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The Efficacy of Common Windmill Butterfly's Silver Nanoparticles against Bacterial Pathogens

Sadia Sadiq[1] and Shareen Akhtar [1*]

1. Department of Zoology, Women University of Azad Jammu and Kashmir, Bagh, Pakistan

***Corresponding author e-mail:** shareenakhtar94@gmail.com

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SUMMARY

Due to their powerful antibacterial qualities, silver nanoparticles (AgNPs) are being employed more and more in a variety of industries, including medicine, nutrition, healthcare, and industry. The aim of this study was to synthesize, characterize, and assess the antibacterial and antibiofilm properties of *Byasa polyeuctes*-derived AgNPs. AgNPs were identified by characterization using ultraviolet-visible (UV-Vis) spectroscopy, which also demonstrated their suitability for diverse applications thanks to their distinctive surface plasmon absorption peak at 450 nm. At a dosage of 0.3 mg, BPAgNPs and BPAqu both displayed efficient suppression of bacterial pathogens like *Proteus mirabilis, Staphylococcus aureus*, and *Klebsiella pneumoniae*. It is noteworthy that BPAqu demonstrated a greater zone of inhibition against *Pseudomonas aeruginosa* (7.666±0.577mm), but not against *Staphylococcus aureus*. BPAgNPs, on the other hand, had a more pronounced inhibitory impact against *Klebsiella pneumoniae* (8.421±1.103mm), while it had little effect on Staphylococcus aureus. This study demonstrates the potential of *Byasa polyeuctes'* aqueous extract and silver nanoparticles as sources for medicinal medicines.

Keywords: Butterfly, Nanoparticle, nanomedicine, *Byasa polyeuctes*

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INTRODUCTION

Technology and research progress at tiny, macromolecular and molecular rank occupy by the source of a length scale of just about one to one hundred nm in any dimension is known as nanotechnology. The arrangement and structures devices uses and since of their small size this systems that have narrative properties and functions and the ability to supervise or plant matter or organisms matter on an atomic scale (Manzoor and Safeer, 2022). Nanoparticles show unique characteristics that depend on their size and shape, which are capable to intermingle with viruses, plants and wildlife. The antibacterial characteristics of silver nanoparticles have displayed excellent in contradiction of a vast variety of bacteria (Siddiqi and Husen, 2016a, b, c).

In the last ten years, silver (Ag) has been used in the production of nanoparticles, whose size range from 1 to 100 nm. Due to the minor size of nanoparticles, the whole surface area of nanoparticle is maximizing that leads to maximum value. Because of this property they are particularly dissimilar from the

bulk material, the applications of silver nanoparticles in different fields involves bioengineering, biotechnology (Niemeyer, 2001), textile (Lewis, 1993), water management, optics (Murphy et al., 2005), electronics (Lee and Jeong, 2005), catalysis (Lewis, 1993) and in medicines (Salata, 2004). Moreover, now AgNPs are extensively used against bacteria and fungi and in a various range of consumer yield which includes wet pipes, tooth pastes, food storage containers, soaps, slippers, air sanitizer sprays, shampoo, vacuum cleaners, mobile, washing machines, socks, respirators, detergents, coating of refrigerators and pillows (Buzea, 2007).

Insects are very important for medicine and they play important role in immune regulation, anti-sensitivity reactions, reducing blood sugar, anti-bacterial activity, anti- oxidant activity and anti-inflammatory activity. Protein, hormones, steroid, polysaccharide, terpenes, anti-bacterial peptide, peptide quinine, alkaloids, lipids and chitin were obtained from insects which are pharmaceutically important compounds (Chen and Feng, 2009). For the treatment of cancer and tumors *Mylabris*was used. From cantharis, cantharidin show good activity against cancer (*Mylabris*spp.) (He et al., 2005; Shi et al., 2005). Regulation of immune system and for controlling mutation *Ericerus pela* eggs and adults was used (Feng et al., 2006a, b). Immune modulatory activities of insects are due to extract of polysaccharides from insects.

