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Arun Kumar Khajuria
Department of Botany, H.N.B. Garhwal University, Pauri Campus, PAURI (Garhwal), Uttarakhand, India

NS Bisht
Department of Botany, H.N.B. Garhwal University, Pauri Campus, PAURI (Garhwal), Uttarakhand, India

Goutam Kumar
Department of Chemistry, H.N.B. Garhwal University, Pauri Campus, PAURI (Garhwal), Uttarakhand, India

Correspondence
Arun Kumar Khajuria
Department of Botany, H.N.B Garhwal University, Pauri Campus, PAURI (Garhwal), Uttarakhand, India

Synthesis of Zinc oxide nanoparticles using leaf extract of *Viola canescens* Wall. ex, Roxb. and their antimicrobial activity

Arun Kumar Khajuria, NS Bisht and Goutam Kumar

Abstract
In the present study, Zinc oxide nanoparticles have been synthesized using leaf extract of *Viola canescens*. Synthesized nanoparticles were subjected to UV- Vis, XRD, FTIR and SEM analysis for further characterization. The average calculated size of ZnO nanoparticles was <26 nm and found to be hexagonal in structure recorded from XRD analysis. FTIR spectra showed the presence of -OH, C=C, C=O, and other groups which may involved in reduction and capping of synthesized nanoparticles. Potential of ZnO nanoparticles as an anti-bacterial agent has been studied against two clinical isolates *Staphylococcus aureus* and *E. coli* and the zone of inhibition observed for both isolates clearly indicated their potential as an anti-microbial agent.

Keywords: *Viola canescens*, antimicrobial activity, *Staphylococcus aureus*

Introduction
Nanotechnology one of the modern techniques of material science based on the manipulation of individual atom or molecule to produce new material at nanoscale level (1-100 nm) for function well below sub microscopic level [1-3]. Nanoparticles with a large surface area relative to their volume can show atom like behavior which results from higher surface energy and wider band gap between valence and conduction band when they are divided to near atomic size. Green synthesis of nano particles has gained great importance over physical and chemical methods due to many advantages. In recent years, use of non-toxic plant material for the synthesis of ZnO nanoparticles has been gained importance due to their simplicity, non-toxic, non-hazardous, biodegradable and eco-friendly in nature. Further, ZnO nanoparticles have unique properties and potential application in optics, electronic, biomedical, agriculture areas, inexpensive, gene delivery, drug delivery and nano-medicine [4-6]. Besides, US FDA, considered and enlisted ZnO nanoparticles as “Generally recognized as safe” (GRAS) metal oxide. ZnO is II-IV binary compound, semiconductor crystallize in either hexagonal wurtzite or cubic Zinc blende structure [7-8].
Plant are integral part of human life and used to treat number different ailament [9-11]. *Viola canescens* member of Violaceae is an important medicinal herb with a wide range of its use in curing different ailament from the common cold to lethal diseases such as cancer. Besides, the plant is also known to have carminative, antipyretic, purgative, anticancerous properties moreover also used to treat the nervous and respiratory disorders [12-14]. Thus in present paper attempt have been made to synthesized Zinc based nano particle using the leaf of *Viola canescens* as plant source.

Material and Methods
Plant Extract preparation
Plants collected from wild were washed under tap water for 10 min to remove all adhering soil followed by washing with double distilled autoclaved water. Any infected leaf found was discarded and only healthy shade dry for 15 days leaves were used for extract preparation. Dry leaves used for the preparation of extract homogenized using mortar and pestle. 5 gm of dry powder of leaves were used for preparing the extract. These were soaked in 100 ml double distilled autoclaved water in Erlenmeyer flask and boiled at 60^0C for 15 min. Extracts were allowed to cool and then filtered using Whatman filter paper

Green synthesis of Zinc oxide nano particles
Green synthesis of ZnO nanoparticles was carried by mixing 100 ml of the plant extract with 100 ml of zinc nitrate hexahydarate extract in 500 ml flask.

