V.H.N. Senthikumara Nadar College (*Autonomous*),

(Accredited with 'A' grade by NAAC) VIRUDHUNAGAR– 626 001.

RESEARCH DEPARTMENT OF BOTANY

(DST-FIST Sponsored)

NOTIFICATION OF ONLINE Ph.D. PUBLIC VIVA- VOCE EXAMINATION

As per the regulations of Madurai Kamaraj University, Madurai, **Mr. A. MANIRAJ (F9513)**, Research Scholar, Department of Botany (DST-FIST Sponsored) VHNSN College, (Autonomous), Virudhunagar will defend his thesis at a Public Viva-Voce Examination through **Video Conference mode** using **Google Meet Platform**.

Title of the Thesis

"STUDIES ON THE EFFECT OF GREEN SYNTHESIZED SILVER NANOPARTICLE AGAINST DRUG RESISTANT BACTERIAL PATHOGENS"

Date & Time

03.12.2021 (Friday) at 11.00 AM

Venue

Research Centre in Botany, VHNSN College (Autonomous),

Virudhunagar-626001.

Video Conference Platform

Google Meet

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The Synopsis of the thesis is available in the College Website and a copy of the thesis is available in the Department Library, for reference. Faculty members, Scholars and Students are most welcome to attend the Viva-Voce Examination and take part in the discussion.

ALL ARE CORDIALLY INVITED

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Studies on the Effect of Green Synthesized Silver Nanoparticle against Drug Resistant Bacterial Pathogens

Synopsis submitted to the Madurai Kamaraj University,
in partial fulfilment of the requirements for the award of the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

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Synopsis

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General Introduction

The serendipitous discovery of wonder drug penicillin [1] in the year 1929 provide impetus for the finding of newer drugs, as a result, number of new antibiotics were discovered between the year 1950 and 1970. This period was golden era for antibiotics. In past 40 years, the discovery of new antibiotics becomes zero, which led us towards the post-antibiotic era. At the same time, common pathogenic microbes became resistant to existing drugs, which resulted in high rate of morbidity and mortality.

The development of drug resistance among microbes could be natural (genetic mutation) or acquired one, which spreads among microbial consortia through horizontal and vertical transfer of resistant genes or transposons. To combat the drug resistant problem, researchers explored nano strategies, which showed a greater promise and viable alternative to alleviate this emerging problem of drug resistance amongst microbes [2]. Silver nanoparticles (AgNPs) exhibited a good antimicrobial activity and less toxic to mammalian cells. For these reasons, biosynthesized AgNPs explored for treating drug resistant pathogens.

AgNPs synthesis either follow a top down or bottom up approach. In the "top down" approach, bulk material is broken down into nanoparticles by using various physical techniques whereas in the "bottom up" approaches, nanoparticles are synthesized by addition of atom by atom in a controlled manner either chemical or biological methods into nuclei which further grow into nanoscale particles. The synthesis of AgNPs is accomplished by various routes such as

chemical methods, physical methods and biological methods (Fig. 1.). Among these biological methods exhibited greater promise due to its simplicity, cost effectiveness, less toxic to mammalian cells and no involvement of toxic chemicals in the synthetic process. Hence, biological synthesis of AgNPs gain popularity to combat the drug resistant pathogens

In biological synthesis, the microorganism and plants are used for synthesis of AgNPs. In microbial synthesis of AgNPs, researchers utilized bacteria [3,4], actinomycetes [5,6] and fungi [7–9]. The biomolecules and enzymes found in the microorganism are responsible for synthesis of AgNPs [10]. Similarly, the extracts of plants/ plant parts were also employed for synthesis of AgNPs. The reduction of Ag^{+} ions into Ag^{0} and their stabilization into Ag nanoparticles using plant extracts either done by primary metabolites (carbohydrates, fats, enzymes) or secondary metabolites (terpenoids, flavonoid, tannins, steroids, phenolic, saponins, tannins and saponins) or combination of both [14]. The recent advancement in the biogenic synthesis of AgNPs is their stabilization or immobilization of AgNPs on various materials surfaces [11] to assed its antimicrobial potential against biofilm forming organisms in the biomedical implants.

