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Antibacterial activity of biogenic synthesized silver nanoparticles using skin of Kashmir Nadi Frog *Paa barmoachensis*

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SUMMARY

Nano-technology is used to synthesis drugs which has resistance against the antibiotics. Due to best antimicrobial efficacy silver nanoparticles are known most effective. At minimum concentration, the antimicrobial properties of silver nanoparticles are nontoxic to individuals. Antibacterial properties prevent the bacteria which are reproduce that is microorganism. The Kashmir Nadi Frog (*Paa barmoachensis)* sample was collected from Dhirkot, Bagh, Azad Jammu and Kashmir, Pakistan. In current research the synthesis, characterization and biological activities of biogenic silver nanoparticles via skin of *paa barmoachensis* extract were performed. The characterization of silver nanoparticles was conceded out by Ultra Violet Visible (UV-Vis) spectrophotometer. The efficiency of synthesized silver nanoparticles were evaluated by antibacterial and antibiofilm assay. The synthesized silver nanoparticles used as source of beneficial drug.

Keywords: Kashmir, Frog, Nanotechnology, ethnomedicine

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INTRODUCTION

The word “nanotechnology” is at the present usually used to refer to the production of novel nano-chemical of range from 1 to 100 nanometers (Thrall, 2004). The different sizes of nanoparticles have different biomedical purposes. To enhance the contrast of image of MRI and ultrasound the nanoparticles are used. Nanoparticles are used in ultrasound that may help to enhance the auditory reflectivity. Nanoparticles enable to enhance the image quality of MRI technology. They enhance the clearness of the image (Liu *et al.*, 2006).

A “Nanotechnology” is currently in use as a tool to explore the darkest avenues of sciences in a lot of way like sensing, and imaging embattled medicine preparation (Langer, 2001) artificial implants and gene delivery systems. The novel medicines are nanoparticles of metals, ceramics or polymers, which can fight circumstances e.g. fight human pathogens like bacteria as well as cancer (Blanco *et al.*, 2011). It helps to improve the efficiency of treating cancer in assured conditions. By utilize of friendly super paramagnetic “nanoparticles” to make “magnetic field

hyperthermia" is extra efficient. Nanoparticles support heat tissue, especially tissue of cancer, by producing fluctuations that produce heat. These heats will the disruption of the tissue that may help to treat the cancer (Liu *et al.*, 2006).

Ethnoherpetology is a branch of "ethnozoology" that studies the interactions between *Homo sapiens*, reptiles, and amphibians. Ethnozoological studies can aid in assessing human population impacts on local animal species and improving manageable management plans. As a result, they are critical to management works (Alves and Souto, 2015; Alves *et al.*, 2018; Altaf *et al.*, 2020). Herpetofauna are widely used in medicine (Altaf *et al.*, 2018), but there is a scarcity of comprehensive data on the scope of this research area and its impact on the species involved (Mendonça *et al.*, 2014; Altaf *et al.*, 2020). The objectives of the present work are to synthesize silver nanoparticles by using skin of *Paa barmoachensis,* to synthesized nanoparticles, to analyze antimicrobial activity and to evaluate Anti biofilm assay of synthesized nanoparticles.

MATERIALS AND METHOD

CHEMICAL APPARATUS

Reagents and Chemicals got from sigma Germany Aldrich Switzerland and Merck.Equipment like shaking incubator, Analytical balance, Weighing machine, Laminar flow, Autoclave and Ulta violet visible spectrophotometer were used during lab research work.

COLLECTIONANDIDENTIFICATION OF FROG

Paa barmoachensis was collected from "Bagh, Azad Jammu and Kashmir, Pakistan". The frog was verified by Ethno-zoologist, "Department of zoology, Faculty of Science, Women University of Azad Jammu and Kashmir, Bagh, Pakistan".

PREPARATION OF EXTRACT

By using modified method of (Singh *et al.,* 2014) extract was prepared. The skin of *Paa barmoachensis* (Kashmir Nadi Frog) sample was superbly pulverized by mortar grinding machine. The extract was made up to "100 ml" using double distilled water. To separate tissue rubbles, the extract was strained through "Whatman NO1 filter paper" and a pure extract were obtained.

SYNTHESIS OFSILVER NANOPARTICLES

The skin filtrate of the Kashmir Nadi Frog (*Paa barmoachensis*) was used to make nanoparticles. To prevent auto-oxidation of silver stored in yellowish-brown bottles, 1 mM silver nitrate (0.0421 g AgNO3) was dissolved in 250 ml purified H2O. For the synthesis of silver nanoparticles, 10 mL PBAqu is mixed with 90 mL 1Mm silver nitrate solution. The formation of silver nanoparticles is indicated by the brown colour.

CHARACTERIZATION OF SILVER NANOPARTICLES

The presence of silver nanoparticles was confirmed using an ultra violet visible spectrophotometer. At 200 nm to 800 nm, the spectrums were recorded.

