International Journal of Forest Sciences (2021). 1(4):114-123

Research Paper **Open Access**

Synthesis of ecofriendly silver nanoparticles from gills of (*Cirrhinus mrigala*) and analysis its antibacterial activity and biofilm reduction assay

Sumaira Bibi[1] and Maryam Faiz[1*]

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

***Corresponding author e-mail:** maryamfaiz1996@gmail.com

SUMMARY

Nanoparticles are small particles with a diameter of 10 to 1000 nanometers. The extract of Cirrhinus mrigala was utilised to make nanoparticles. The colour altered from light color to dark brown color, showing the first appearance of AgNPs (i.e. silver nanoparticles). The nanoparticles were filtered by 15,000 rpm centrifugation for 30 minutes. Antibacterial activity was carried out three pathogens were used *Pseudomonas aeruginosa, Klebsiela pneumonia* and *Staphylococcus aureus* it was concluded that *C. marigala* AgNps showed stronger bactericidal activity compared *C. marigala* aqueous extract**.** The highest significant effective antibacterial was shown by AgNPs and extract of *C. marigala* against *Pseudomonas aeruginosa* (24 mm ± 0 mm and 21 mm ± 0 mm respectively). The principle component analysis (PCA) first two axes explained 91.375 of the variation in the sampled avian community (Component 1: 76.311 percent; Component 2: 15.064 percent).Variables loading onto Component 1 included AgNP-1 (r = 5.8427), AgNP-2 (r = -0.74325), AgNP-3 (r = 0.29089), Ext-1 (r = -1.8331), Ext-2 (r = -1.6154) and Ext-3 (r = -1.9419). AgNP and extract also loaded onto component 2 (AgNP-1: r = -0.67209; AgNP-2: r = 1.8906; AgNP-3: r = 1.5096 and Ext-1: r = -0.90355; Ext-2: r = -0.93844 and Ext-3; -0.8861).

Keywords: Ethnomedicine, Nanomedicine, Nanoparticles

Citation: Bibi, S., M. Faiz. 2021. Synthesis of ecofriendly silver nanoparticles from gills of (*Cirrhinus mrigala*) and analysis its antibacterial activity and biofilm reduction assay. International Journal of Forest Sciences. 1: 114-123.

INTRODUCTION

Nanotechnology is an exciting field of study with a wide range of applications in the community, industry, business, and homoeopathy. Nanotechnology is a non-polluting technology that allows for the creation of a wide range of environmentally benign and safe nanomaterials (Anuradha *et al.*, 2014). For the somatic and biochemical production of nanoparticles, numerous chemicals used are poisonous and cause severe ecological contamination. As a consequence, organic sources, rather than somatic and biochemical methods, may be employed to make nanoparticles for applications such as medicine transfer, optics, catalysis, bio sensing, and antimicrobial activity. By eliminating the complex process of cell culture, the use of florae for the creation of nanoparticles can be more

advantageous than employing extra organic techniques. The use of extracts of plant also lowers the rate of "nanoparticles" as compared to made by microorganisms (Esumi *et al.*, 2001; Prathna *et al.*, 2011).

From early time to recent, human beings used the different parts of animal for folklore (Riaz and Altaf, 2021) and modern pharmaceutical purpose (Akhtar, 2021; Nazir, 2021; Zainab, 2021). Different parts and products of animals like egg (Tariq, 2020), father (Adil and Tariq, 2020), fats (Ijaz and Faiz, 2021), meat (Mughal *et al.*, 2020; Haidar and Bashir, 2021), milk (Aslam and Faiz, 2020), venom (Altaf and Faiz, 2021), horn, skin, excrement and corporal secretion are use for the synthesis of well known medications (Altaf *et al.*, 2017; Altaf *et al.*, 2018; Altaf *et al.*, 2020; Saleem *et al.*, 2021).

Metallic nanoparticles have been use in an extensive application in several fields (Firdhouse and Lalitha, 2015). Specifically, as compositions forms, and dimensions, (Lee and El-Sayed, 2006) of Metallic nanomaterial's are significantly related to their somatic, biochemical, and visual properties (Liu *et al.*, 2005; Luo *et al.*, 2008), technologies based on nano-scale constituents have been subjugated in a many types of fields from chemistry to drug (Singh *et al.*, 2008; Murphy *et al.*, 2015).

