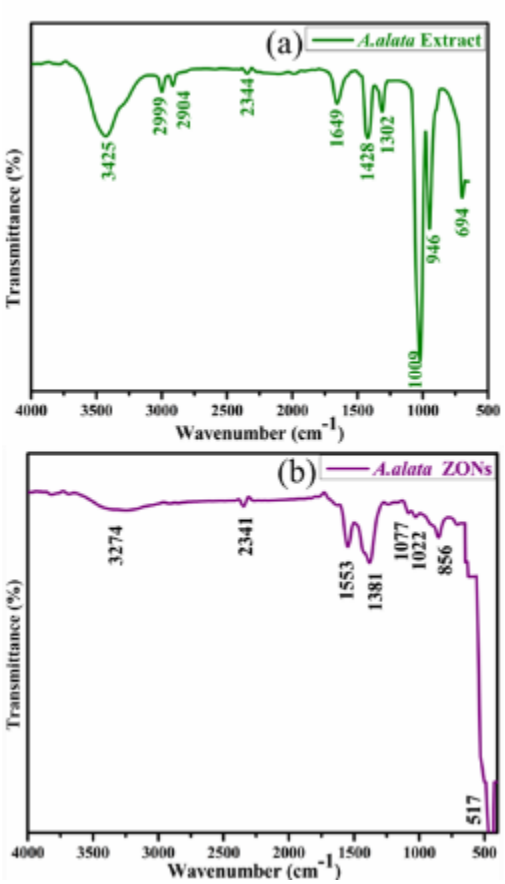


Figure 7 Fourier Transform Infrared (FTIR) spectra of *Andrographis alata* (a) ethanol extract and (b) synthesized ZONs.

3.9. Dynamic light scattering (DLS)

The Brownian movement of the nanoparticles in the colloidal suspension based on the hydrodynamic diameter of synthesized particles was determined by DLS. The average size of the *Andrographis alata* NPs in the aqueous medium was 22nm shown in the Figure 8, this is relatively larger than the theoretical size of the NPs calculated using XRD [10], because of the hydrodynamic shell. Particle shape, structure and roughness are the responsible for the size of the hydrodynamic shell [32]. The agglomeration of nanoparticles related to polydispersity index (PDI) which is associated with the nanoparticle size variation[33]. PDI measures were found to be 1.000 indicates the monodispersity in the medium.

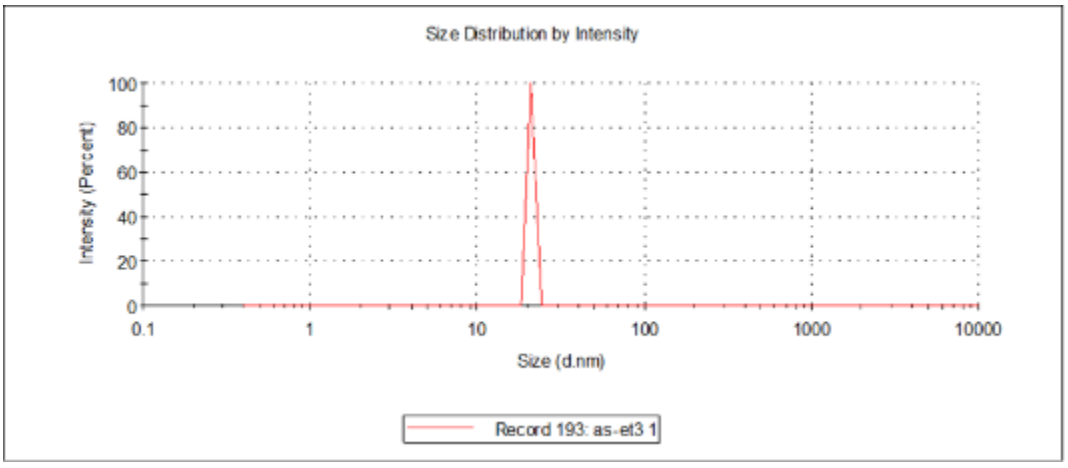


Figure 8 Dynamic Light Scattering (DLS) analysis of synthesized ZnO NPs of *Andrographis alata* ethanol extract

