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ANTIMICROBIAL ACTIVITY OF *AJUGA PARVIFLORA* LEAF EXTRACT MEDIATED ZINC OXIDE NANOPARTICLES

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Abstract

Nanoscience and Nanotechnology has attracted a lot of attention because of its wide variety of application. Living beings are known to be susceptible to microbial attack and this necessitates the production of antimicrobial agents. That's why, in recent years, plant mediated synthesis of metallic nanoparticles is an interesting issue of the nanoscience and nanotechnology. In the present investigation, antimicrobial activity of ZnO nanoparticles synthesized from leaf extract of *Ajuga parviflora* were evaluated against the selected bacterial (*Streptococcus pneumonia, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa*) and fungal (*Aspergillus fumigatus* and *Trichoderma*) strains by using well diffusion method. The present study was intended to evaluate the antimicrobial activity of leaf extract mediated ZnO nanoparticles against different pathogenic bacteria and fungus. These results have indicated that these small sized nanoparticles showed good antibacterial and antifungal activity.

Keywords: *Ajuga parviflora* Zinc oxide nanoparticles, Antibacterial activity, Antifungal activity.

Introduction

Nanoscience and Nanotechnology is one of the very encouraging and emerging field of science and engineering with its marvelous applications in physics, chemistry, biology and material science (Iravani *et al.,* 2014; Pal *et al.,* 2011; Kalyani *et al*., 2019). Nanoparticles have been widely used in variety of potential applications in optical, electronics, photocatalytic activity, biomedical, food technology, cosmetics, sensors, energy and environmental & health science because of its enhanced properties like high surface area to volume ratio, high reactivity and tunable pore size (Gondwal and Joshi nee Pant, 2018; Fu and Fu, 2015; Anbuvannan *et al*., 2015). Nanoparticle can be synthesized by various methods including physical, biological and chemical. The chemical and physical methods have some serious disadvantages as these involve toxic chemicals and high radiations which are very harmful to the environment (Tran and Le, 2018; Khandel and Shahi, 2018). Biological methods using plant extracts are much superior to physical and chemical methods because these are clean, non-toxic, ecofriendly and cost effective (Kandwal *et al*., 2019a). Therefore, researchers have shifted their focus towards plant mediated synthesis of metallic nanoparticles (Heera and Shanmugam, 2015; Jana *et al.,* 2000). Phytochemicals like alkaloids, terpenoids, flavonoids, proteins, polysaccharides, polyphenolic acids etc., present in plant extracts play a key role not only in synthesis of metallic nanoparticles but also prevent their further agglomeration (Liu *et al.,* 2017; Ovais *et al.,* 2018).

Microbes are tiny living organisms which are the integral part of life having dual effect in nature i.e. some of them are beneficial to mankind and others are highly pathogenic. Disease caused by microbes like bacteria, viruses, fungi etc. are of great concern worldwide, which results in the millions of death per year due to diseases. The increased prevalence of microbes to synthetic drugs raised the broad spectra of "Untreatable diseases" of infectious agents, which therefore, demanded some alternative treatments worldwide. Now-a-days, environmentally benign methods for the synthesis of metallic nanoparticles (Ag, Au, ZnO, CuO etc.) using plant extract have been widely used for its antimicrobial activities (Brayner *et al.,* 2006; Pirtarighat *et al.,* 2019; Varhese *et al*., 2015). Metallic nanoparticles have comparatively larger surface area to volume ratio, thus enhances the reactivity of metallic nanoparticles as a result of this they can penetrate and readily interact with the biomolecules on the surface of cells and inside the cells (Elumalai and Velmurugan, 2015; Khajuria *et al*., 2019a). In recent times, plant mediated zinc oxide nanoparticles have drawn much interest of the researchers for effectively fighting against multi drug resistant microbes. Zinc oxide nanoparticles are of antibacterial and antifungal activities even at lower concentrations hence suitable for thin coating applications (Sharma *et al*., 2010; Azizi *et al*., 2016). The antifungal activity of ZnO nanoparticles does not affect soil fertility compared to the conventional antifungal agents. Based on literature, zinc oxide nanoparticles using various plants extract shows potential antimicrobial activities against various microbial strains viz. *Pseudomonas aeruginosa*, *Escherichia* coli, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans* (Badoni *et al.,* 2017; Prachi *et al.,* 2019; Ahmad *et al*., 2019).