Decisions have been made to investigate their remarkable qualities and put them to good use in antibacterial and anti-cancer therapies, among other things (Le Ouay and Stellacci, 2015) optoelectronics and diagnostics (Jeong *et al.*, 2015), aquatic sterilization (Gangadharan *et al.*, 2010) and other clinical and pharmacological applications (Thirumurugan and Dhanaraju, 2011). Silver has attractive substantial properties and is a "low-cost" and "plentiful natural source", yet the usage of silver-based nanomaterial has been incomplete due to their unpredictability, for example the oxidation in an oxygen-containing fluid (Wang *et al.*, 2002). Silver nanoparticles, therefore, have an unrealized prospective compared to gold nanoparticles comparatively stable (Desireddy *et al.*, 2013). The size of silver nanoparticles can be changed depending on the application. Silver nanoparticles created for medication delivery, for example, are typically larger than 100 nanometers to accommodate the amount of drug to be supplied. Silver nanoparticles may be produced into a variety of forms, including rods, triangles, rounds, octahedral, polyhedral, and other shapes, depending on their surface qualities (Heiligtag and Niederberger, 2013).

Biofilm denotes to the difficult populations of microorganisms that may be found close to a superficial or may form groups without adhering to a superficial, as understood in bacteria and microorganism (Dufour *et al.*, 2010; Ansari *et al.*, 2015) and suppressed firmly in an extracellular environment (Hawser *et al.*, 1998; Borlee *et al.*, 2010). Main objectives of the study were to synthesize silver nanoparticles, evaluate antibacterial activity of silver nanopacticales of *C. marigala* and evaluate antibiofilm assay of silver nanopacticales of *C. marigala*.

MATERIALS AND METHOD

PREPARATION OF *CIRRHINIUS MRIGALA* EXTRACT

To eliminate dust, the Cirrhinus mrigala was totally cleaned with tap water and dehydrated in the oven for a few days. With the use of a grinder, the dried

specimens were pulverised. For the extract, “50ml” of distilled water was mixed with “0.1 g” of fish powder, agitated for “20 minutes” at “60°C”, and then heated for “2 minutes”. The mixture was boiled, then lower temperature and filtered with “Whatman paper No. 1”. A sample of the “filtrate” was chosen and marked (Figure: 1).

SYNTHESIS OF SILVER NANOPARTICLES

The AgNO3 was employed as a metal source and *Cirrhinus mrigala* extract was used for nanoparticle formation. Silver nanoparticles were synthesized using the technique given, with minor alterations. While “20 mL” *Cirrhinus mrigala* aqueous extract was mixed with “5 mL” AgNO3 for this experiment “1 mM”. The mixture was warmed to “100°C” until the colour altered from light to dark brown, indicating that silver nanoparticles were forming. The nanoparticles were purified by centrifugation at “15,000 rpm for 30 minutes”. The nanoparticles were rinsed three times with distilled water before being dried overnight in an oven (Figure 1).

AGAR WELL DIFFUSIONASSAY

The agar well diffusion technique was used to determine antibacterial activity. The bacterial culture was done on nutrient broth media, and the antibacterial activity was done on Muller Hinton agar. Inoculating a strain in “25ml” of “nutrient broth medium” and at “37°C” on a rotary shaker for “24hours” activated the bacteria. At “45°C”, the overnight culture was combined with new “Muller Hinton agar media” and placed into “sterile Petri plates”. For solidification, all “Petri dishes” were held at “room temperature” in a “laminar flow”. Three 5 mm diameter wells were formed in each plate with a 1 ml sterile micropipette tip, and the agar plug was removed with a sterilised needle. Each prepared well was filled with about 30 l of extract, 30 l of manufactured nanoparticles dissolved in DMSO (Dimethyl sulfoxide), and a control solvent sample (DMSO). As a negative control, DMSO was employed, while discs of “chloramphenicol (10 g/ml)” were used as a positive control. The antibacterial action was assessed by measuring the diameter of the inhibitory zone in millimetres after “24 hours”. With a scale, the diameter of the “clear zones” around every well was estimated (Hammer *et al.*, 1999). Antimicrobial effect was graded based on the inhibition area size: insensitivity was attributed to the size “0 mm”, low sensitivity to the size “1-3 mm”, moderate sensitivity to the size “>3-6 mm”, and high sensitivity to the size “> 6-10” micrometres.