Peptide are extracted from insects, Lepidoptera, Hymenoptera and Diptera are used for antitumor, antibacterial and anticancer activity (Pullet and Stoklin, 2005). In China insects are conventionally used in drug to treat cancer or tumor. The insects which show good activity against cancer are bees, grubs, ants, cantharis *Mylabris spp*. house flies *Musca domestica*, Silkworms *Bombyxmori*, wasps, caterpillar fungi, (Chen and Feng, 2009). Arthropods have segmented bodies, jointed limbs and are invertebrates. The cuticles of insect consist of chitin. With the calcium carbonate millipedes, crustaceans, beetle and mites, cuticles are biomineralized (Cutler, 1980). The aims of this study are as follows; to synthesize the AgNPs using common windmill (*Byasa polyeuctes)* butterfly extract, to characterize the AgNPs by using Ultra Violet-VisibleSpectroscopy, to access Antimicrobialactivity and to evaluate Antibiofilmassay.

MATERIALS AND METHODS

In the Microbial Biotechnology laboratory, research work was done in the Department of Zoology, Women University of Bagh, Azad Jammu and Kashmir Pakistan.

IDENTIFICATION AND COLLECTION OF *BYASA POLYEUCTES*

The common windmill butterfly (*Byasa polyeuctes*) was accumulating from district "Bagh, the area of Azad Jammu and Kashmir Pakistan". The butterfly was recognized from book "Butterflies of the world" by (Lewis, 1973).

PREPARATION OFEXTRACT

Reena et al. (2014) used the modified method for the preparation of extract. The butterfly sample was dried at 60ºC using a dry oven. The sample of butterfly was carefully crushed by utilized pestle and mortar. The extract was made up to 100mL

using double- distilled Milli-Q water. Then extract were filtered by using Whatman No. 1 filter paper, after filtration we get a clean extract.

SYNTHESIS OF SILVERNANOPARTICLES

The common windmill butterfly (*Byasa polyeuctes*) extract was utilized for the synthesis of silver nanoparticles (Figure 1). In order to stop silver from auto-oxidizing, 250 ml of distilled water was added to 1 mM silver nitrate (0.0421 g of AgNO3) before being put in an amber-colored bottle. This produced nanoparticles. For the standard creation of silver nanoparticles, 10 ml of the aqueous extract were combined with 90 ml of a 1 mM AgNO3 solution and kept at room temperature in the lab. As a result, the emergence of a brown color was initially used to detect the formation of silver nanoparticles.

SILVERNANOPARTICLES CHARACTERIZATION

For the characterization of silver nanoparticles of *Byasa polyeuctes* was done by Ultra violet–Visible spectrophotometer. From 200 to 800 nm, the spectrum (or absorption spectra) was measured. We examined the size and shape of the silver nanoparticles we created using a specialized microscope called a field emission-scanning electron microscope (SEM). In order to comprehend the pellets containing Ag nanoparticles from the *Byasa polyeuctes* and extract better, we additionally used a device called the SHIMADZU FTIR-8400S.

