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Synthesis of zinc oxide nanoparticles using leaf extract of *Ajuga parviflora* Benth. In Wall and their characterization

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Abstract
Ajuga parviflora is one of the potent medicinal herb of Himalaya belongs to Lamiaceae family used in number of different folk medicine time immemorial. In this work, we design to synthesize zinc oxide nanoparticles from zinc nitrate via green route using the leaf extracts of *Ajuga parviflora*. Zinc oxide nanoparticles were characterized by XRD, EDX, TEM and FTIR analysis. Zinc oxide nanoparticles showed UV-visible spectral bands between 310-330 nm. EDX analysis of nanoparticles confirms the presence of zinc and oxygen. TEM analysis revealed that nanoparticles are spherical in shape with the average particle size less than 20 nm. X-Ray Diffraction (XRD) structural analysis affirms the synthesis of crystalline zinc oxide nanoparticles. FTIR spectra confirms the presence of phytochemicals of plant extract which played a key role in the synthesis of zinc oxide nanoparticles.

Keywords: Zinc oxide nanoparticles, *Ajuga parviflora*, TEM, XRD, crystalline

Introduction
Ajuga parviflora is an important medicinal herb of Himalaya belongs to Lamiaceae family commonly used to control hypertension, hepatitis and diabetes [1, 2]. It exhibited a variety of biological activities, such as antibacterial, antifungal, antioxidant, insecticidal [3-5]. *Ajuga parviflora* is a short-lived perennial herb with its stem branched from base, pink purple to blue, short flowers and leaves, sessile, hairy, ovate and tinged with purple on lower surface [6, 7].
Nano science is a new and advanced inter-disciplinary field under discussion which is based on the elementary properties of Nano-size materials [8, 9]. Metallic nanoparticles can be synthesized by various methods including physical, chemical and biological. However, green synthesis of metallic nanoparticles is more stable, non-hazardous, ecofriendly, efficient and cost effective than the long established chemical and physical methods [10, 11]. Therefore, researchers are largely focusing on the green routes of synthesis of metallic nanoparticles. Zinc oxide nanoparticles have drawn the attention of many researchers owing to their several applications to the electronics, communications, UV-protection cosmetics, sensors, environmental protection, biological and medicinal industries [12, 13]. Zinc oxide nanoparticles can absorb sulphur and arsenic from water therefore also used as a water purifier [14]. In the past few years, metallic nanoparticles have significantly drawn interest of researchers due to their diverse applications ranging from material science to biological science. Phytochemicals present in the extracts of various parts plants (roots, leaf, fruits, seeds and bark) acts as capping and stabilizing agent of zinc oxide nanoparticles [15-17].
Various researchers have reported synthesis of zinc oxide nanoparticles using different plant materials [18-22]. Due to large surface area to volume ratio and high catalytic activity of zinc oxide nanoparticles, they are used in several catalytic processes. Moreover, antimicrobial activities of zinc oxide nanoparticles have also been reported [23-25]. After reviewing data, it has been found that no report has been texted regarding the green synthesis of ZnO nanoparticles by using aqueous leaf extract of *Ajuga parviflora*. The objective of present study is to synthesize zinc oxide nanoparticles through the leaf extracts of *Ajuga parviflora* and its characterization by FTIR, XRD, EDX and TEM analysis.

Material and Methods
Collection of plant materials
Fresh leaves of *Ajuga parviflora* were collected from Kandolia, Pauri Garhwal, Uttrakhand and authenticated from Herbarium Jammu University and its accession no. HBJU 16003 was collected.

Plant Extract preparation

Fresh leaves of *Ajuga parviflora* were thoroughly washed under tap water for 15 min followed by washing with double distilled autoclaved water. Then, only healthy leaves were shade dried until the constant weight of leaves were achieved. Then, these dry leaves were grinded into fine powder using mortar and pestle and 5 g of finely powdered *Ajuga parviflora* was taken with 100 ml double distilled water and heated at 65 °C for 25 minutes. Then, the extract was allowed to cool at room temperature and filtered using Whatman Fitter paper No. 1.

Green synthesis of Zinc oxide Nano particles

50 ml of *Ajuga parviflora* leaf extract was taken in a 250 ml of Erlenmeyer conical flask. It was heated on magnetic stirrer at 60 °C for 15 minutes. Then, 50 ml of 100 mM zinc nitrate hexa hydrate solution was poured drop by drop to it with continuing stirring. Few drops of 1 M NaOH solution was also added to it in order to maintain pH ranging 9-12. The change in colour of solution from brown to yellow was considered as a visual marker for the synthesis of nano particles. Then, the solution was centrifuged for 10 minutes at 7500 rpm and washed with distilled water followed by acetone to remove the impurities. The obtained material was dried at 40 °C for 24 hours in oven followed by mashing in mortar-pestle to get fine powdered nanoparticles and stored in air tight bottles for further characterization and anti-microbial activities.