3.10. Antimicrobial activity

The *In vitro* biological application of phyto-fabricated ZnO NPs was investigated against Gram (+) *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis* and *Corynebacterium diphtheriea*, Gram (-) pathogens like *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhii* i.e. four Gram (+) and four Gram (—) microorganisms. It is observed that, the antibacterial activity of pure plant extract was very trace and the synthesized ZONs at 100µL shown significant against Gram(+) *Corynebacterium* and *Bacillus* was found to be 17mm and 13mm and Gram(-) *K.pneumonia* and *P.aeruginosa* shows 9mm and 16mm but very least in *Staphylococcus* and in Gram(—) *E.coli* and *Salmonella typhii*. The inhibition zones of plant extract and synthesized nanoparticles 50µL and 100µL of eight bacteria are shown in the Figure 9.

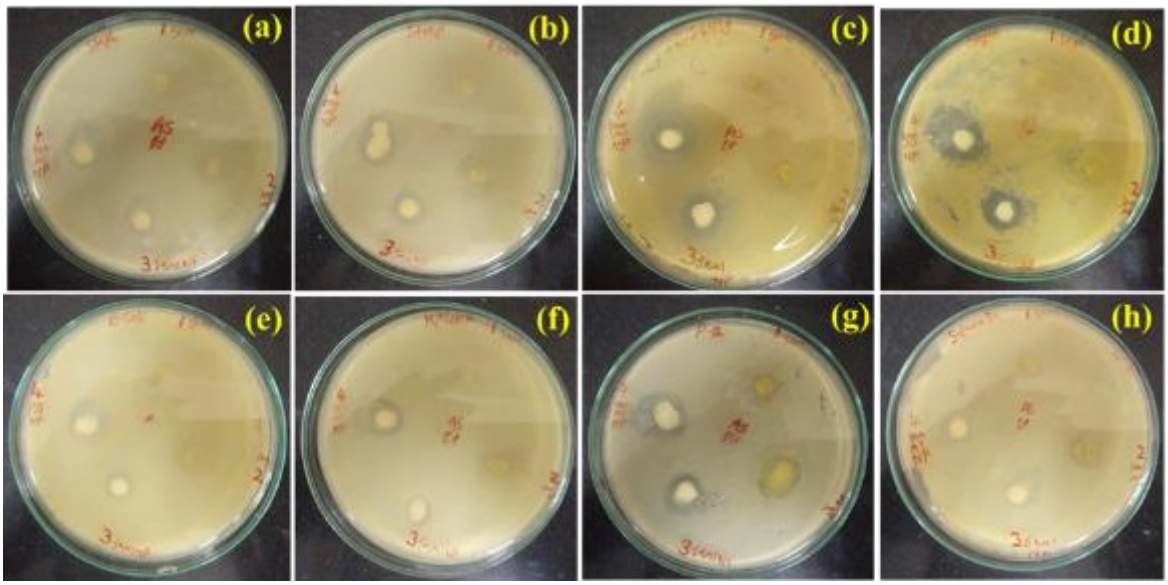


Figure 9 Kirby-Bauer disc diffusion method for analysis of the antibacterial activity of *Andrographis alata* whole plant ethanol extract and synthesized ZONs to Gram (+) bacteria: (a) *Staphylococcus aureus*,(b) *Streptococcus pyogenes* (c) *Bacillus subtilis* (d) *Corynebacterium diphtheriea* and Gram(—) bacterium (e) *Escherichia coli*,(f) *Klebsiella pneumonia*,(g) *Pseudomonas aeruginosa* and (h) *Salmonella typhii*.(1. 50µL extract; 2. 100µL extract; 3. 50µL ZONs; 4. 100µL ZONs).