IN VITRO ANTIBACTERIAL ACTIVITY

The bacteria which were used as *Klebsiella pneumonia, Proteous Mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus.* All these strain of bacteria obtained from Pathological laboratory, from Al-Shifa Hospital Islamabad Pakistan. Nutrient agar (NA) media (Oxide: CMOO3) and Nutrient broth (NB) media (Oxide: CM1) were utilized for bacterial culturing. A 25ml nutrient broth containing bacteria was grown using an inoculating loop and maintained at a temperature of 37°C for 24 hours in a rotary shaker. We combined the sterilized bacteria culture with freshly made nutritional agar in Petri dishes, and the temperature was set at 45 °C. We put the Petri dishes in a laminar flow at room temperature to solidify them. We made three tiny, 5 mm-diameter wells in each of the plates using a sterile micropipette tip (1ml). We used sterile needles to extract a tiny bit of agar. Then, we filled each prepared well with approximately 30 l of BPAgNps and BPAqu solvent, and we incubated the plates at 37 °C for 24-48 hours. Following the procedure outlined by Seeley and VanDemark in 1962, we determined the diameter of the inhibitory zones in millimeters (mm) to determine the bacterial growth after 24 hours. We applied Hammer's approach from 1999 to measure the diameters of the zones if they were larger than 5mm. The results (sensitivity tests) were expressed as ***(>10- 25 mm) for high sensitivity, **(>5- 10 mm) for moderate sensitivity *(1-5 mm) for low sensitivity and zero (0) for no sensitivity,.

ANTI-BIOFILMASSAY

Crystal violet assay with little modification was used to measure the antibiofim

activity (O'Toole, 2011). In Boro-silicate tubes (Minitek, USA) bacterial growth was done each tube contained 2 ml of nutrient broth medium and 30 mg/ml of BPAgNPs and BPAqu and then kept in incubator at "37°C" for whole night. Silver nitrate, chloramphenicol, and nutritional broth were utilized as the positive and negative controls, respectively. We took out the broth medium the next day. We added 0.1% crystal violet to 125 l of water to stain the affected cells. After 10 to 15 minutes of incubation at room temperature, the tubes were washed with water to get rid of any loose cells. We next dissolved these biofilms in a 30% acetic acid solution and gave them another 10 to 15 minutes to incubate at room temperature. The dissolved crystal violet at 550 nm was measured using a spectrophotometer, and the reference sample was a solution of 30% acetic acid in water.

RESULTS AND DISCUSSION

SYNTHESIS OF AgNPs OF *BYASA POLYEUCTES*

A reduction process happened when silver ions were added to an aqueous extract of the common windmill butterfly (*Byasa polyeuctes*). Before adding AgNO3, the *Byasa polyeuctes* Aqu extract had a light yellow tint. However, the color turned dark brown after boiling for 15-20 minutes, suggesting the development of silver nanoparticles (Ag nanoparticles). *Byasa polyeuctes* silver nanoparticles (BPAgNPs), which were made utilizing the BPAqu extract, may be seen in Figure 1. The production of BPAgNPs is indicated by a shift in color from yellowish-brown to dark brown; surface plasmon resonance is thought to be responsible for the dark brown hue.

Figure 1: Prelaminar synthesis of BPAgNPs using aqueous extract of *Byasa polyeuctes*

CHARACTERIZATION OFBPAgNPs

Additionally, by looking at their UV-Vis spectrum at wavelengths about 450nm, we were able to validate the production of BPAgNPs.

UV-VISIBLESPECTROMETRY

UV-Vis spectroscopy is an extremely helpful and safe method of characterizing newly formed nanoparticles (Figure 2), which is often utilized to check the stability and synthesis of silver nanoparticles (Sastry et al., 1998).