SPREAD PLATE COUNT

The bacterial colonies number on a “Petri dish” is counted using the plate approach. Each and every one pathogens were treated with silver nitrate for an overnight period at 37°C for the “spread plate count procedure”. All of the treated samples were distributed uniformly over the solidified agar the next day and kept at “37°C” whole night. Colonies were counted after a period of incubation.

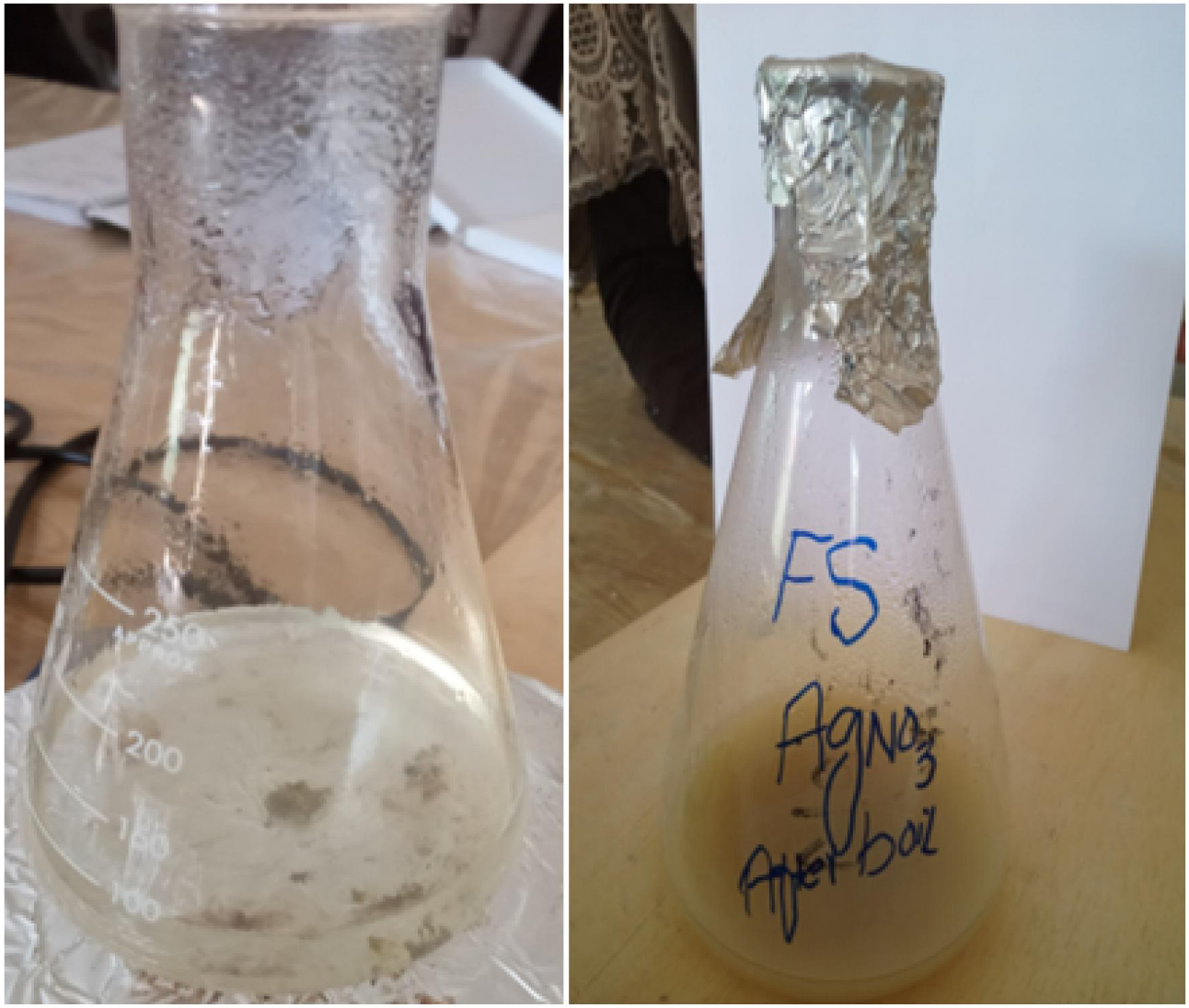

Figure 1: Color change before (CMAqu) and after the formation of CMAgNps.