Prepared leaf extract was heated at 60 ^{0}C with constant stirring using magnetic stirrer for 10 min, before adding ZnO extract in the flask slowly. After complete pouring of the ZnO extract in the flask having plant extract, the mixture of both solutions was kept for vigorous stirring at 60 ^{0}C for 4 hrs. The change in colour of the solution was considered as a visual marker for the synthesis of nano particles. Further, the precipitate starts appearing after 3 hrs of vigorous stirring at 60 ^{0}C. Precipitate formed in the reaction was allowed to settle at room temperature for 24 hrs. Supernatant was discarded and crude was centrifuged for 20 min at 5000 rpm. Finally, pellets were oven dried at 60 ^{0}C for 10 hrs followed by mashing in mortar and pestle to get a fine powder and stored in air tight bottles for further characterization and anti-microbial activities.

Characterization of green ZnO nanoparticles

Synthesized nano particles were subjected to UV-Vis absorption spectra to study its optical properties. XRD (X-Ray Diffraction) analysis of nano particle was recorded in the range of 2θ from 0^0 to 65^0 using powder diffractometer. FTIR (Fourier Transform Infrared Spectroscopy) in the range of 4000-500 cm^{-1} was used to identify various phytochemicals present in the reduction and capping of ZnO nanoparticles. Surface morphology of ZnO nanoparticles was analyzed with SEM (Scanning Electron Microscopy).

Anti-bacterial assay

In ordered to study the effect of synthesized nano particles of *Viola canescens* against clinically isolated *Staphylococcus aureus* and *E. coli* bacterial strains were assayed by Agar well diffusion method. Muller Hinton Agar Medium (HI-Media) was used in bacterial assay and prepared by dissolving 33.9 gm into 1000 ml of distilled water, this dissolved medium then subjected to the autoclave at 121 ^{0}C temperature and 15 Pascal pressure for 15 minutes. The autoclaved media then poured into 20×90 mm Petri plate (Borosil) under the laminar air flow chamber and were subjected to incubation for 24 hrs at 30 ^{0}C in a bacteriological incubator to observe any contamination if present. Sterilized plates were then subjected to inoculation of bacterial strains. Suspensions of bacterial strains were prepared by using sodium chloride (HI-Media) dissolved with the required amount of distilled water by dissolving a loop of bacteria from prepared slant. Suspension of the bacteria was then loaded on MHA plates, which further swapped gently over the surface of the plate with the help of the slider. Finally, wells were made by using sterilized cork borer, in which prepared samples solution (50µl-100 µl) were poured with the help of micropipette. Each plate then incubated at 37 ^{0}C for 24 hrs and the diameter of the zone of complete inhibition was measured with the help of scale.

Results

(i) Visual observation UV- visible analysis

Change in colour is the preliminary test for nanoparticles synthesis, colour change from brown to yellow evident the formation of ZnO nanoparticles using leaf extract of *Viola canescens*. Further, confirmation for synthesis was carried out by using UV-vis analysis, which showed characteristic peak at 340 nm, which could be attributed to the ZnO nanoparticles.

(ii) XRD (X-ray diffraction)

XRD spectra gave the insight of the synthesized nanoparticles, which includes crystallinity, structure and size of the nanoparticles. The prominent peaks at 31.879, 34.499, 36.350, 39.120, 47.610, 56.650 and 62.890 (Fig 1) which corresponded to lattice planes of 100, 002, 101, 102, 112, 300 and 103 respectively (Table 1). Confirming the hexagonal size of ZnO nanoparticles, the average nanoparticles size was calculated by using Scherrer's equation. The recorded average size of synthesized nanoparticles was less than 26 nm [15-16].

$$D = \frac{0.9\lambda}{\beta \ cos\theta}$$

Where D is crystallite size, $\boldsymbol{\lambda}$= is wavelength, θ= is Bragg angle and $\boldsymbol{\beta}$= is Full width at half maxima.

(iii) FTIR analysis

FTIR spectra showed the presence of characteristic bands for several functional groups which may involved in the synthesis and capping of nano particles. Broad band at 3476.41cm^{-1} corresponds to the stretching vibrations of -OH group. The peak at 2432.03 cm^{-1} is attributed to the phosphin (P-H). Further, peaks at 2102.01, 1634.22, 1548.07, 1384.65 and 871.06 cm^{-1} are due to C≡C, C=C, C=O, C-O and CH_2 groups stretching vibrations respectively. But the band at 691.05 cm^{-1} is due to C-H bending vibrations. Besides, band at 513.87 cm^{-1} corresponds to ZnO hexagonal structure [17-18] (Fig 2).