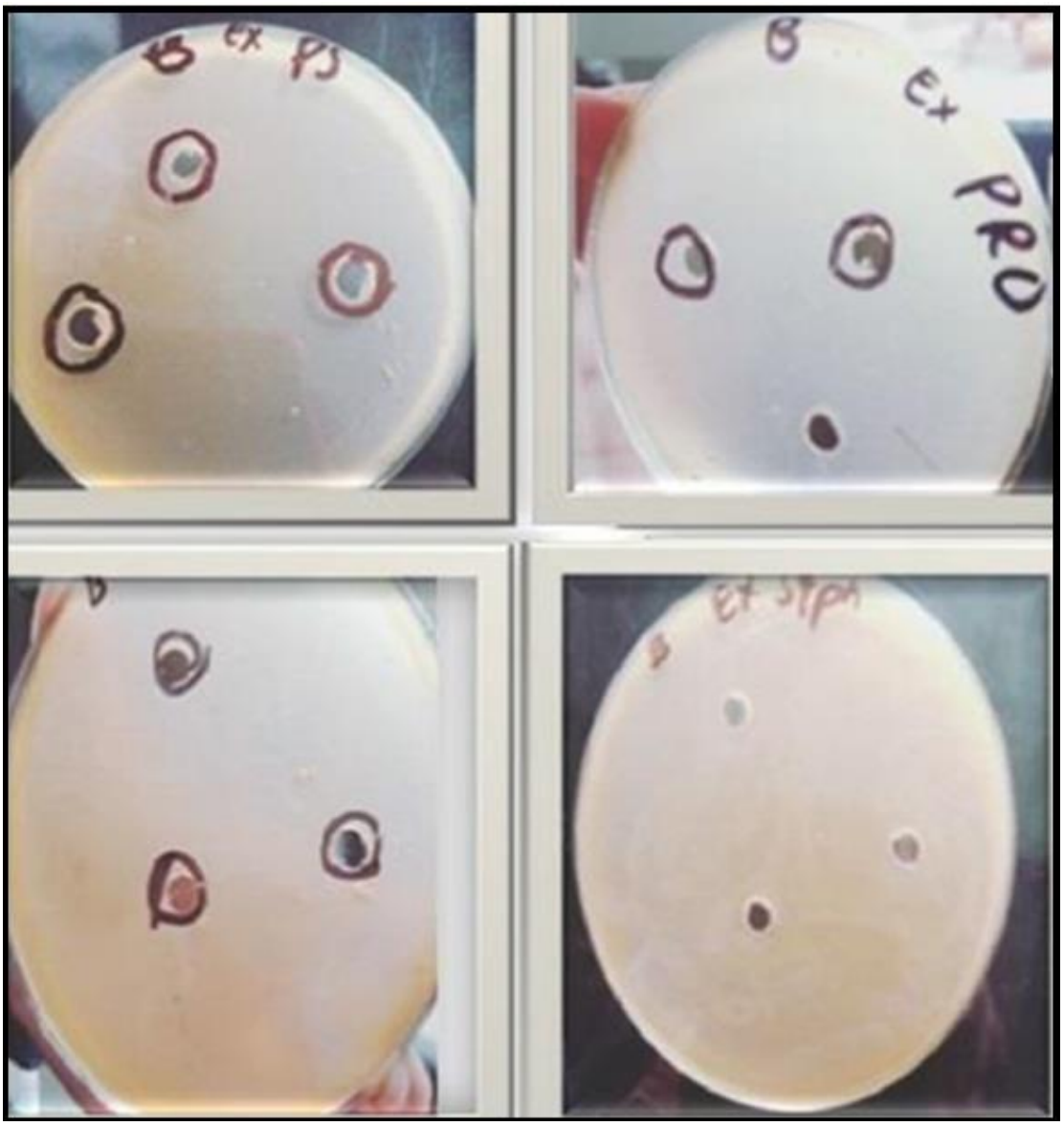

Figure 2: Visualization of the impact of BPAqu extract against bacterial pathogens.

AgNPs have distinctive ocular properties that allow them to interact strongly with precise light wavelengths. UV-Vis spectroscopy is sensitive, quick, clear, easy, particular for diverse kinds of nanoparticles and requires simply a small interval of

clock for conclusion and measurement, for the characterization of a colloidal suspension calibration is not needed. In silver NPs, valence band and the conduction band are much close to each other; electrons can pass liberally among these bands. These liberally-moving electrons, because of the mutual electron oscillation of silver nanoparticles with the light wave, lead to the increase in the absorption band of SPR (Das et al., 2009). The dielectric medium, chemical atmosphere and particle size are very important in the absorption of AgNPs (Das et al., 2009). On the surface Plasmon, the examination of this peak, is effectively known for a variety of metallic NPs from 2 to 100 nm in sizes (Sastry, 1998).