CHARACTERIZATION OF NANOPARTICLES

The starting nanoparticles characterization of was completed using "UV Visible spectrophotometer". "Absorption spectra" were noted at "200 nm, 400 nm, 600 nm and 800 nm".

ANTIBACTERIAL EFFECTS OF SILVER NANOPARTICLES AND EXTRACT

Human clinical four bacterial pathogens i.e. "*Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus*" were utilized.

Antibiofilm Assay

The anti-biofilm activity was determined using a slightly modified crystal violet test. Bacteria were cultured overnight at "37°C" in "Borosilicate tubes" containing "2 ml" of nutritional broth medium, "30 mg/ml" Cirrhinus mrigala

extract, and manufactured nanoparticles. As a control, silver nitrate and chloramphenicol were utilised. Only nutritional broth was used in the negative control tubes. Following the incubation period, the broth medium was withdrawn and the adhered cells were stained with 125 l of "0.1 percent crystal violet:. To eliminate extra unattached cells and dye, "borosilicate tubes" were incubated at "room temperature" for "10-15 minutes" before being washed with water. After that biofilm staining, solubilized crystal violet with "30 percent acetic acid" and incubated for "10-15 minutes" at "room temperature". A "spectrophotometer" was utilized to note the "solubilized crystal violet" at "550 nm". The blank was "30 percent acetic acid" in H_20.

STATISTICAL ANALYSIS

The data were analysis with the help of MS Excel and Past software (version 3.0).

RESULTS AND DISCUSSION

In the present study in vitro activities of synthesized silver nanopartical (DhAgNPs) using *Cirrhinusmrigala* (ABAqu) were carried out.

Antibacterial Activity

In this work, pathogenic bacterial strains were demonstrated to have antibacterial action against green produced silver nanoparticles, as measured by the width of the inhibitory zone, but every aqueous extract of C. marigala and AgNO3 had very modest antimicrobial activities. Larger inhibitory zones were seen against "Gram-negative" and "Gram-positive" bacteria, which might be related to phytochemical constituents chemicals in the *C. marigala* extract implicated in nanoparticle production. In comparison to silver salt and *C. marigala* aqueous extract, green produced silver nanoparticles demonstrated greater bactericidal activity (Figure 2 and Table 1).

The inhibition diameter was noted both the extract and synthesized silver nenoparticles showed activity against the pathogen sued here. Four pathogens were used named *Pseudomonas aeruginosa, Klebsiela pneumonia* and *Staphylococcus aureus.* The effective antibacterial was shown by AgNPs and extract of *C. marigala* against *Pseudomonas aeruginosa*was 24 mm ± 0 mm and 21 mm ± 0 mm respectively. The silver nanoparticles and extract showed significant inhibitory effect against *Staphylococcus aureus* 22.66 mm ± 3.78 mm and 20 mm ± 0 mm respectively. AgNPs and extract of *C. marigala* also showed effective antibacterial activity against *Klebsiela pneumonia* was 21.33mm ± 0.57 mm and 21.66 ± mm. Silver nanoparticles have a higher bactericidal action cause of their huge area, that due to increase with contact with microorganisms. These outputs are very much supported with Nazir (2021) study.

Biofilm Reduction Assay

The purpose of the test was to see if Silver nanoparticles could stop microorganisms from forming biofilms. The most prevalent pathogens involved in biofilm formation, according to scientists, are Cirrhinus marigala and Staphylococcus aureus. Silver nanoparticles have the ability to disrupt harmful

bacteria' biofilms. The inhibitory effects of the produced nanoparticles were investigated utilising four clinical bacterial isolates in the current study. To produce a biofilm, test bacteria were cultured with and without silver nanoparticles (Figure 3 and Table 2). When compared to antibiotics and *C. marigala* aqueous extract, nanoparticles demonstrated a considerable reduction in biofilm development. As a result, the results of this study clearly suggest that the produced nanoparticles can be employed as biofilm breaking agents against certain bacterial pathogens (Nazir, 2021).

