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Antimicrobial Activity of *Rhus parviflora* Roxb.: Leaves Extract Mediated Synthesized ZnO Nanoparticles

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Abstract : Human beings are known to be susceptible to microbial attack and this necessitates the development of antimicrobial agents, to counter the microbial attack. That's why, in recent years, plant mediated synthesis of metallic nanoparticles is an interesting issue of the nanoscience and nanotechnology. Many biochemical methods are used for synthesis of the nanoparticles, in order not to harm the human kind. Therefore, the present work deals with the investigation of antimicrobial activity of Zinc Oxide (ZnO) nanoparticles, synthesized from 0.1M solution of Zinc acetate dihydrate {$[Zn(CH_3COO)_2].2H_2O$} and aqueous extract of leaves of *Rhus parviflora* Roxb. (Anacardiaceae) at controlled temperature and pH range. The resulted ZnO nanoparticles as potential antibacterial agents have been studied on *Staphylococcus aureus* (Gram positive bacteria) and *Pseudomonas aeruginosa* (Gram negative bacteria) strains. Another study has indicated that these small sized nanoparticles showed good antifungal activity against *Aspergillus niger* and *Candida albicans* at concentration 200µg/ml.
Keywords : Antimicrobial agents, Anacardiaceae, Nanoparticles, Antimicrobial activity.

Introduction

The belief that nanotechnology is an ultramodern combination of science, chemistry, engineering, biology and medicine, is accepted between the scientists. Evaluation has shown that whatever the size of the smaller nanoparticles, new and different activity and characteristics of their own. The use of these materials in order to fight against pathogenic microorganisms can be an appropriate choice. Nanotechnology is expected to be the basis of many of the main technological innovations of the 21st century research and development. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. The source of the plant extract is known to influence the properties of the nanoparticles. This is because different extracts contain different concentrations and combinations of organic reducing agents. Typically, a plant extract mediated bioreduction involves mixing the aqueous extract with an aqueous solution of the relevant metal salt. The reaction occurs at room temperature and is generally completed within a few minutes. In view of the number of different chemicals involved, the bioreduction process is relatively complex. The number of methods including physical and chemical methods, electrochemical reduction, photochemical reduction and heat evaporation have been used for the synthesis of ZnO nanoparticles[1].

The biosynthesized ZnO nanoparticles are trusted to be friendly with environment, non toxic, bio-safe and biocompatible. ZnO nanoparticles posses antibacterial and antifungal activity 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[2]. The bacterium and fungal lipid bi-layers get ruptured due to cytotoxic behavior of ZnO nanoparticle resulting in the drainage of the cytoplasmic contents[3]. The

antimicrobial effect of ZnO nanoparticles is also investigated and antibacterial agents were developed against a wide range of microorganisms to control the bacterial infection[4,5].

Rhus parviflora Roxb. belongs to the family Rosaceae and commonly known as *Tungla* or *Tintideek*, abundant as gregarious shrub or small trees, to 4m high; bark grey, smooth; on open, exposed, slopes of sub montane zones, ascending to 1800m, and found in W. Himalaya, C. India, Nepal and Sri Lanka[6]. In some tribal areas, infusions of leaves were given in cholera. Phytochemicals like gallic acid, some flavones viz., rutin, myricetin, quercetin, myricitrin, quercitrin, kampferol and some glycosides (isorhamnetin-3-α-L-arabinoside) have been isolated from the plant[7,8]. In vitro anti-HIV activity and preliminary safety profile of the extracts prepared from the leaves of *R. parviflora* were also studied[9]. Plants are looked for their medicinal value since aeon, which they accumulate with their past experience with native flora[11]. And the present study is intended to study antimicrobial activity of the synthesized ZnO nanoparticles from aqueous leaves extract of this valuable medicinal plant shown in Figure 1.

Figure 1 *Rhus parviflora* Roxb. plant

Materials and Methods

Preparation of leaves extract and Zinc Oxide nanoparticles :

R. parviflora 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 70^0C. Then the leaves extract was collected in separate conical flask by filtering it through Whatman filter paper no.1 and stored for further studies.

For the synthesis of ZnO nanoparticles, 50 ml of leaves extract was taken and heated to 60-70^0C 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 yellow colored precipitate. The colored precipitate was centrifuged and washed with deionized distilled water. The washing and centrifuging was repeated several times using ethanol and water. The obtained material was dried at 30^0C for 12 hours in oven. Finally, to get a finer and uniform nature for characterization, the light yellow colored dried material was mashed in a mortar-pestle[13].