Objectives of work

1. Greener synthesis of AgNPs by using microbes (*Bacillus licheniformis, Thermomonospora sp* and *Fusarium solani*) and plant (*Ficus benghalensis*).

2. To standardize the biosynthetic process of AgNPs, altering the silver ion concentration, volume of extract, pH and time.

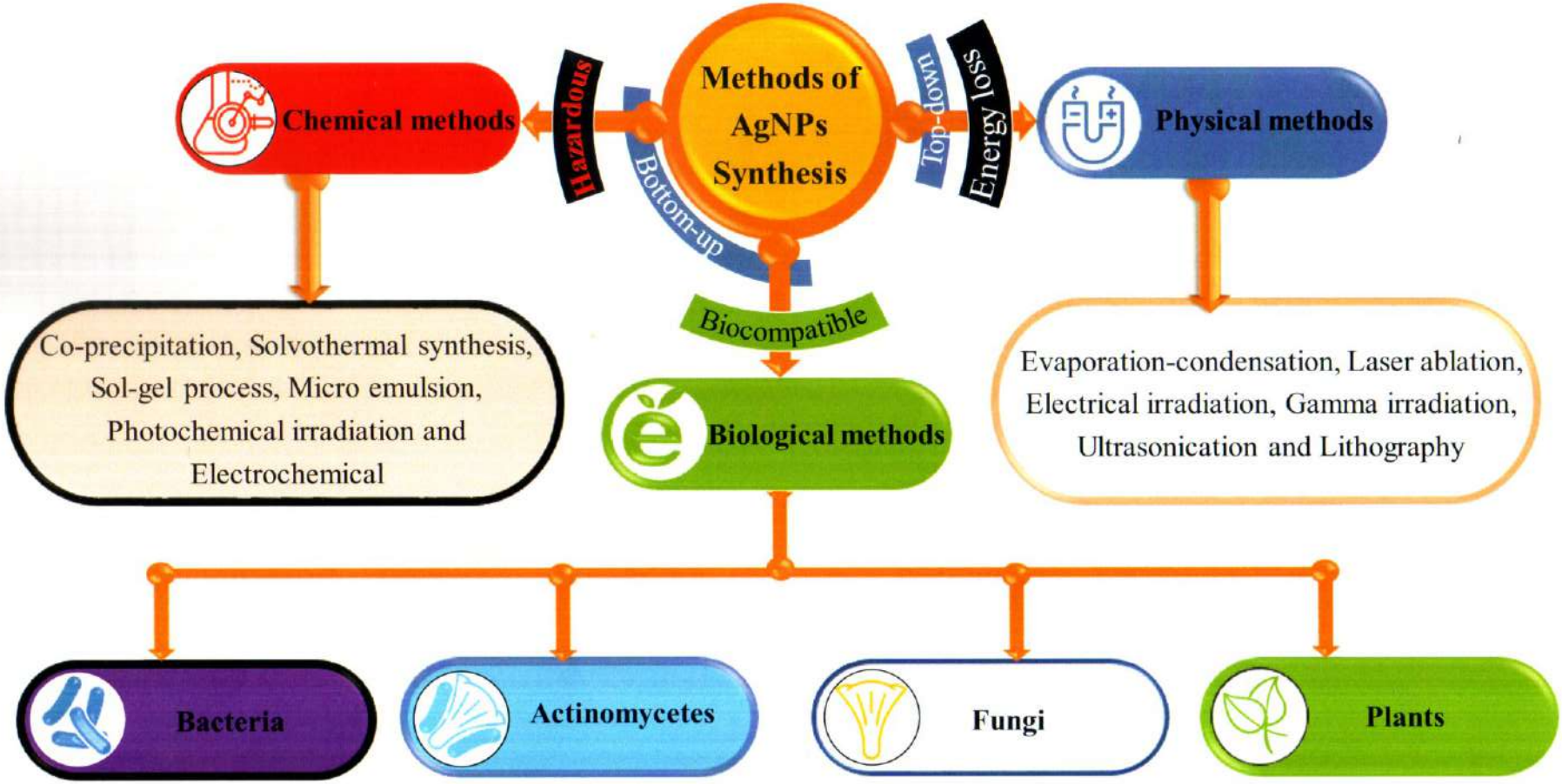

Figure. 1. Schematic representation about synthesis of AgNPs by various routes.