Figure 2: Zones of inhibition of CMAqu and CMAgNps against *Pseudomonas aeruginosa, Klebsiela pneumonia* and *Staphylococcusaureus*.

The synthesized silver nanoparticles were most effective against pathogens as compared to antibiotic used (Chlp). AgNPs were highly effective against *S. aureus*. AgNPs also showed effectiveness against *P. aeruginosa.* AgNPs showed less effectiveness against *K. Apnemunia*.

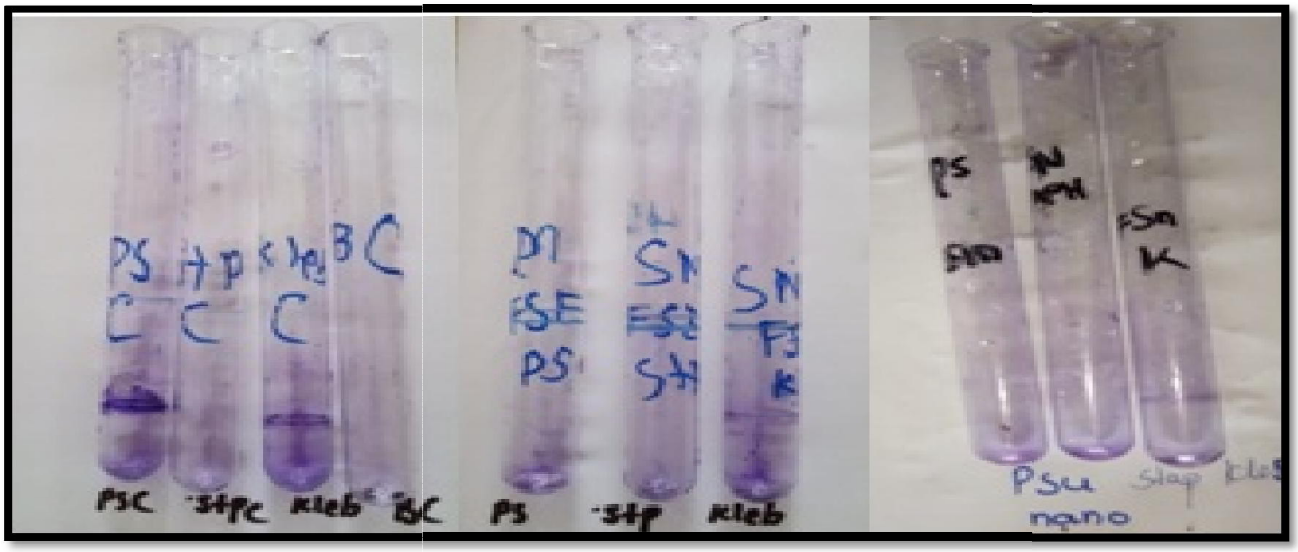

Figure 3: Assessment of antibiofilm assay of CMaqu and CMAgNps against *Pseudomonas aeruginosa, Klebsiela pneumonia* and *Staphylococcus aureus*.

PRINCIPAL COMPONENT ANA LYSIS

The first "two axes" of the "principal component analysis" explained 91.375 of variation in sampled ("component 1": 76.311 %; "component 2": 15.064 %). Variables loading onto "component 1" included AgNP-1 (r = 5.8427), AgNP-2 (r = -0.74325), AgNP-3 (r = 0.29089), Ext-1 (r = -1.8331), Ext-2 (r = -1.6154) and Ext-3 (r = -1.9419). AgNP and extract also loaded onto "component 2" (AgNP-1: r = -0.67209; AgNP-2: r = 1.8906; AgNP-3: r = 1.5096 and Ext-1: r = -0.90355; Ext-2: r = -0.93844 and Ext-3; -0.8861 (Figure 4).