(iv) SEM analysis

In order to visualize the surface morphology of ZnO nanoparticles, SEM analysis was carried out and obtained monograph clearly demonstrate the presence of agglomerations of nanoparticles with spherical morphology (Fig 3).

(v) Antimicrobial assay

Agar well diffusion method was used to perform the assay against two bacterial strain i.e, *Staphylococcus aureus* and *E. coli* while sodium chloride solution was used as control and Amoxicillin was used as the drug for the comparative response. Results clearly indicate that nano particles have anti-microbial activity (Table 2). Maximum zone of inhibition was observed against gram –ve bacteria *E.coli* (10.33±0.58 mm), the minimum zone of inhibition was observed against gram +ve bacterial *Staphylococcus aureus* (7.33±1.53 mm). Zone of inhibition was somewhat correlation with zone of inhibition in case of drug used (Fig 4). Thus ZnO nanoparticles showed their potential for using as anti-microbial agent.

Conclusion

Plant mediated synthesis of ZnO nanoparticles is an alternative and beneficial technique, which proved better over conventional physical and chemical methods and have many advantages over the later. Plant mediated synthesis is both cost effective and safe (biodegradable, non-toxic, non-hazardous etc) practice. Functional groups (hydroxyl, phosphin, and carbonyls) present in leaf extract effectively acted as reducing and capping agent which lead to the synthesis of ZnO nanoparticles. XRD pattern suggested hexagonal structure with <26 nm average size of nanoparticles. Besides, spherical morphology was observed in SEM analysis, moreover, nanoparticles showed anti-microbial activity.

Table 1: Peak list for average size calculation of nanoparticles

Pos. [°2Th.]	hkl	FWHM Left [°2Th.]	d-spacing [Å]
31.899	100	0.31	2.80324
34.519	002	0.41	2.59622
36.369	101	0.36	2.46827
39.110	102	0.17	2.30116
47.640	112	0.55	1.90724
56.684	300	0.33	1.62259
62.921	103	0.52	1.47593

Table 2: Antimicrobial activity of synthesized ZnO nanoparticles

Sample name		Zone of inhibition (mm)			
	Amoxicillin	**Control**	**50µl**	**75µl**	**100µl**
E. Coli	11	0.00	8.33±0.58	9.67±1.53	10.33±0.58
Staphylococcus aureus	9	0.00	7.33±1.53	7.67±1.53	9.33±1.15

