Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2585
DOI URL: http://dx.doi.org/10.21474/IJAR01/2585
ISSN NO. 2320-5407

RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED ZnO NANOPARTICLES AGAINST GRAM POSITIVE (STAPHYLOCOCCUS AUREUS) AND GRAM NEGATIVE (PSEUDOMONAS AERUGINOSA) BACTERIA: A COMPARATIVE STUDY

P. P. Badoni[1], Goutam Kumar[2*], Reena Purohit[2], Mahender Singh[2] and Arun K. Khajuria[3].

1. Associate Prof., Department of Chemistry, HNB Garhwal University, Campus Pauri, Uttarakhand, India.
2. Research Scholar, Department of Chemistry, HNB Garhwal University, Campus Pauri, Uttarakhand, India.
3. Research Scholar, Department of Botany, HNB Garhwal University, Campus Pauri, Uttarakhand, India.

Manuscript Info

Manuscript History

Received: 27 October 2016
Final Accepted: 25 November 2016
Published: December 2016

Key words:-
Nanotechnology, Antibacterial behavior, *Staphylococcus aureus, Pseudomonas aeruginosa.*

Abstract

Nanotechnology has attracted a lot of attention from every generation: either its new or old generation. Biosynthesized nanoparticles have been used mainly because of their medicinal properties. An important medicinal property of these nanoparticles is their antibacterial behavior against Gram positive and Gram negative bacteria. ZnO nanoparticles of *Arisaema tortuosum* (Wallich) Schott, and *Rhus parviflora* Roxb., leaves extract were synthesized by biochemical (precipitation) method and characterized by X-ray diffraction and UV-Visible spectroscopic analysis. At concentration 200 µg/ml, their antibacterial activity was evaluated against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacteria and the results showed the antibacterial activity order as:
ZnO nanoparticles (*R. parviflora*) > ZnO nanoparticles (*A. tortuosum).*

Introduction:-

Nature has designed various methods for the generation of nano and micro sized inorganic materials which have contributed to the development of relatively new and largely unexplored area of research. And in last few years, it has been observed that researchers have attempted to synthesize nanoparticles within the size range of 100 nm and these extensive attempts and concerns on nanoparticles is widening due to their vast potential applications in wide areas of science and technology.

It has been reported that inorganic materials interact with biological materials and established a series of nanoparticle/ biological interfaces that depend on colloidal forces as well as dynamic bio-physicochemical interactions. These interactions lead to the formation of new nanomaterial with controlled size, shape, surface chemistry, roughness and surface coatings[2]. The synthesis of nanoparticles with specific morphologies and properties is one of the most important aspects of nanoscience which studies materials whose size lies within the nanometer range[3]. While chemical synthetic procedures can lead to the generation of toxic chemical by-products or require high temperature and/or pressure, biosynthesis of metallic nanoparticles using plant extracts provides a facile and green method of nanoparticle synthesis.

Corresponding Author:- Goutam Kumar
Address:- Research Scholar, Department of Chemistry, HNB Garhwal University, Campus Pauri, Uttarakhand, India.

Several physical and chemical procedures have been reported for the synthesis of ZnO nanoparticles and currently, biosynthesis method is widely utilized. Plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness[1]. Applications of these nanostructures are seen in catalysis, sensors, water purification, antimicrobial activities. The use of non toxic materials like plant extract for the synthesis of ZnO nanoparticles offers numerous benefits of medicinal applications.

ZnO nanoparticles are of antibacterial and antifungal activities even at lower concentrations hence suitable for thin coating applications. The antifungal activity of ZnO nanoparticles does not affect soil fertility compared to the conventional antifungal agents[11]. The bacterium and fungal lipid bi-layers get ruptured due to cytotoxic behavior of ZnO nanoparticles resulting in the drainage of the cytoplasmic contents[12]. Even antibacterial agents were developed against a wide range of microorganisms to control the bacterial infection[8].

At last, in the present work, ZnO nanoparticles of *Arisaema tortuosum* (Wallich) Schott, and *Rhus parviflora* Roxb., leaves extract were synthesized by biochemical (precipitation) method and characterized by X-ray diffraction and UV-Visible spectroscopic analysis. These nanoparticles showed wonderful antibacterial activity against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacteria.

Fig. 1(a) *Arisaema tortuosum* Fig. 1(b) *Rhus parviflora*

Fig. 1:- showing images of the plants (a) *Arisaema tortuosum* and (b) *Rhus parviflora* used in this work.