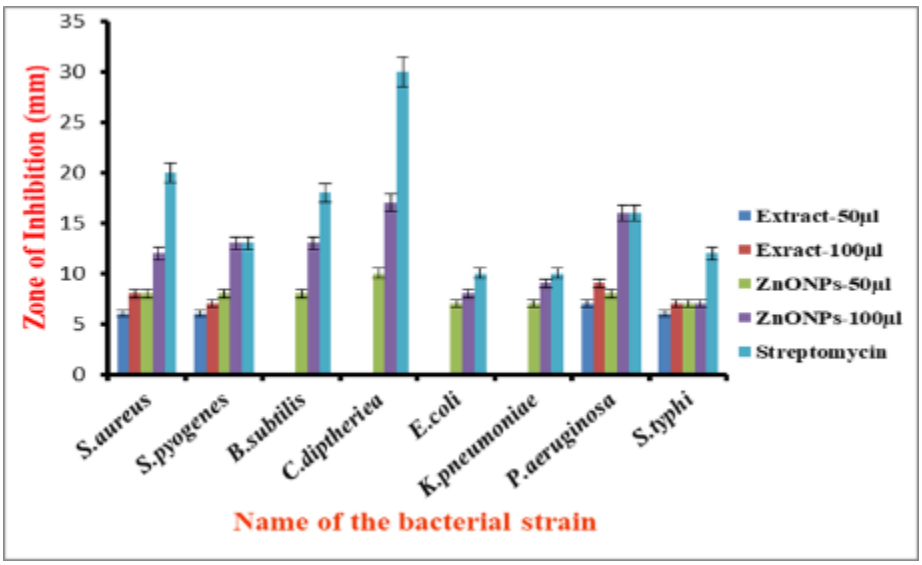


Figure 10 Antibacterial activity of whole plant extract and synthesized ZONs of *Andrographis alata*

Table 2 Inhibition zones of *Andrographis alata* ethanol extract and synthesized ZONs against eight bacterial strains

Samples	Concentrations (µg/mL-1)	Zone of Inhibition (mm)							
		Gram (+)				Gram (–)			
		S.a	***S.p***	***B.s***	***C.d***	***E.c***	***K.p***	***P.a***	***S.t***
Andrographis alata	50	06	06	00	00	00	00	07	06
ethanol extract	100	08	07	00	00	00	00	09	07
Andrographis alata	50	08	08	08	10	07	07	08	07
ZONs	100	12	13	13	17	08	09	16	07
Streptomycin	100	20	13	18	30	10	10	16	12

S.a- Staphylococcus aureus, S.p- Streptococcus pyogenes, B.s- Bacillus subtilis, C.d- Corynebacterium diphtheria, E.c- Escherichia coli, K.p- Klebsiella pneumonia, P.a- Pseudomonas aeruginosa, S.a- Salmonella typhii and streptomycin is used as a positive control

The bacterial activity was allied with the structure of bacterial cellwall, mesosome and ribosomal subunit. Noteworthy variations might be taken place in the shape of the bacterial cell due to cell surface packed by the ZONs. By the adsorption, penetration and release of Zn+2 ions in to the cytoplasm, binds to sulphur containing aminoacids and intrusive the bio-signals which affects electron transport chain inactivation, inhibits energy metabolism, reduce ATP synthesis and disrupt DNA/damage that leads to mitochondrial oxidative stress and mesosomal oxidative stress. Wholly, these results in biological electrolites exuded out and cause cell death [29,34]. In addition to these, hydroxyl groups in polyphenols trigger hydroxylation of cellwall that turns into lethal to pathogenic cell [35].

3.11. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of phyto-synthesized ZONs for *S.aureus, S.pyogenes, B.subtili, E.coli* and *K.pneumonia* were shown at 62.5µg/mL and for C.diphtheriea and P.aeruginosa were at 31.25µg/mL and Gram negative organism *S.typhii* shown at 250µg/mL. This is because of the resistance developed by the microorganisms. Previous paper with antibacterial activity of green synthesized ZONs also reported that organisms shown little bit high concentrations were need for inhibition i.e about 3000µg/mL [36].

3.12. Minimum bactericidal concentration (MBC)

MBC is observed for *S.aureus, S.pyogenes, B.subtilis, E.coli* and *K.pneumonia* were at 250µg/mL, and for *C.diphtheriea* and *P.aeruginosa* at 62.5µg/mL and for Gram negative bacterium *S.typhii* shown at 500µg/mL of phyto-synthesized ZONs.