3. The biosynthesized AgNPs were characterized by using various spectroscopy and microscopic techniques (UV-Visible spectroscopy, FTIR spectroscopy, XRD pattern analysis, SEM with EDAX & TEM with SAED pattern images analysis and DLS studies)

4. To evaluate the antibacterial activity of AgNPs against drug resistant uropathogenic bacterial isolates from clinical samples (urine and catheter tips).

5. Functionalization of glass substrate and catheter tube with bio-fabricated AgNPs and their assessment against drug resistant uropathogenic *Proteus mirabilis*.

Review on biological synthesis of AgNPs

In general, microbes (bacteria, actinomycetes and fungi) and plants were explored for the green synthesis of AgNPs. The vast array of biomolecules (enzymes, saccharides, proteins, amino acids, vitamins, hormones and secondary metabolites: alkaloids, flavonoids, terpenoids, tannins, saponins, phenolic component and steroids) found in biological organisms responsible for the reduction of silver ion ($Ag^{+1/2/3}$) to stable AgNPs (Ag^0). These biomolecules took part in the reduction of silver ion and determine the degree of uniqueness towards its physiochemical properties. The biomolecules/ bio-constituents coated on the surface of AgNPs renders good stability as well as prevent agglomeration, which enhance the formation of AgNPs with uniform size and shape and excellent antimicrobial too (Fig. 2).

Recently, biosynthesized AgNPs was employed to combat the infectious microbial pathogens. Previous studies exhibited that AgNPs had lot of application potential in various fields, notably in biomedical application [2] such as drug delivery systems, surface modification

of catheter [12], dental implants [13], wound healing [14], bone healing and other medical applications. Many researches work on AgNPs focused at antimicrobial, prevention of biofouling, antioxidant and anticancer activity. Recently, researchers infused AgNPs with clinically relevant materials (cotton fabrics, natural and artificial fibers, thin polymer films, and wound healing) and for fabrication of AgNPs surface coated medical devices (implant and catheters), to prevent drug resistant nosocomial infections [8]. Due to the biocompatibility nature, AgNPs implanted materials help us to reduce the treatment duration and side effects of systemic treatments. Perusal of literature revealed that functionalized material surfaces proved strong inhibitory activity against biofilm forming multidrug resistant pathogens. These approaches foresee to production of newer anti biofilm materials in greener way.

Methodology to be adopted

Preparation of Bio Extracts: The microbes (***Bacillus licheniformis, Thermomonospora sp., Fusarium solani***) was inoculated in medium and it kept in a shaker incubator for 3- 4 days. The biomass was harvested from culture medium by using centrifugation and washed thrice with sterile deionized water under aseptic condition. Afterwards, microbial biomass was suspended in sterile distilled water and incubated for 2-3 days followed by centrifugation (12,000 rpm) to obtain the cell free extract. Fresh and healthy leaves of ***Ficus benghalensis*** chopped leaves were boiled with 100 ml of distilled water at 100° C for 15 min in water bath. This extract was filtered through nylon mesh, followed by Whatman No. 1 filter paper.