Materials and Methods:-

Materials: Collection:-

The leaves of both plants were collected from Hilly area of District Pauri (Uttarakhand), India. Zinc acetate dihydrate and sodium hydroxide pellets were purchased from Merck-Millipore (India).

Methods: Preparation of Leaves Extract and Zinc Oxide Nanoparticles:-

The leaves extract was prepared by mixing 05 gm of dried leaves powder with 100 mL deionized water in 250 mL of Erlenmeyer flask and boiled for 20-30 minutes at 70^{o}C. Then the leaves extract was collected in separate conical flask by filtering it through Whatman filter paper no.1 and stored for further studies.

But for the synthesis of ZnO nanoparticles, 50 ml of leaves extract was taken and heated to 60-70^{o}C using a magnetic stirrer heater. 0.1M Zinc acetate dihydrate and 1M NaOH solution were added to it with constant stirring. This mixture is then boiled until it reduced to a deep color precipitate. The color precipitate was centrifuged and washed with deionized distilled water. The washing and centrifuging was repeated several times using water and ethanol. The obtained material was dried at 30^{o}C for 12 hours in oven. Finally, to get a finer and uniform nature for characterization, the color dried material was mashed in a mortar-pestle .

Characterization:-

The synthesized ZnO nanoparticles were characterized by XRD and UV-Vis spectroscopic analysis from USIC, HNBGU and SAIF & CIL, PU, India, respectively.

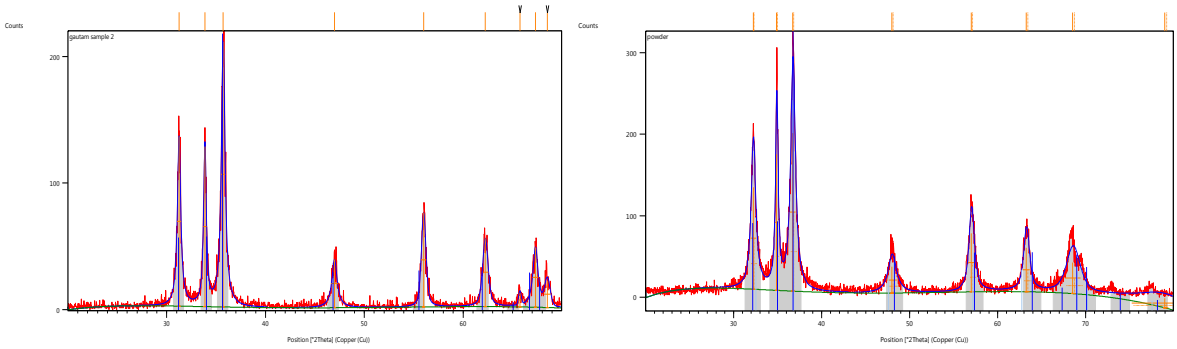
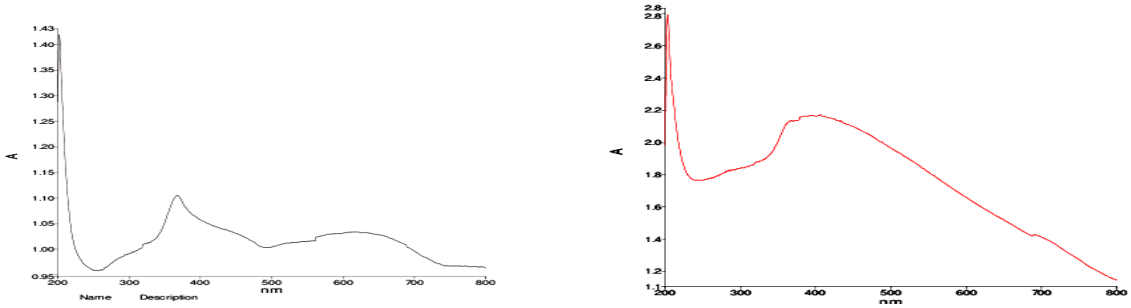


Fig. 2(a) Fig. 2(b)

Fig. 2;- XRD patterns of ZnO nanoparticles of a) *A. tortuosum* and b) *R. parviflora* leaves extracts showed crystalline nature of these nanoparticles and the average size of nanocrystal was estimated according to Scherer's equation:

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

Where, 'd' is the crystallite size, 'λ' the X-ray wavelength (CuKα), 'K' is the shape factor, 'β' the Full Width Half Maximum (FWHM) and 'θ' is the diffraction angle. It is calculated that the average crystallite size of ZnO nanoparticles of *A. tortuosum* and *R. parviflora* leaves extracts are <20 and <25 nm, respectively.