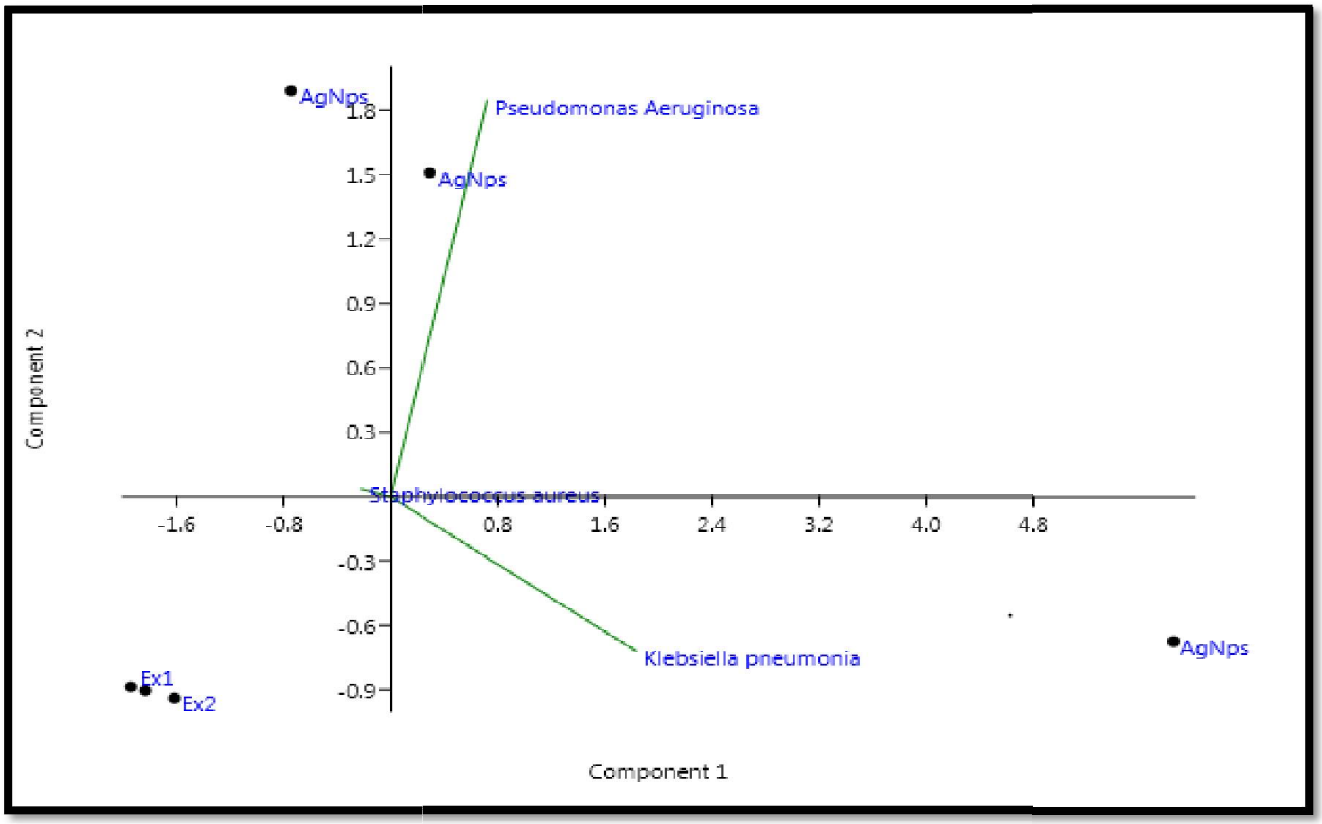

Figure 4: PCA showing the variation among pathogens efficiency.

Table 1: Antibacterial activity of CMAqu and CMagNpsExtract against *Pseudomonas aeruginosa, Klebsiela pneumonia Staphylococcus aureus*.