Characterization of green ZnO nanoparticles

Formation of zinc oxide nanoparticles was preliminary examined by using Elite-double beam –UV-visible spectrophotometer. Then, zinc oxide nanoparticles was subjected to X-ray diffraction (XRD) analysis (CuK_α radiation, λ_{max} = 1.54 Å) and its spectra was reported in the range of 2θ from 0° to 75°. FTIR analysis ranging from 4000-500 cm^{-1} was to recognize the phytochemicals present in plant extract responsible for capping and stabilizing nanoparticles. The average size of ZnO nanoparticles was calculated by using Debye Scherrer's equation:

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

Where D is average crystallite size, K is the Scherrer's constant,
λ is the X-ray wavelength, β is the full width at half maxima (FWHM) and θ is the Bragg's diffraction angle.
EDX was performed for determining the composition of synthesized nanoparticles. TEM analysis was used to determine the surface morphology zinc oxide nanoparticles.

Result And Discussion

In the present work, aqueous leaf extract of *Ajuga parviflora* was used to synthesize zinc oxide nanoparticles. Bio-active components present in the leaf extract performed a key role in the synthesis of ZnO nanoparticles.

Change in the colour of solution from brown to yellowish may be due to the formation of zinc oxide nanoparticles. Fig. 1 shows the UV-visible spectrum of the solution having a broad absorption peak at λ_{max} = 310-330 nm which confirmed the presence of ZnO nanoparticles which may be attributed to the ZnO nanoparticles and its calculated energy band gap is between 3.77 -4.01 eV.

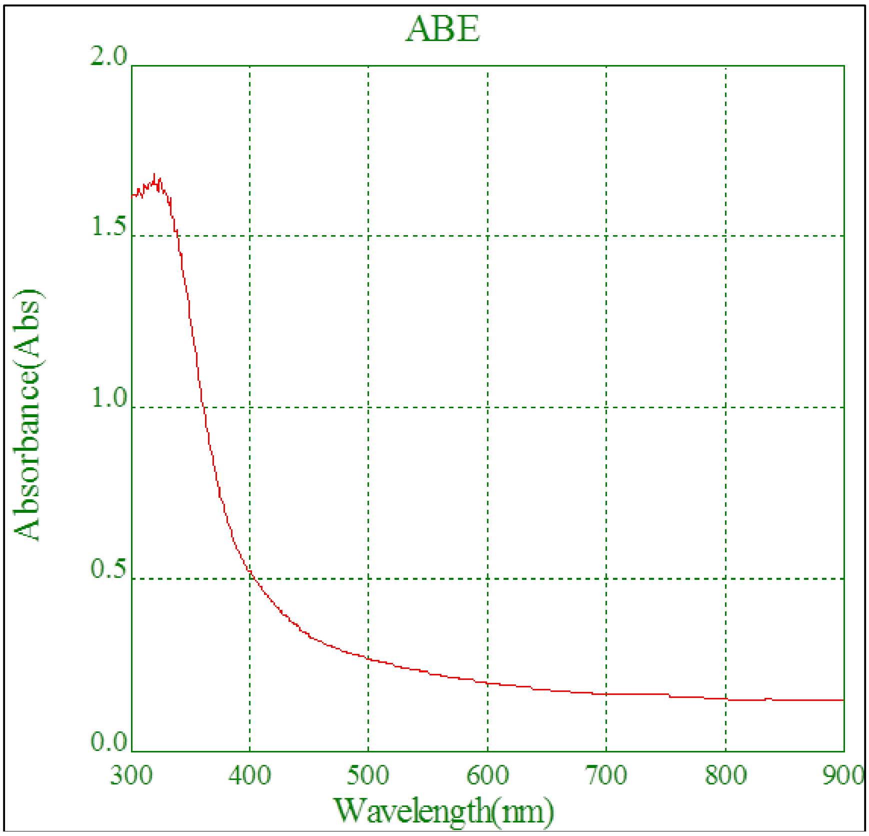

Fig. 1: UV-visible spectrum of ZnO nanoparticles

EDX analysis

EDX was carried out for the composition of nanoparticles. Fig. 2 shows the presence of ZnO with sharp signals of Zn and O at around 1 keV and 0.5 keV respectively. Peak of C may be attributed due to the bioactive components present in plant extract.