Determination of antimicrobial activity by well diffusion method:

For antibacterial test, Soyabean Casein Digest agar/broth and Sabouraud's dextrose agar/broth were used for antifungal test. The pure cultures of test organisms viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Local Isolated Culture), *Candida albicans* (Local Isolated Culture) and *Aspergillus niger* (Local Isolated Culture) were used.

Then the bacteria were inoculated into Soyabean Casein Digest broth and incubated at 37^0C for 18 hours and suspension was checked to provide approximately, 10^5 CFU/ml. The same procedure was done for fungal strains and the strains were inoculated into Sabouraud's dextrose broth but the fungal broth cultures were incubated at 48-72 hours.

The agar well diffusion method was modified. Soyabean Casein Digest agar medium (SCDM) was used for bacterial cultures. The culture medium was inoculated with the bacteria separately suspended in nutrient

broth. Sabouraud's dextrose agar/broth was used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with extracts (200µg/ml), positive controls and solvent blanks. Conventional antibiotic viz. Erythromycin (1 mg/ml) was used as standard positive control and antibacterial agent and Flucanazole (1 mg/ml) was used as standard positive control and antifungal agent. The plates were then incubated at 37^0C for 18 hours for determination of antibacterial activity and 28^0C for 48-72 hours for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

Results and Discussion

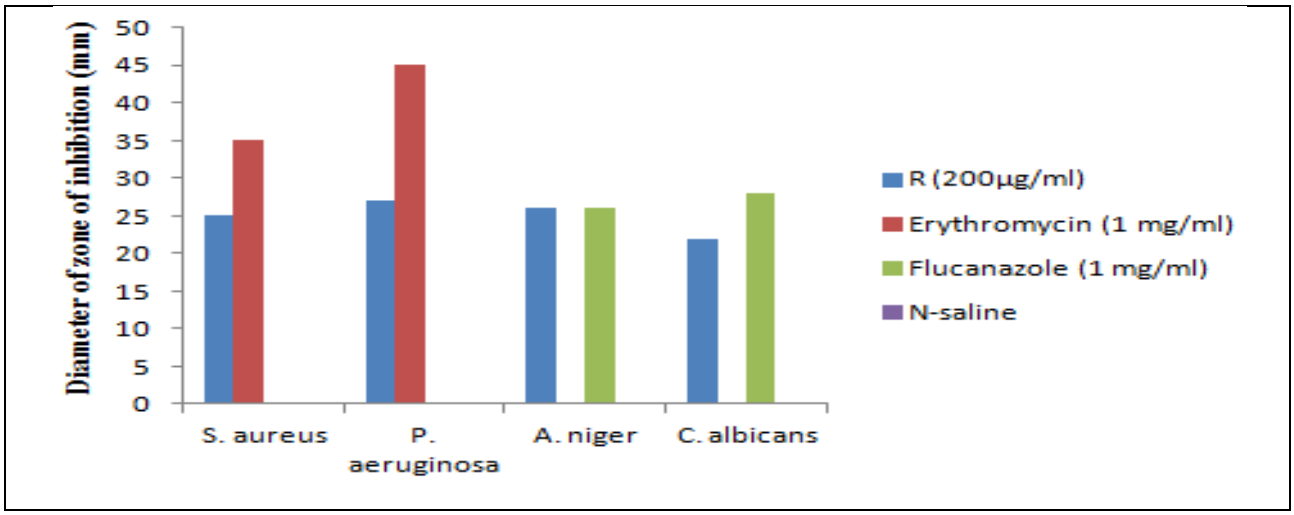
Biosynthesized ZnO nanoparticles were characterized by X-Ray Diffraction (XRD), Fourier transform Infrared (FTIR) and UV-Visible spectroscopic techniques and results of XRD confirmed the formation of nanoparticles,having size less than 20nm. FTIR analysis revealed the presence of polyphenolic compounds and UV-Visible spectra showed the presence of ZnO phase.