Test Sample Pathogen	**Silver nanoparticles**	**Extract**	**DMSO**	**Chloramphenicol (10 ug)**
Pseudomonas aeruginosa	24 mm ± 0 mm	21 mm±0mm	-	27.0 mm ±0.0 mm
Klebsiela pneumonia	21.33mm±0.57mm	21.66mm±1.52mm	-	35.0mm±0.0 mm
Staphylococcus aureus	22.66mm±3.78mm	20mm ± 0mm	-	29.0mm±0.0 mm

Table 2: Antibiofilm assay of CMAqu and CMAgNps against Chloramphenicol (CHL) was taken as positive control and broth was taken as negative control.

Test organism	CM ex	CMAgNPS	Pathogens	Chlps	Broth
Pseudo	0.025	0.013	0.044	0.0193	0.002
Kleb	0.032	0.022	0.032	0.0152	0.002
S. aureus	0.022	0.024	0.025	0.0186	0.002

REFERENCES

Adil, S., S. Tariq. 2020. Study of traditional and modern applications of feathers-a review. Journal of Wildlife and Ecology. 4: 141-150.

Akhtar, S. 2021. Antimicrobial effect of silver nanoparticles synthesized from skin of *Laudakia agrorensis*. Journal of Wildlife and Ecology. 5: 176-185.

Altaf, M., A.M. Abbasi, M. Umair, M.S. Amjad, K. Irshad, A.M. Khan. 2020. The use of fish and herptiles in traditional folk therapies in three districts of Chenab riverine area in Punjab, Pakistan. J Ethnobiol Ethnomed. 16: 1-21.

Altaf, M., M. Faiz. 2021. Snake venom-a review. Journal of Wildlife and Ecology. 5: 146-158.

Altaf, M., A. Javid, M. Umair, K.J. Iqbal, Z. Rasheed, A.M. Abbasi. 2017. Ethnomedicinal and cultural practices of mammals and birds in the vicinity of river Chenab, Punjab-Pakistan. Journal of ethnobiology and ethnomedicine. 13: 41.

Altaf, M., M. Umair, A.R. Abbasi, N. Muhammad, A.M. Abbasi. 2018. Ethnomedicinal applications of animal species by the local communities of Punjab, Pakistan. Journal of ethnobiology and ethnomedicine. 14: 55.

Ansari, M.A., H.M. Khan, A. Khan, S. Cameotra, M. Alzohairy. 2015. Anti-biofilm efficacy of silver nanoparticles against MRSA and MRSE isolated from wounds in a tertiary care hospital. Indian journal of medical microbiology. 33: 101.

Anuradha, G., B. Syama Sundar, J. Sreekanth Kumar, M. Ramana. 2014. Synthesis and characterization of silver nanoparticles from Ocimum basilicum L. var. thyrsiflorum. Eur. J. Acad. Essays. 1: 5-9.

Aslam, H., M. Faiz. 2020. Ethnopharmacological and modern applications of milk of various mammalian species-a review. Journal of Wildlife and Ecology. 4: 211-226.

Borlee, B.R., A.D. Goldman, K. Murakami, R. Samudrala, D.J. Wozniak, M.R. Parsek. 2010. Pseudomonas aeruginosa uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. Molecular microbiology. 75: 827-842.

Desireddy, A., B.E. Conn, J. Guo, B. Yoon, R.N. Barnett, B.M. Monahan, K. Kirschbaum, W.P. Griffith, R.L. Whetten, U. Landman. 2013. Ultrastable silver nanoparticles. Nature. 501: 399.

Dufour, D., V. Leung, C.M. Lévesque. 2010. Bacterial biofilm: structure, function, and antimicrobial resistance. Endodontic Topics. 22: 2-16.

Esumi, K., A. Kameo, A. Suzuki, K. Torigoe. 2001. Preparation of gold nanoparticles in formamide and N, N-dimethylformamide in the presence of poly (amidoamine) dendrimers with surface methyl ester groups. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 189: 155-161.

Firdhouse, M.J., P. Lalitha. 2015. Biosynthesis of silver nanoparticles and its applications. Journal of Nanotechnology. 2015.

Gangadharan, D., K. Harshvardan, G. Gnanasekar, D. Dixit, K.M. Popat, P.S. Anand. 2010. Polymeric microspheres containing silver nanoparticles as a bactericidal agent for water disinfection. Water research. 44: 5481-5487.