IN VITRO ANTIBACTERIAL ACTIVITY

The antibacterial activities of synthesised silver nanoparticles were tested against four bacteria pathogens using the agar well diffusion method (Rios *et al.*, 1988). Four human pathogens that are used *Staphylococcus aureus, Klebsiella pneumonia, proteus mirabilis, Pseudomonas aeruginosa.* Microbial Bio-technology Laboratory, "Department of Zoology, Women University of Azad Jammu and Kashmir, Bagh, Pakistan", provided these bacterial strains. Bacterial culturing was carried out. The inoculating loop was used to grow bacteria in nutrient broth 2ml for bacterial nourishment. At room temperature, the bacteria were incubated overnight in a rotary shaker. The nutrient agar was combined with a 45^{o}C overnight culture. These mixtures were then transferred into Petri plates. Petri plates were set sideways in laminar flow at room temperature to solidify. Three of the wells are 5mm in diameter. Each plate was made with a sterilised micropipette tip (1ml) and then the agar plug was removed with sterilised needles. Thirty micro litres of PBAqu and PBAgNps, the control solvent, were placed in each prepared well. Then leave it in the incubator for 24-48 hours at room temperature. After 24 hours the growth of bacteria was calculated zone diameter in milli- meter (Seeley Jr and VanDemark, 1962). Scale was used to calculate the diameters of zones (Hammer *et al.*, 1999). There is no sensitivity if the values are all zero. Low sensitivity ranges from 1 to 5 millimetres, medium sensitivity ranges from 5 to 10 millimetres, and high sensitivity ranges from 10 millimetres.

ANTI-BIOFILMACTIVITY

Crystal violet assay were used for anti-biofilm activity with little bit alterations (O'Toole, 2011). In tubes containing 3 0 m g/m l PBAqu and PBAgNPs, as well as 2 ml N B, an equal amount of PBAqu and PBAgNPs were present. Place these tubes in an incubator at room temperature overnight. Silver nitrate was used as a control for Chloramphenicol. Only N B was kept as a negative control and discarded the next day. The cells were then stained with crystal violet. Tubes were incubated at room temperature for ten to fifteen minutes before being discarded. A spectrophotometer was used to calculate the amount of dissolved crystal violet. As a blank acetic acid, water was used.

RESULTS AND DISCUSSION

YNTHESIS OF PBAgNPS

When *paa barmoachensis* (PBaqu) was mixed by the ions of silver, the process of reduction were happened. Before the addition of silver Nitrate PBaqu has yellowish color whereas after boiling for fifteen to twenty minute the yellowish color altered to dark brown color, which showed the confirmation of silver nanoparticles PBAgNPs via PBaqu extract. The changing of color showed that indication of biosynthesis of ABAgNPs. The solution which has Brown color is due to Surface Plasmon Resonance (Singh *et al.*, 2014).