Antimicrobial activity:

It was carried out on four clinically isolated strains namely *Staphylococcus aureus* (Gram positive bacteria), *Pseudomonas aeruginosa* (Gram negative bacteria), *Aspergillus niger* (fungus) and *Candida albicans* fungus). The antimicrobial activity of samples ofnanoparticles (R) were tested against these microbial cultures by well diffusion method. The value of diameter of zone of inhibition (mm) is given in Table 1. And Table 2 lists the MIC and MLC values observed for various test microbes. All the bacterial and fungal microbes showed good sensitivity towards these ZnO nanoparticles at concentration 200µg/ml. The efficiency of these nanoparticles is better than the Erythromycin (used against bacterial infection) and Flucanazole (used against fungal infection)**.**Thus, in agreement with currently available results, ZnO nanoparticles were found to posses good antibacterial and antifungal activity against all the tested microbes.

Table 1 Antimicrobial activity of nanoparticles

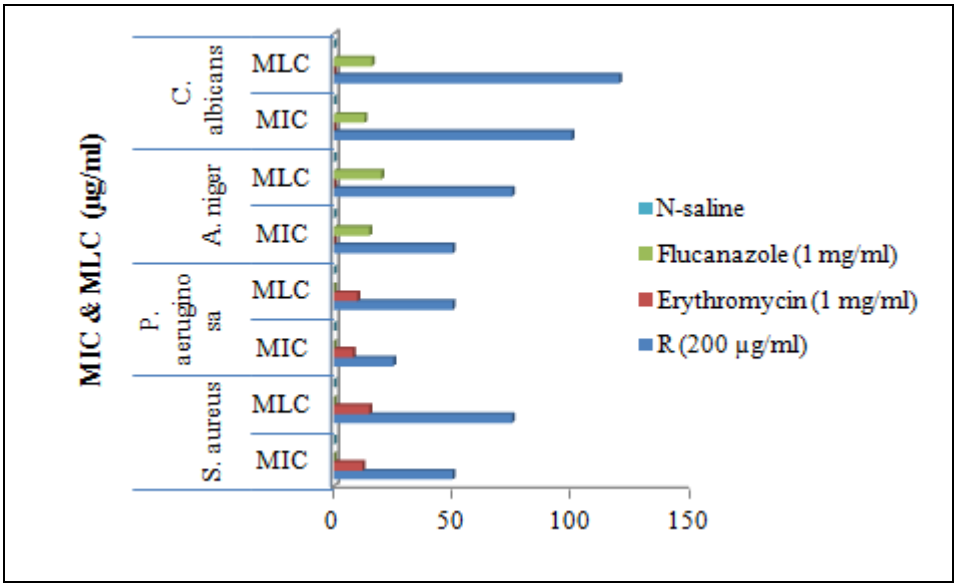
Samples	Diameter of zone of inhibition (mm)			
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Aspergillusniger***	***Candida albicans***
R(200µg/ml)	25.0	27.0	26.0	22.0
Erythromycin (1 mg/ml)	35.0	45.0	NT	NT
Flucanazole (1 mg/ml)	NT	NT	26.0	28.0
N-saline	NA	NA	NA	NA



Graph 1 Graphical representation of antimicrobial activity of nanoparticles.

Table 2: Minimum inhibitory concentration (MIC) and Minimum lethal concentration (MLC) of the nanoparticles.

Samples	MIC & MLC (µg/ml)							
	Staphylococcus aureus		***Pseudomonas aeruginosa***		***Aspergillus niger***		***Candida albicans***	
	MIC	**MLC**	**MIC**	**MLC**	**MIC**	**MLC**	**MIC**	**MLC**
R (200 µg/ml)	50	75	25	50	50	75	100	120
Erythromycin (1 mg/ml)	12	15	08	10	NT	NT	NT	NT
Flucanazole (1 mg/ml)	NT	NT	NT	NT	15	20	13	16
N-saline	NA	NA	NA	NA	NA	NA	NA	NA



Graph 2 Graphical representation of Minimum inhibitory concentration (MIC) and Minimum lethal concentration (MLC) of the nanoparticles.

Conclusion

In the present work, ZnO nanoparticles were synthesized using Zinc acetate dihydrate and leaves extract of *R. parviflora* Roxb. The synthesized ZnO nanoparticles were highly crystalline and showed antimicrobial activity against *Staphylococcus aureus* (Gram positive bacteria), *Pseudomonas aeruginosa*(Gram negative bacteria), *Aspergillus niger* (fungus) and *Candida albicans* (fungus) and it was found that these nanoparticles inhibit their growth at concentration200µg/ml. Thus, it can be concluded that the explored eco-friendly, highly efficient ZnO nanoparticles prepared from *R. parviflora* Roxb., leaves extract are expected to have more extensive applications in biomedical fields.

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