ANTIBACTERIAL EFFECT

The goal of our study was to use the agar well diffusion experiment to ascertain the lowest concentrations at which green-synthesised nanoparticles (BPAgNPs) and their aqueous extract (BPAqu) might prevent the growth of four disease-causing bacteria (Figure 3). According to our research, BPAgNPs were remarkably effective at eliminating every tested bacterial pathogen. With the exception of 0.03 mg/ml, which demonstrated the least inhibition and no antibacterial action (as indicated in Table 1), BPAgNPs exhibited substantial antibacterial activities at all concentrations.

INIHIBITORY EFFECT OF BPAqu ANDBPAgNPs

In the current research the antibacterial activity revealed that synthesized silver nanoparticles were very active against gram-negative bacteria i.e. *P. aeruginosa, K. pneumonia, Proteus mirabilis* and gram positive bacteria i.e. *S. aureus*. BPAgNPs showed highest activity against *Klebsiella pneumonia* (8.421±1.103mm). The moderate activity of BPAgNPs was reported against *Pseudomonas aureginosa* with inhibition zone of (7.333±0.210 mm) and lowest antibacterial activity of BPAgNPs was recorded against *Proteus mirabilis* (6.433±2.213 mm).

The results revealed that aqueous extract of common windmill butterfly (BPAqu) showed the highest activity against *Pseudomonas aeruginosa* with zone of (7.666±0.210 mm) whereas BPAqu displayed mild activity against *Klebsiella pneumonia* with zone of inhibition (6±2.00 mm) as well as BPAqu showed minimum activity against *Proteus mirabilis* (4.333±2.886 mm) respectively (Table 1). However BPAgNPs and BPAqu show no activity against *Staphylococcus aureus.* Plaza et al. (2010) and Lee et al (2012) described the tetraterpenoid nature of palmitate and the fatty nature of oleic acid showed antimicrobial activity against both Gram-positive and G-negative organisms (Plaza et al., 2010). Previous study revealed that antimicrobial activity of two different concentrations of three extracts of *Polyclinum madrasensis* has been evaluated in vitro against gram positive and negative bacteria that are isolated from infected patients showed appreciable growth inhibition to microorganisms.

ANTIBIOFILMASSAY

In our most recent research, we used a crystal violet assay to examine the impact of *Byasa polyeuctes* Aqu and *Byasa polyeuctes* silver nanoparticles on biofilm formation. The encouraging findings demonstrated that BPAqu, BPAgNPs, or chloramphenicol exposure prevented the pathogens we investigated from forming

biofilms (Figure 4). Contrary to popular belief, *Byasa polyeuctes* Aqu and BPAgNPs both revealed amazing potential for dramatically reducing biofilm development. *Byasa polyeuctes* Aqu, however, had a marginally stronger capacity to reduce biofilms than did BPAgNPs. This provides a potential strategy for resolving problems with biofilm (Table 2).