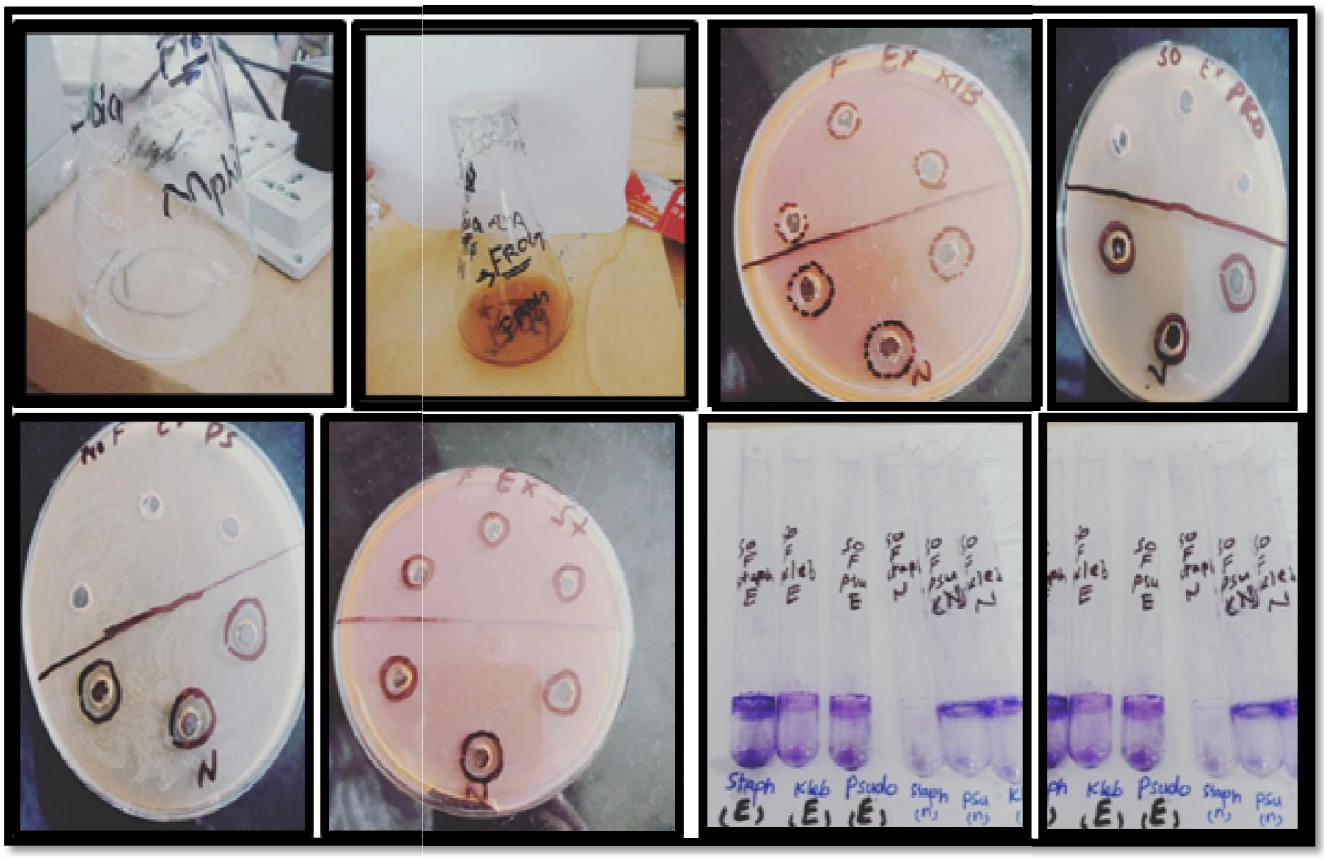

Figure 1: Synthesis of silver-nanoparticles and antibacterial and antibiofilm activity.

CHARACTERIZATION OF PBAgNPs

The synthesis of PBAgNPs was established by Ultra Violet Visible spectrum. at a range from 200-800 nm wavelengths. To confirm the synthesis of PBAgNps, the absorbance was recorded at 400 nm.

ANTIBACTERIAL ACTIVITY

The present study was started to assess the antibacterial activity of PBAgNPs and PBAqu against 4 germ causing bacteria. This assessment was done through agar well diffusion method. In the presentstudy, it was notified that PBAgNPs showed major antibacterial activity in contradiction of Gram negative bacteria *Proteus mirabilis* (10±0). *While* PBAqu showed no inhibition zone *against proteus mirabilis. The PBAgNPs,* showed moderate inhibition zone that was (8.666±3.785) against *Staphylococcus aureus.* Although the PBAqu, showed low inhibition zone that was 5±0 against *Staphylococcus aureus.* The PBAgNPs showed moderate inhibition zone that was 9.666±0.577 against *Klebsiella pneumoniae* In PBAqu, showed no inhibition zone against *Klebsiella pneumoniae*. PBAgNPs showed moderate inhibition zone that was 6±1.732 which have against *Pseudomonas aeruginosa.* In PBAqu shows no inhibition zone against *Pseudomonas aeruginos*. It is concluded that PBAgNPs showed high antibacterial activity as compared to PBAqu (Table 1 and Figure 1). Boman (2000) reported that the antimicrobial activity of frog skin peptides is tested

against a small number of pathogenic bacteria e.g. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Table 1: Antibacterial activity.

Test sample	PBAqu	PBAgNPs	Chloramphenicol (10ug)
Staphylococcus aureus	5±0	8.666±3.785	0.027
Klebsiella pneumonia	9.333±4.041	9.666±0.577	0.023
Pseudomonas aeruginosa	0±0	6±1.732	9.999
Proteus mirabilis	0±0	10±0	1.430

ANTIBIOFILM ACTIVITY

In the current research, by using crystal violet staining the impact of PBAqu and PBAgNPs on biofilm formation was tested. Result exposed that tested gram negative bacteria pseudo were able to form bofilm in the presence of PBAgNPs, but in PBAqu pseudo showed quite adherence. Outcome showed the PBAqu has not probable to decrease as related to PBAgNPs, but the all assemblies should high prospective to decrease development of biofilm (Table 2 and Figure 1). Christensen *et al.* (1985) demonstrated that the anti-biofilm activity of AgNPs was partitioned against main biofilm forming bacteria *P. aeruginosa* and *S. aureus* and adherence capability were compare with the earlier study showed the biofilms were relatively adherence. The synthesized nanoparticles inhibited formations of biofilm by inhibit the production of exopolysachharides.

CONCLUSION

The present study demonstrates that an essay and efficient path of silver nanoparticles synthesis using extract of *Paa barmoachensis* (Kashmir Nadi Frog). AgNPs confirmed by using UV-vis spectroscopy. The PBAgNPs showed good antibacterial activity as compared to the PBAqu as exposed by Disc Diffusion method. PBAqu were able to form biofilm as compared to the PBAgNPs. It can be used as therapeutic drugs.

Table 2: Antibiofilm activity.

Test	PBAgNPs	PBAqu	Pathogen	Chloramphenicol
Staph	0.274 ±0.045	0.265±0.053	1.45	0.12
Pseudo	0.185±0.002	0.244±0.009	1.45	0.023
Proteus	0.449±0.005	0.269±0.034	1.05	1.430
Kleb	0.446±0.004	0.220±0.006	1.542	0.027

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