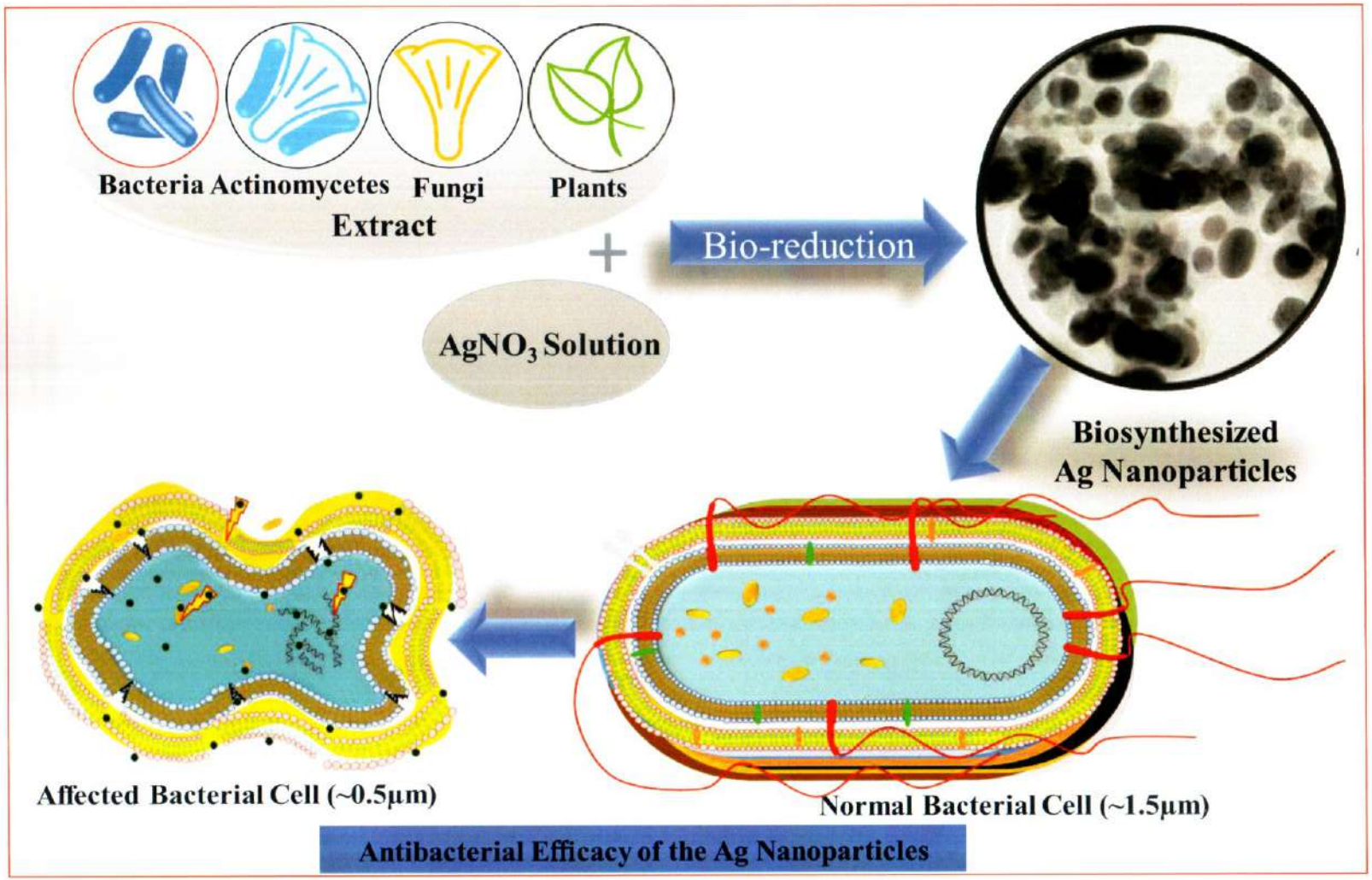

Figure. 2. Proposed bactericidal mechanisms of biological synthesized AgNPs.

Synthesis of Biogenic AgNPs: The required amount of $AgNO_3$ was added to pure distilled water prior to experiments. Further, the prepared $AgNO_3$ solutions were mixed with the respective bio-extracts (bacteria, actinomycetes, fungi and plant) at ambient condition, which effectively resulted in the formation of AgNPs.

Optimization of Experimental Conditions: The concentration of $AgNO_3$ solution, volume of bio-extracts pH and time were optimized for above mentioned life forms (bacteria, actinomycetes, fungi and plant) in this study.

Characterization Techniques of AgNPs: Ultraviolet - Visible Spectroscopy, Fourier Transform Infrared (FT-IR) Spectroscopy, Powder X-Ray Diffraction (XRD), Scanning Electron Microscopy with EDAX (SEM), Transmission Electron Microscopic (TEM) and Dynamic light scattering (DLS) and zeta potential studies.

Antibacterial Activity Studies: To evaluate the antibacterial activity of AgNPs by using Turbidimertic assay and Agar Well Diffusion Method against drug resistant uropathogenic bacteria isolates from clinical samples.