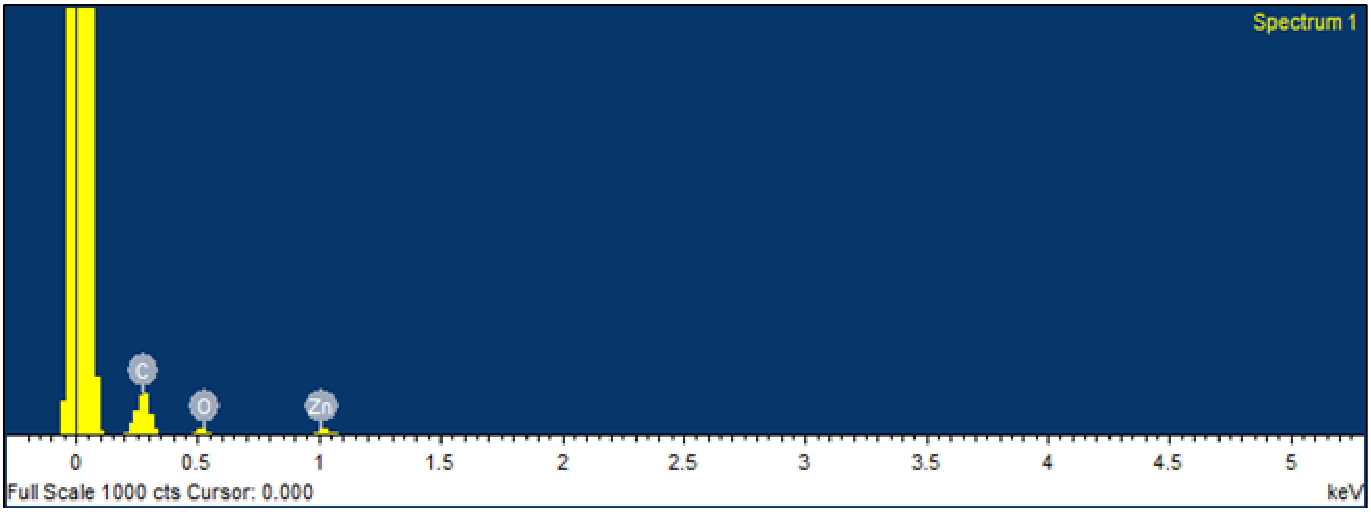

Fig. 2: EDX pattern of ZnO nanoparticles

XRD analysis

XRD spectra showed the crystalline nature of ZnO nanoparticles and the average crystallite size was calculated using Debye-Scherrer's equation [26-28]. The average calculated size of ZnO nanoparticles is less than 13 nm. XRD spectra of synthesized nanoparticles showed sharp peaks at 2θ = 32.00°, 34.62°, 36.47°, 47.72°, 56.79°, 63.01° and 68.25° which are indexed to (100), (002), (101), (102), (110), (103) and (112) respectively (Table 1) of hexagonal closed packed nano crystals (Fig. 3).