Haidar, R., S.M. Bashir. 2021. Chemical composition, traditional and modern uses of meat of animals-a review. Journal of Wildlife and Ecology. 5: 47-55.

Hammer, K.A., C.F. Carson, T.V. Riley. 1999. Antimicrobial activity of essential oils and other plant extracts. Journal of applied microbiology. 86: 985-990.

Hawser, S., G. Baillie, L.J. Douglas. 1998. Production of extracellular matrix by Candida albicans biofilms. Journal of medical microbiology. 47: 253-256.

Heiligtag, F.J., M. Niederberger. 2013. The fascinating world of nanoparticle research. Materials Today. 16: 262-271.

Ijaz, S., M. Faiz. 2021. Chemical composition, folk and modern uses of fats and oil-a review. Journal of Wildlife and Ecology. 5: 104-110.

Jeong, S.H., H. Choi, J.Y. Kim, T.W. Lee. 2015. Silver-Based Nanoparticles for Surface Plasmon Resonance in Organic Optoelectronics. Particle & Particle Systems Characterization. 32: 164-175.

Le Ouay, B., F. Stellacci. 2015. Antibacterial activity of silver nanoparticles: a surface science insight. Nano today. 10: 339-354.

Lee, K.-S., M.A. El-Sayed. 2006. Gold and silver nanoparticles in sensing and imaging: sensitivity of plasmon response to size, shape, and metal composition. The Journal of Physical Chemistry B. 110: 19220-19225.

Liu, F.-K., F.-H. Ko, P.-W. Huang, C.-H. Wu, T.-C. Chu. 2005. Studying the size/shape separation and optical properties of silver nanoparticles by capillary electrophoresis. Journal of Chromatography A. 1062: 139-145.

Luo, W., W. Hu, S. Xiao. 2008. Size effect on the thermodynamic properties of silver nanoparticles. The Journal of Physical Chemistry C. 112: 2359-2369.

Mughal, S., M. Pervaz, S.M. Bashir, S.S. Shamashad. 2020. Assessment of diversity and ethnopharmacological uses of birds in Chakar, Hattian Bala district, Azad Jammu and Kashmir -Pakistan. Journal of Wildlife and Ecology. 4: 35-44.

Murphy, M., K. Ting, X. Zhang, C. Soo, Z. Zheng. 2015. Current development of silver nanoparticle preparation, investigation, and application in the field of medicine. Journal of Nanomaterials. 2015: 5.

Nazir, N. 2021. Biosynthesis of silver nanoparticles from skin of Hazara toad (*Bufo melanostictus*) and assessment of antibacterial activity. Journal of Wildlife and Ecology. 5: 111-119.

Prathna, T., N. Chandrasekaran, A.M. Raichur, A. Mukherjee. 2011. Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size. Colloids and Surfaces B: Biointerfaces. 82: 152-159.

Riaz, T., M. Altaf. 2021. Diversity and cultural uses of mammals in Dhirkot, Azad Jammu and Kashmir, Pakistan. Journal of Wildlife and Ecology. 5: 159-167.

Saleem, R., M. Altaf, M. Umair, M.S. Amjad, A.M. Abbasi. 2021. Ethnopharmacological applications of the amphibians and reptiles among the people in the vicinity of Margalla Hill National Park, Islamabad, Pakistan. Journal of Wildlife and Ecology. 5: 13-25.

Singh, M., S. Singh, S. Prasad, I. Gambhir. 2008. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest Journal of Nanomaterials and Biostructures. 3: 115-122.

Tariq, S. 2020. Chemical composition and traditional uses of eggs of different avian species-A review. Journal of Wildlife and Ecology. 4: 45-50.

Thirumurugan, G., M. Dhanaraju. 2011. Novel biogenic metal nanoparticles for pharmaceutical applications. Advanced science letters. 4: 339-348.

Wang, S., N. Mamedova, N.A. Kotov, W. Chen, J. Studer. 2002. Antigen/antibody immunocomplex from CdTe nanoparticle bioconjugates. Nano letters. 2: 817-822.

Zainab, S. 2021. Antibacterial and antibiofilm activity of Bull frog *Hoplobatrachus tigerinus* skin extract. Journal of Wildlife and Ecology. 5: 32-37.