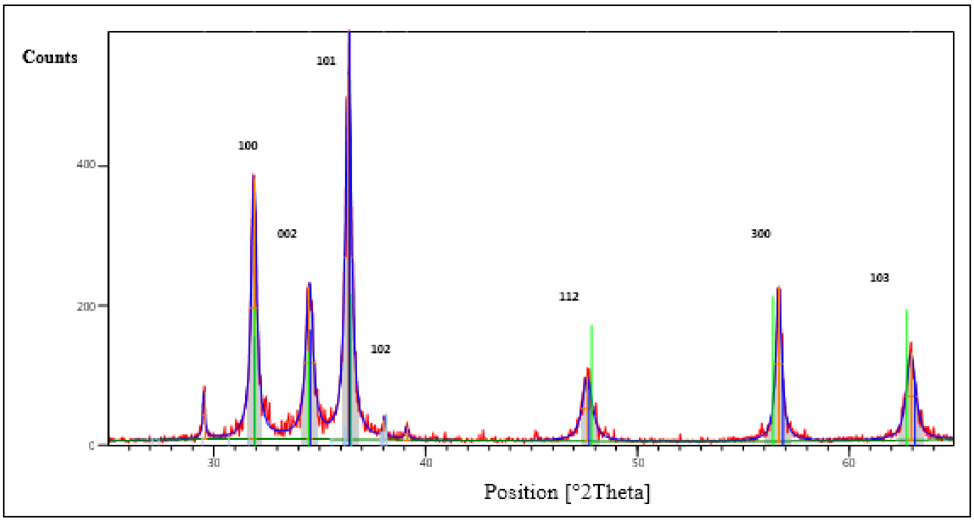

Fig 1: XRD spectra of synthesized ZnO nanoparticles

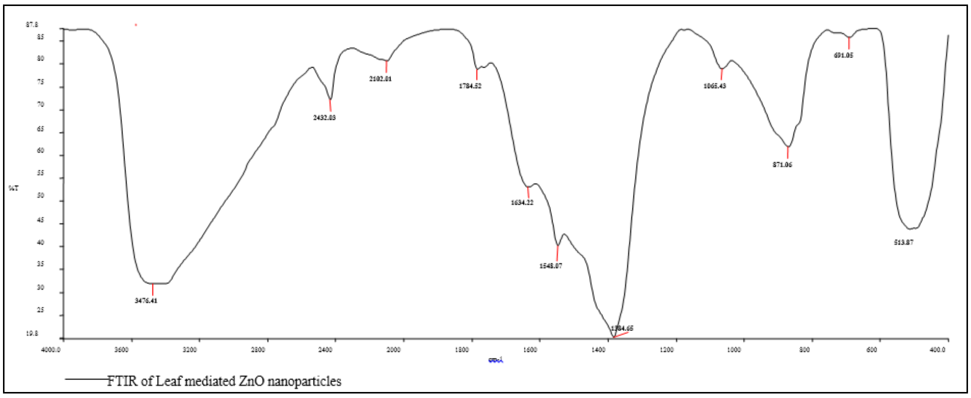

Fig 2: FTIR of leaf mediated ZnO nanoparticles

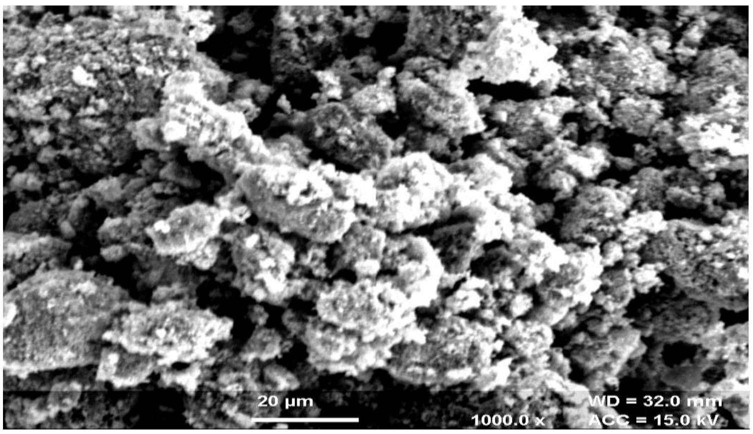

Fig 3: SEM image of ZnO nanoparticles

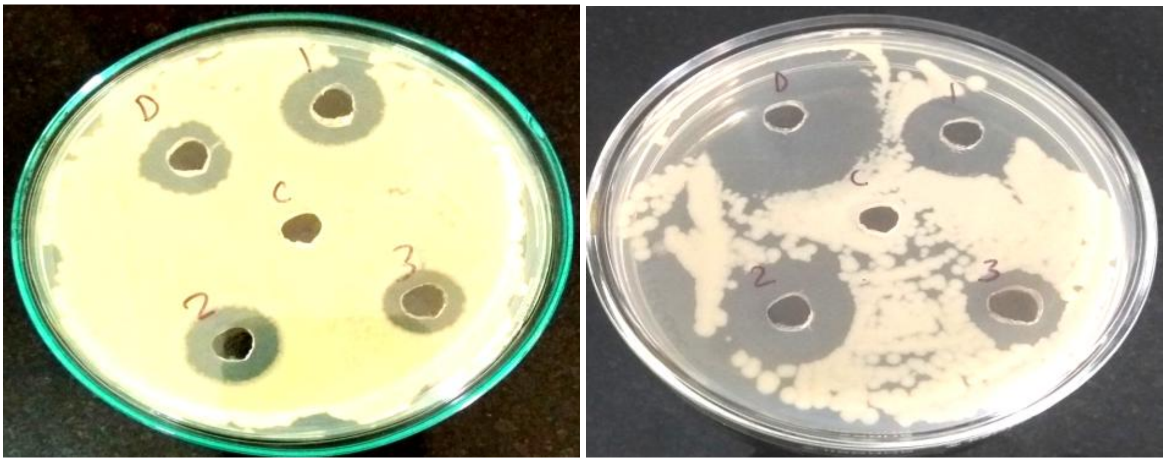

Fig 4: Anti-microbial effect of ZnO nanoparticles on *E. coli* and *S. aureus*

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