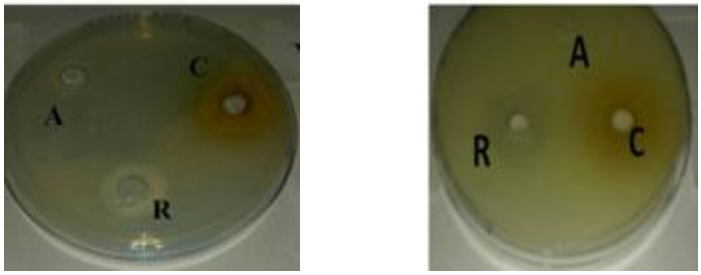


3(a) *A. tortuosum* 3(b) *R. parviflora*

Fig. 3:- UV-Visible spectra of ZnO nanoparticles of a) *A. tortuosum* and b) *R. parviflora* leaves extracts showed absorbance in the range of 355-375 nm for both nanoparticles. It can be predicted from the spectra that the formation of ZnO nanoparticles of both plants has been taken place.

Antibacterial Activity:-

In the following Fig. 4 (a & b), '**A**' and '**R**' terms are used for *A. tortuosum* and *R. parviflora* leaves extract mediated synthesized ZnO nanoparticles for the evaluation of their antibacterial activity.



4(a) *Staphylococcus aureus* 4 (b) *Pseudomonas aeruginosa*

Fig. 4:- The minimum diameter of zone of inhibition of ZnO nanoparticles of *A. tortuosum* leaves extract can be seen against *Staphylococcus aureus* and *Pseudomonas aeruginosa* where as maximum for *R. parviflora* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* pathogens at concentration 200 µg/ml.

Table 1:- Antibacterial Activity Nanoparticles.

Nanoparticle	Diameter of zone of inhibition (mm)	
	Staphylococcus aureus (Gram positive)	*Pseudomonas aeruginosa* (Gram negative)
A (200 µg/ml)	18.0	15.0
R (200 µg/ml)	25.0	27.0

Table 1. Values of diameter of zone of inhibition in mm of both nanoparticles showing the efficiency of 'R' nanoparticle over the other i.e. 'A' against the *Staphylococcus aureus*-Gram positive and *Pseudomonas aeruginosa*-Gram negative bacteria.

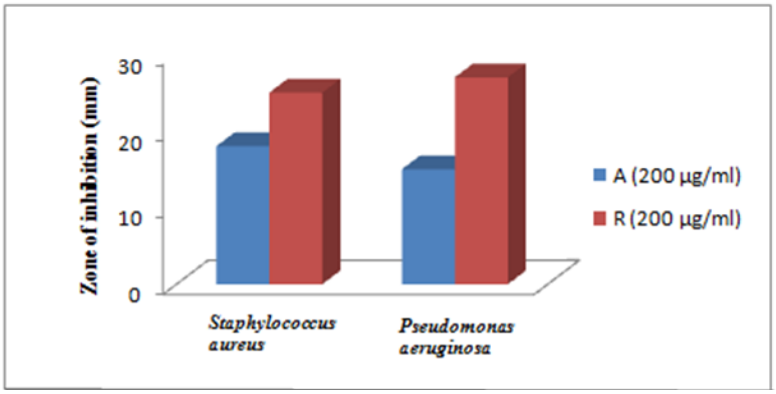


Fig. 5:- Histogram of antibacterial activity of ZnO nanoparticles of *A. tortuosum* (A) and *R. parviflora* (R) is describing the potential of both nanoparticles. Clearly, it can be seen that the antibacterial potential of 'R' nanoparticles is much higher than the potential of 'A'.

Conclusion:-

From the above results, it is concluded that these nanoparticles are crystalline in nature and of average size in nanometer range. At concentration 200 µg/ml, antibacterial activity of these biosynthesized ZnO nanoparticles against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacteria showed the following order:

ZnO nanoparticles (*R. parviflora*) > ZnO nanoparticles (*A. tortuosum)*

Acknowledgment:-

Gratefully acknowledge the financial support from the University Grants Commission (UGC), New Delhi in the form of Junior Research Fellowship.

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