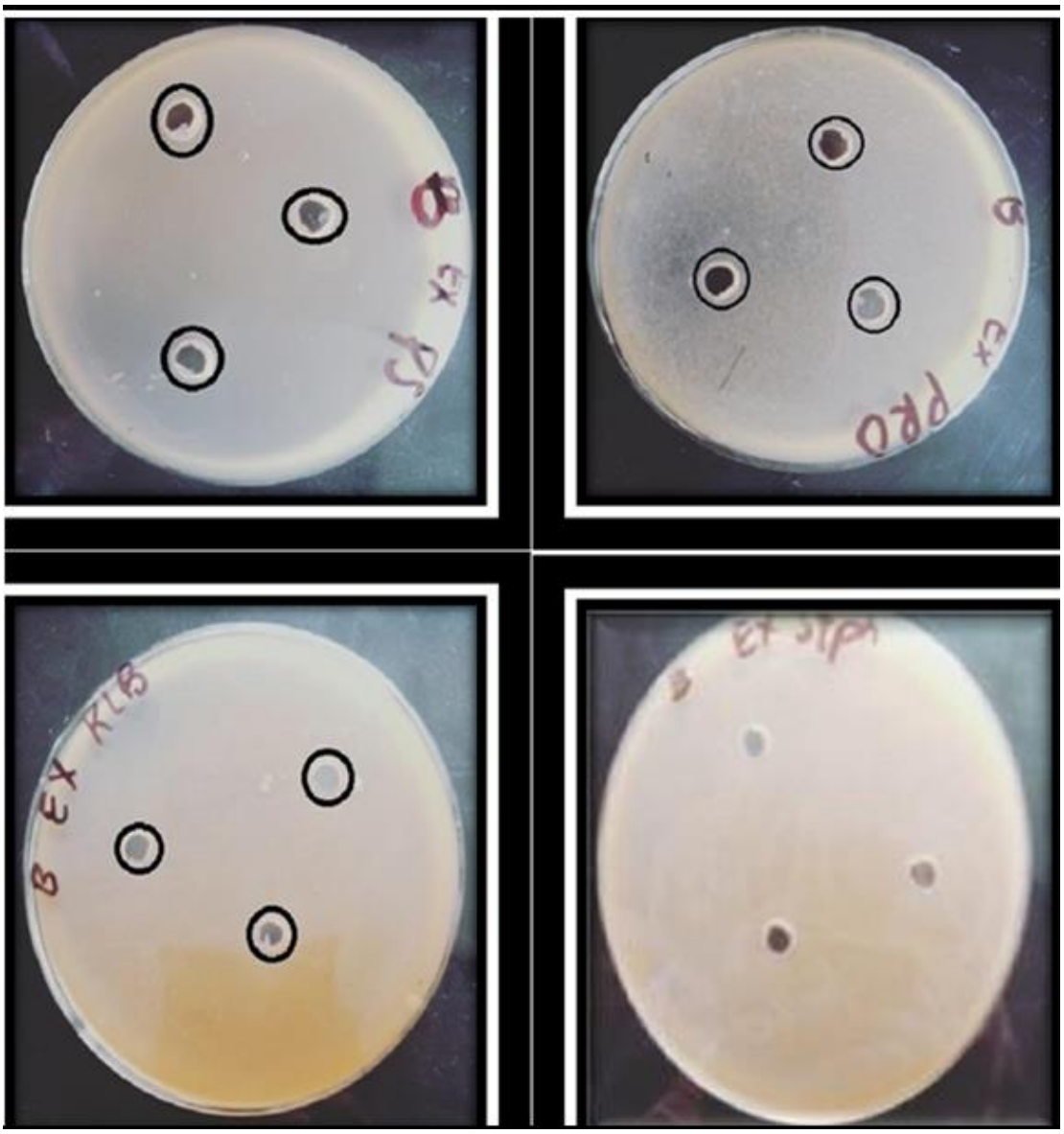

Figure 3: BPAgNPs against bacterial pathogens.

Antibiotic resistance and bacterial pathogenicity are both influenced by biofilm development, hence specific approaches are required to address this issue. The usage of foreign objects is a significant contributor to the rise of biofilm infections. Therefore, it is essential to remove the indwelling medical equipment, replace it with new, clean equipment, and administer antibiotics with caution and vigilance in order to treat biofilm-associated illnesses. Previous studies have shown that younger biofilms respond better to antibiotic treatment than older biofilms. The

most important factor in the formation of clinical disorders that are mostly associated with the mature form of biofilms is the inability to diagnose premature biofilms in the body (Hengzhuang et al., 2011).