Functionalization of Glass Slide and Catheter with AgNPs: Green synthesis of AgNPs and their effective utilization for fabrication of AgNPs over the surface of glass slide and catheter tube to explored its antimicrobial potency against the drug resistant nosocomial uropathogens. The major nosocomial uropathogenic infections are caused by Gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis* and Gram-positive bacterium *Staphylococcus aureus.* These organisms have the capacity to form biofilms on biotic and abiotic surfaces and provide 1000-fold enhanced resistance to antibiotic agents. Hence, it poses greater difficulty in eradication. *Proteus mirabilis*

is one of the predominant biofilms forming microorganisms causes Catheter associated Urinary Tract Infections (CaUTIs), which enhance morbidity and mortality in humans. To alleviate CaUTIs, catheter tubes are coated with AgNPs as an alternative way to reduce the infection. Hence, the present work is carried out for Functionalization of glass substrate and catheter tube with bio-fabricated AgNPs and their assessment against drug resistant uropathogenic *Proteus mirabilis*.

Results

Green synthesis of AgNPs was successfully achieved by using microbes such as *Bacillus licheniformis, Thermomonospora* sp, *Fusarium solani* and plant *Ficus benghalensis* to produce BL-AgNPs, T-AgNPs, FS-AgNPs and FB- AgNPs respectively. For each biogenic process, the reaction kinetics was investigated and optimized the synthesis of crystalline AgNPs by varying the parameters such as Ag ion concentration, amount of leaf extract, pH of the reaction mixture and the time by using UV–Vis spectroscopy. Further, biosynthesized AgNPs were characterized by following techniques such as Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD) pattern, Scanning electron microscopy (SEM) with Energy dispersive spectroscopy (EDAX) and Transmission electron microscopy (TEM) with Selected area (electron) diffraction (SEAD) pattern and Dynamic light scattering (DLS).

The first technique employed after successful biogenic AgNPs was UV-Vis spectroscopy. The UV absorption peak found to be 416 nm, 435 nm, 430nm and 421-422 nm respectively for BL-AgNPs T-AgNPs, FS-AgNPs and FB- AgNPs, which indicate the surface plasmon resonance of AgNPs. FTIR band analysis of all biogenic AgNPs indicated the involvement of various functional group(s) of biomolecules (carbohydrates/ proteins/ amino acids) as reducing, capping

and stabilizing agents. The mean crystal size of all biogenic AgNPs was calculated by using XRD, SEM, TEM and DLS. The values were shown as follows: XRD- 11.24, 13.7 nm, 12.82 nm and 22.8 nm; SEM- 19.53, 17.01, 22. 2 and 27.77 nm; TEM- 17.36, 14.17, 20.23 and 28.69 nm; DLS-37. 33, 50.40, 44.26 and 60 nm; respectively for BL-AgNPs, T-AgNPs, FS-AgNPs and FB-AgNPs. The shape of BL-AgNPs was quasi-spherical in nature, whereas T-AgNPs, FS-AgNPs and FB- AgNPs were found to be spherical in shape. EDAX result revealed that all biogenic AgNPs were composed of 77-85% of silver and remaining elements represented by oxygen, carbon and other organic peaks. The FB-AgNPs had more percentage of silver than the others biogenic AgNPs. The crystalline nature of all biogenic AgNPs was confirmed by SEAD pattern. Hydrodynamic diameter and Zeta potential were exhibited by DLS, FB-AgNPs exhibited larger crystal size and greater zeta potential and confirms the excellent stability than the other biogenic AgNPs.

All the biogenic (BL-AgNPs, T-AgNPs, FS-AgNPs and FB- AgNPs) AgNPs exhibited strong antimicrobial activity, as well as enhanced synergistic activity with streptomycin, against multi drug resistant Gram-positive ((*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus* and *mirabilis*) and Gram-negative (*Staphylococcus aureus*) bacterial pathogens. Due to the proven ability of the biogenic AgNPs as an antimicrobial and antibiofilm agent, it was further explored for functionalization of glass slide (Fig. 3) and urinary catheter