After reviewing data, it has been found that no report has been texted regarding the antimicrobial activity of zinc oxide nanoparticles by using the aqueous leaf extract of *Ajuga parviflora*. *Ajuga parviflora* is one of the potent medicinal herb of Himalaya belongs to Lamiaceae family commonly used against wide spectra of diseases from common body pains to chronic diseases such as diabetes. Plant is also used to cure hepatitis and hypertension (Hamayun *et al.,* 2006; Ahmad *et al.,* 2018). It exhibited

several pharmacological activities, such as antibacterial, antifungal, insecticidal and antioxidant (Rahman *et al.,* 2013; Gulzar *et al.,* 2017; Kumar *et al.,* 2018). Several phytochemicals like alkaloids, essential oils and terpenoids were isolated from the *Ajuga parviflora* plant (Nawaz *et al.,* 2000; Singh *et al.,* 2015; Kumar *et al.,* 2018). *Ajuga parviflora* is a perennial pubescent herb, 10 to 30 cm in height, commonly found in grassy slopes of Himalaya, plant is distributed in entire Himalaya from Kashmir to Uttarakhand in India and mountains of Afghanistan, Nepal and Bhutan. Plant with its stem branched from the base, short flowers with corolla pink purple to blue and leaves, sessile, hairy, ovate and tinged with purple on lower surface (Collett, 1980; Gaur, 1999). In the previous work, we have successfully synthesized spherically shaped crystalline zinc oxide nanoparticles using leaf extract of *Ajuga parviflora* with their average particle size less than 20 nm (Kandwal *et al.,* 2019b). Thus in present work, attempt has been made to evaluate the antimicrobial potential of green synthesized ZnO nanoparticles using *Ajuga parviflora* leaf extract against five bacterial strain *Staphylococcus aureus*, *Streptococcus pneumoniae, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* and two fungal strains *Aspergillus fumigatus* and *Trichoderma Viride*.

Materials and Methods

Collection and authentication of plant materials:

Fresh and healthy leaves of *Ajuga parviflora* were collected from Nagdev forest range, Pauri, Uttarakhand and authenticated from Herbarium Jammu University and its accession number HBJU 16003 was collected.

Preparation of leaves extract and zinc oxide nanoparticles:

Fresh and healthy leaves of *Ajuga parviflora* were entirely washed under tap water for 10 min followed by washing with double distilled autoclaved water in order to remove adhesive dirt. Then, leaves were shade dried until the constant weight of leaves was achieved. Then, these dry leaves were grinded into fine powder using mortar and pestle and 6 g of finely powdered *Ajuga parviflora* was taken with 120 ml double distilled water and heated at 70 °C for 20 minutes. Then, the extract was allowed to cool at room temperature and filtered in a separate conical flask using Whatman filter paper No. 1.

50 ml of *Ajuga parviflora* leaf extract was taken in a 250 ml of Erlenmeyer conical flask and 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 8-12. 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 their anti-microbial activities (Khajuria *et al*., 2017).

Culture media used for the determination of antimicrobial activities of metallic nanoparticles:

Soyabean casein digest agar/broth and Potato dextrose agar/ broth of Hi Media Pvt. Bombay, India were used for antibacterial and antifungal test respectively.

Test organisms:

The pure culture of five bacterial strains viz. *Staphylococcus aureus* (MTCC-1144), *Streptococcus pneumonia* (MTCC-655), *Pseudomonas aeruginosa* (MTCC-2474), *Klebsiella pneumoniae* (MTCC-4040) and *Escherichia coli* (clinical obtained from V.C.S.G Base hospital Srinagar, Uttarakhand, India) and two fungal strains viz. *Aspergillus fumigatus and Trichoderma Viride* were used in present study.

Antibacterial & Antifungal Assay:

In order to study the antimicrobial activity of synthesized ZnO nano particles using *Ajuga parviflora* leaf extract against test organisms, the microbial 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 double distilled water and then autoclaved at 121^0 C temperature and 15 Pascal pressure for 15 minutes. Then the media was poured into petri plate under the laminar air flow chamber and then subjected to incubation for 24 hrs at 30^0 C. The bacterial strains were inoculated into sterilized plates. Finally, 9 mm diameter wells were prepared by using sterilized cork borer and filled with prepared sample solution, positive control and solvent blank with the help of micropipette. Sodium chloride was used as standard positive control. The plates were then incubated at 37^0C for 24 hrs for the determination of antibacterial activity. The diameter of zone of complete inhibition was measured with the help of scale for each of test organisms (Khajuria *et al.,* 2019b).