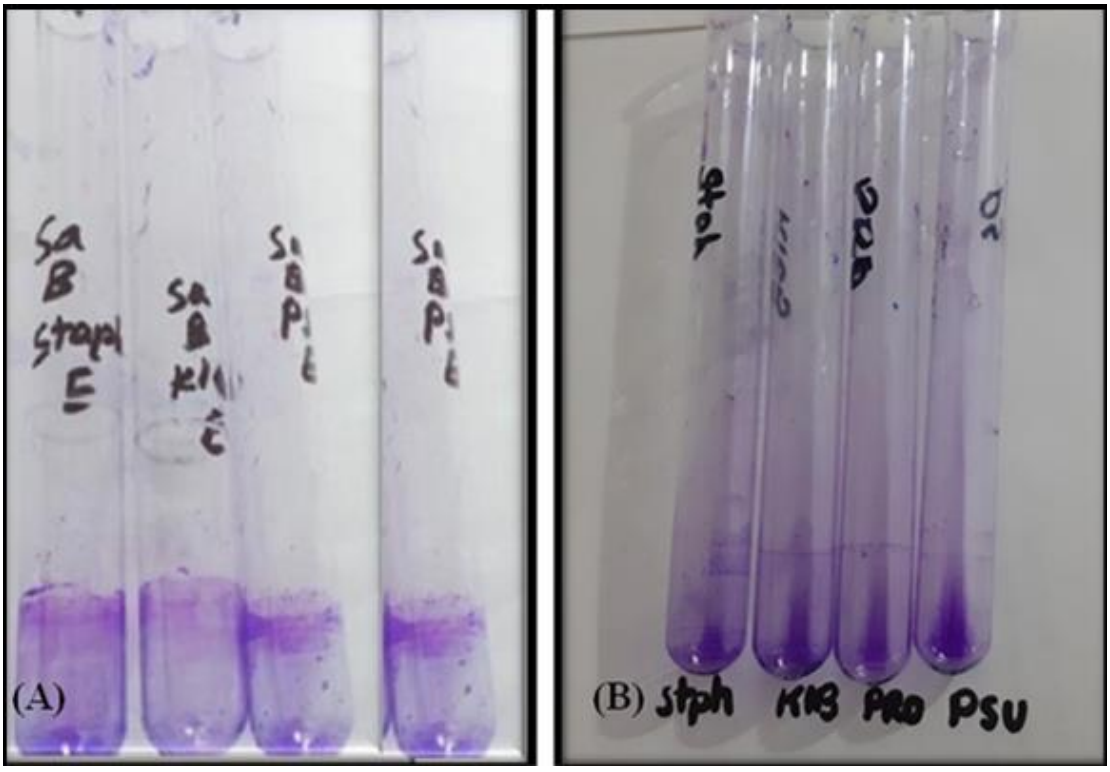

Figure 4: Antibiofilmactivity shown by BPAqu (a) BPAgNPs (b) against bacterial pathogen.

Table 1: Antibacterial activity of BPAgNPs and BPAqu against bacterial pathogens.

Test organisms	BPAqu	BPAgNPs	DMSO	Chloramphenicol (10ug)
Proteus mirabilis	4.333±2.886	6.430±2.213	-	25.00±5.00
Klebsiella pneumonia	6.00±2.00	8.421±1.103	-	21.3±3.21
Pseudomonas aeruginosa	7.666±0.577	7.333±0.210	-	17.3±0.57
Staphylococcus aureus	0	0	-	15.00±0.00

Inhibition zones were examined as zero exhibited no sensitivity, 2-6 represented as moderate sensitivity while 7-8 showed high sensitivity.

Table 2: Mean and STD of anti-biofilm activity of BPAqu and BPAgNPS against bacterial pathogens.

Test organism	Mean and STD of BPAgNPs	Mean and STD of BPAqu	Pathogen	Chloramphenicol
S. aureus	0.008±0.020	0.010±0.0212	1.45	0.12
P. aeruginosa	0.008±0.005	0.006±0.0222	1.45	0.023
P. mirabilis	0.013±0.015	0.014±0.0360	1.05	1.43
K. pneumonia	0.033±0.022	0.003±0.0324	1.542	0.027

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