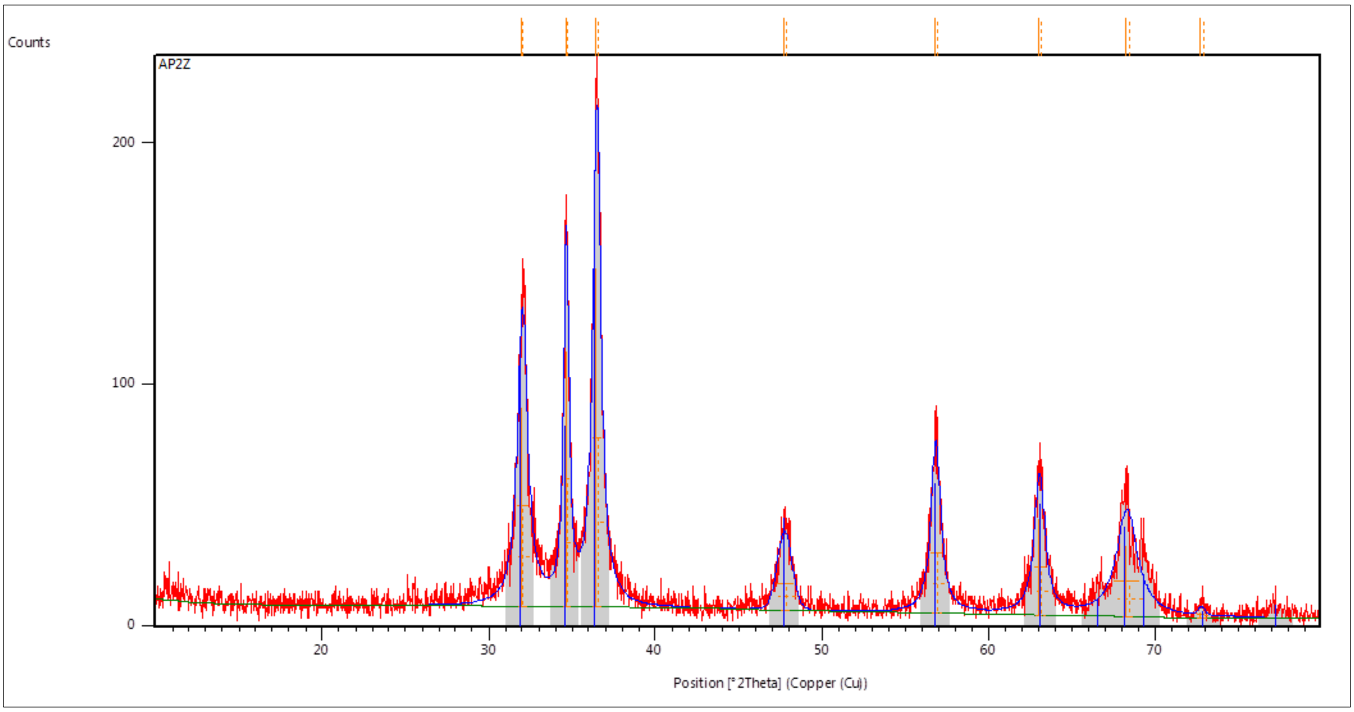

Fig. 3: XRD spectra of ZnO nanoparticles

Table 1: Peak List for Average Size Calculation of Nanoparticles

2θ (°)	hkl	FWHM left	*d*-spacing (Å)	Rel. int. (%)
32.00	100	0.70	2.79	58.91
34.62	001	0.35	2.58	75.54
36.47	101	0.56	2.46	100
47.72	102	1.04	1.90	15.60
56.79	110	0.65	1.62	35.42
63.01	103	0.71	1.47	28.76
68.25	112	1.47	1.37	21.33

TEM analysis

TEM analysis was used to study the surface morphology ZnO nano structures (Fig. 4). These results confirmed that ZnO nanoparticles were spherical in shape with their average size less than 20 nm.