PDA (Potato dextrose agar) was used for the fungal cultures. The culture medium was prepared by dissolving 39 gm into 1000 ml of double distilled water and then autoclaved at 121^0 C temperature and 15 Pascal pressure for 15 minutes. Then the media was poured into petri plate under the laminar air flow chamber and then subjected to incubation for 24 hrs at 30^0 C. The bacterial strains were inoculated into sterilized plates. Finally, 9 mm diameter wells were prepared by using sterilized cork borer and filled with prepared sample solution, positive control and solvent blank with the help of micropipette. DMSO (Dimethyl sulfoxide) was used as standard positive control and flucanazole was used as antifungal agent. The plates were then incubated at 37^0C for 48 hrs for the determination of antibacterial activity. The diameter of zone of complete inhibition was measured with the help of scale for each of test organisms (Rajiv *et al*., 2013).

Results and Discussion

Transmission electron microscope (TEM) and X-ray diffraction (XRD) analysis showed that *Ajuga parviflora* mediated zinc oxide nanoparticles were spherical and crystalline in nature with their average size less than 20 nm, FTIR analysis shows that the peak at 462.7 cm^{-1} is due to the presence of ZnO crystals (Kandwal *et al*., 2019b).

Agar well method was used to perform the antibacterial and antifungal assay and the diameter of zone of inhibition (mm) is measured against *Staphylococcus aureus*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli*, *Aspergillus fumigatus and Trichoderma Viride* (Table 1 and Table 2). The maximum zone of inhibition was recorded for *Klebsiella pneumonia* and *Trichoderma Viride*, 2.4 cm and 1.06 cm respectively, while minimum zone of inhibition was reported against *Streptococcus pneumonia* (1.4 cm), *Escherichia coli* (1.4 cm) and *Aspergillus fumigatus* (0.9 cm). The present work demonstrates that ZnO nanoparticles have antimicrobial effects on the selected test organisms Shah *et al*., (2015) working on antimicrobial activity of ZnO nanoparticles using leaf extract of *Camellia sinesis* against 6 microbial strains reported greater zone of inhibition in *Pseudomonas aeruginosa* than *Staphylococcus aureus* and *Escherichia coli*, supporting the present study. Furthermore, Senthilkumar *et al.,* (2014), working on antibacterial activity of *Camellia sinensis* mediated ZnO nanoparticles against four bacterial strains reported maximum zone of inhibition in *Klebsiella pneumonia*, supporting the present study. The antifungal activity of synthesized nanoparticles revealed that the growth of *Aspergillus fumigatus and Trichoderma Viride* were inhibited even at lower concentrations which are comparable to the antifungal activity reported by Jasim (2010).

Table 1: Antimicrobial activity of Zinc Oxide nanoparticles against different bacterial strains.

Test Organism	Control	ZnO-AP
Staphylococcus aureus (MTCC-1144)	00	1.5
Streptococcus pneumonia (MTCC-655)	00	1.4
Escherichia coli	00	1.4
Pseudomonas aeruginosa (MTCC-2474)	00	1.9
Klebsiella pneumoniae (MTCC-4040)	00	2.4

Table 2: Antimicrobial activity of Zinc Oxide nanoparticles against different fungal strains.

Samples	Diameter of zone of inhibition in 'cm'	
	Aspergillus fumigatus	***Trichoderma Viride***
ZnO-AP (250 µg)	0.9	1.06
Drug Fluconazole	1.40	1.50
Control	00	00

ZnO-AP - *Ajuga parviflora* mediated ZnO nanoparticles; Zone of inhibition in 'cm'

Conclusion

In the present work, *Ajuga parviflora* mediated ZnO nanoparticles showed potential antimicrobial activity against five bacterial strains viz. *Staphylococcus aureus*, *Streptococcus pneumoniae, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* and two fungal strains viz. *Aspergillus fumigatus* and *Trichoderma Viride*. Thus, it can be concluded that the eco-friendly and highly efficient ZnO nanoparticles synthesized from *Ajuga parviflora* leaf extract are expected to have more extensive applications in industrial and biomedicinal fields.

Conflict of Interest

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

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