3.13. *In vitro* antioxidant activity

Free radicals are the resultants of oxidative stress that owing to utmost oxidative diseases [37]. Free radicals like hydroxyl, superoxide and non-radical species like singlet oxygen and hydrogen peroxide are the diverse variability's of activated oxygen constituting ROS (reactive oxygen species) [38]. For the balance of these generated free radicals, an active anti-oxidative defence system is required. Curing of these diseases by antioxidant therapy is a highly important. Redox potential of phytochemicals in plant extract shows the antioxidant activity, that perform a key role in quenching of singlet/triplet oxygen, decompose peroxides and neutralize free radicals [39].

3.14. DPPH (1,1-diphenyl2-picrylhydrazil) assay

Andrographis alata ethanol extract displays DPPH radical scavenging activity, however, synthesized ZONs showed very good activity. At 20µg/mL concentration plant extract shows 30%, ZONs shows 37% and ascorbic acid shows 38%. When the concentration increases scavenging activity also increases. At 100µg/mL concentration scavenging percent was 52%, 67% and 79% for extract, synthesized nanoparticles and ascorbic acid respectively was shown in Figure 11. As the concentrations of plant extract and ZONs raises, antioxidant activity also rises was also recorded previously [40].

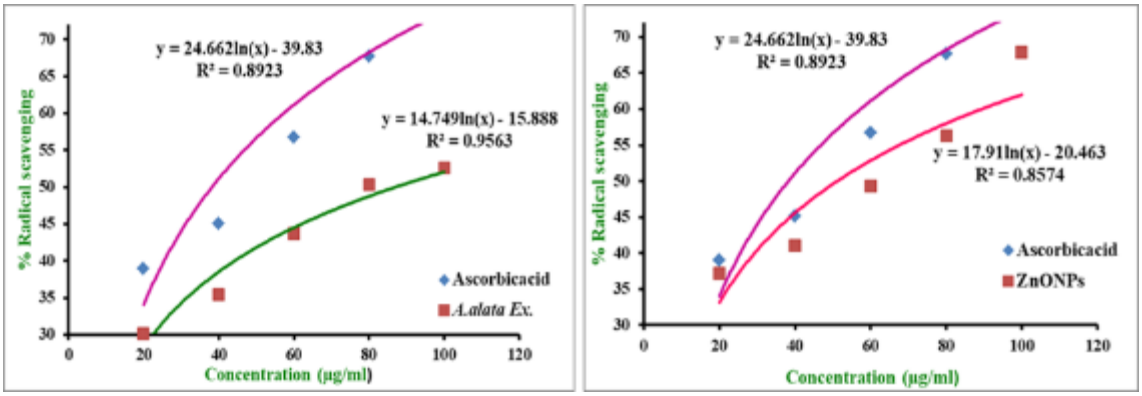


Figure 11 DPPH Radical scavenging percent of *Andrographis alata* ethanol extract and synthesized ZONs

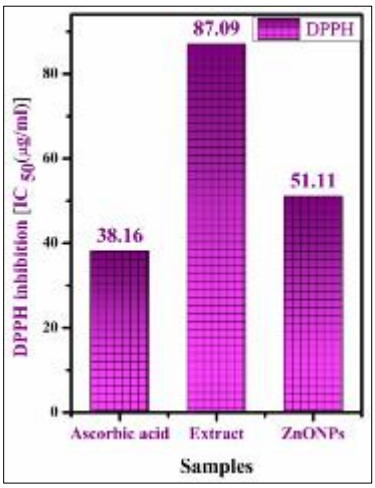


Figure 12 IC_{50} values of *Andrographis alata* DPPH

4. Conclusion

This work displays the green synthesis of *Andrographis alata* ZONs whole plant ethanol extract. The phyto-synthesis provides an eco-friendly, one-step and competent approach for fabricating NPs. In XRD analysis, by using Debey-scherrer's formula, the particle size was 7.85nm, EDAX shows the percentage of Zinc as 70.54% and Oxygen as 20.18% which shows the presence of Zinc and Oxygen in the synthesized *Andrographis alata* ZONs. The ZONs are naturally stabilized by their biological secondary metabolites, which exhibits good antioxidant and antibacterial potential.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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