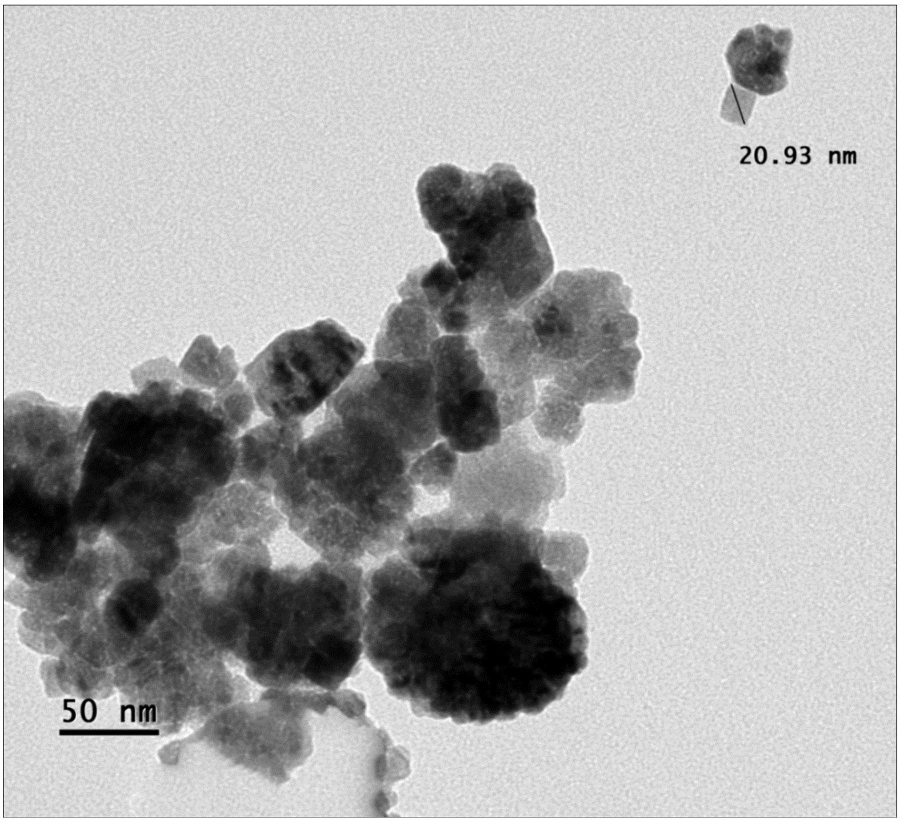

Fig. 4: Transmission electron micro-image of ZnO nanoparticles

FTIR analysis

FTIR peak in Fig. 5 at 3411.7 cm^{-1} corresponds to stretching vibrations of hydroxyl group. Further, peaks at 1571.6 cm^{-1}, 1492.8 cm^{-1} and 1412.9 cm^{-1} may be attributed to C=O frequency of extensively conjugated systems, C=C aromatic stretching and C-O stretching of ArOH respectively. Further, peak at 462.7 cm^{-1} shows the presence of ZnO crystals.

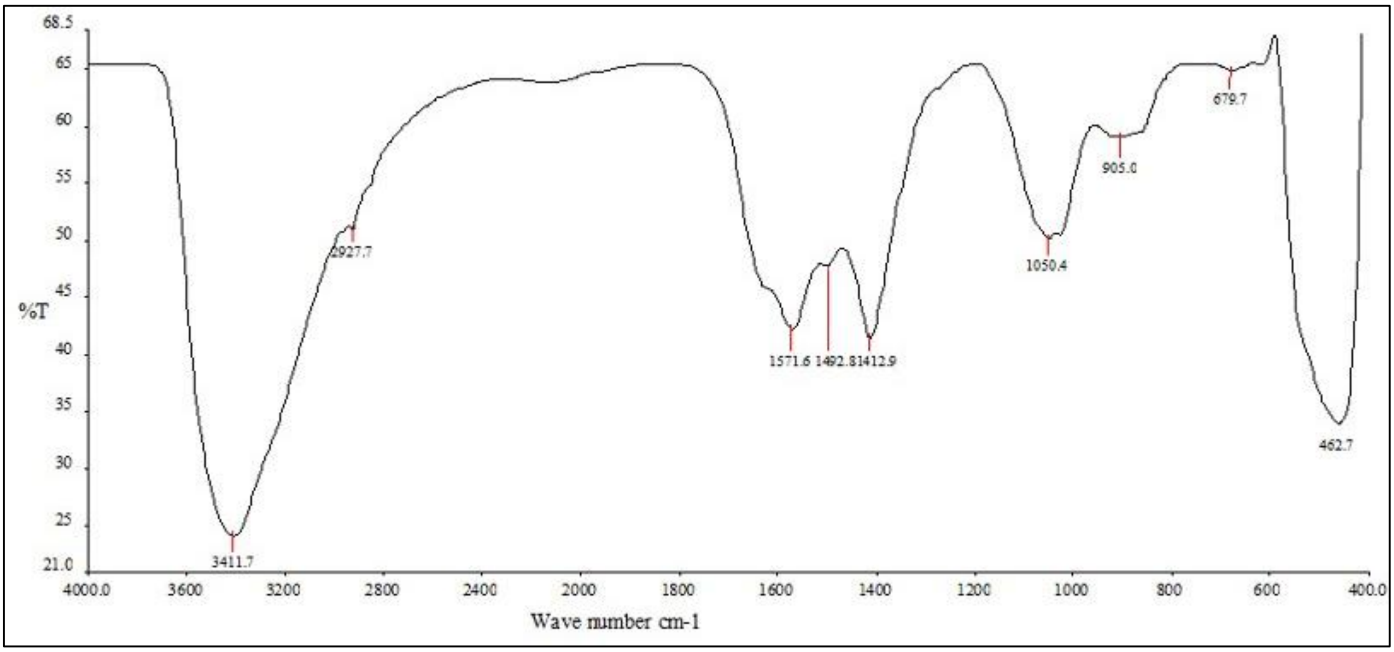

Fig 5: FTIR spectrum of ZnO nanoparticles

Conclusion

It can be concluded that *Ajuga parviflora* leaf extract mediated synthesis of zinc oxide nanoparticles is non-toxic, rapid, non-hazardous and cost effective. Spherically shaped, crystalline zinc oxide nanoparticles with their average size less than 20 nm are synthesized. Phytochemicals presents in leaf extract effectively acts as capping and stabilizing agents of zinc oxide nanoparticles. Thus, it is an alternative, promising and better route for the synthesis of zinc oxide nanoparticles than the conventional physical and chemical methods.

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Conflict of Interest
The authors declare that there is no conflict of interests regarding the publication of this article.

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