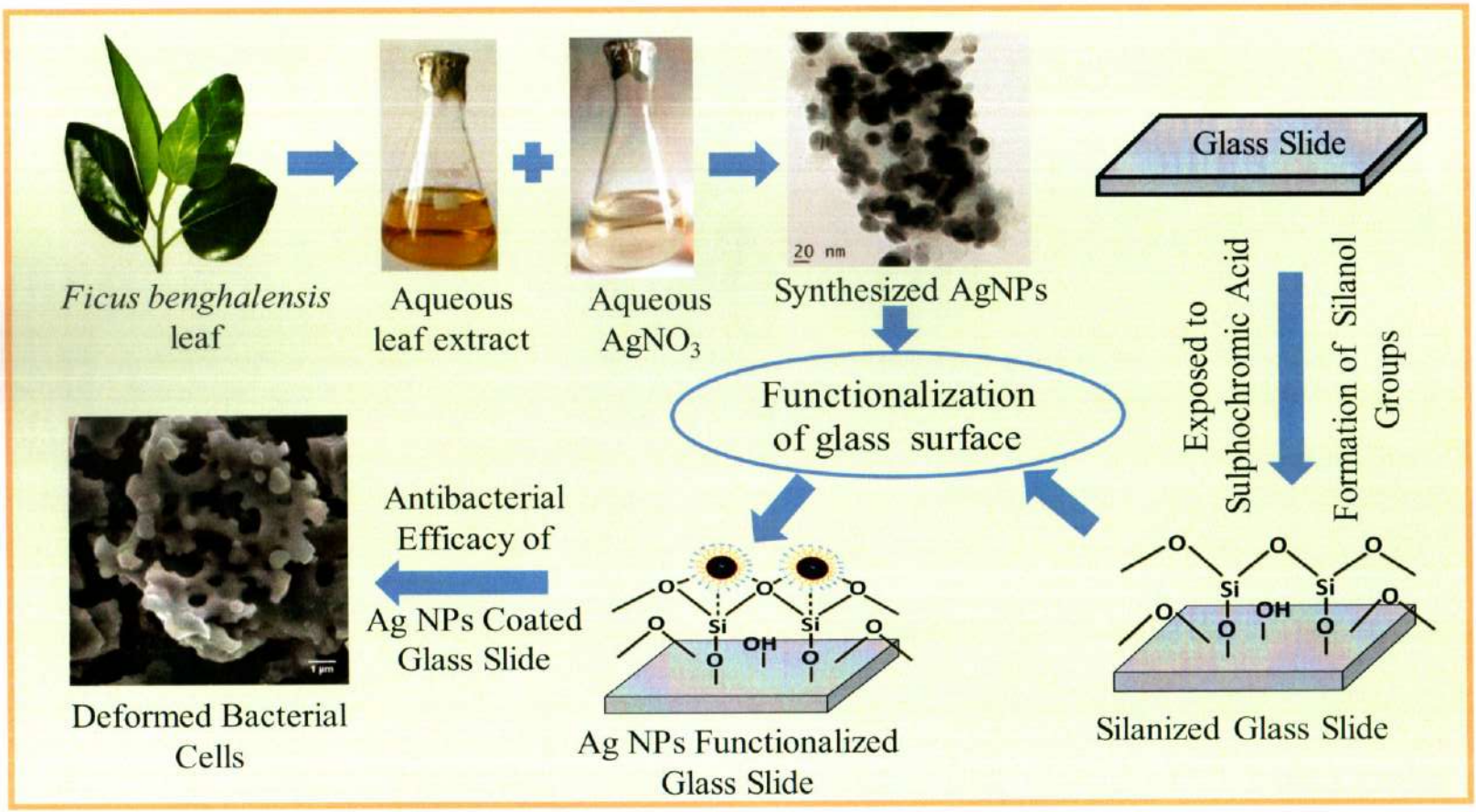

Figure. 3. Schematic representation of Functional Surface processes involved in the salinization and functionalization of glass slide with FB-AgNPs and Antibacterial Activity against clinical isolated Multi-Drug Resistant *Proteus mirabilis*.

surface. A review of physiochemical characterization and antimicrobial potential of biogenic AgNPs revealed that FB-AgNPs found to be a potential candidate especially combat the drug resistant Proteus mirabilis. Hence, FB-AgNPs was used for fabrication of antibiofilm surfaces. The FB-AgNPs was functionalized over the glass and silicone catheter to evaluate its anti-biofilm potential. FB-AgNPs functionalization of glass slide and urinary catheter surfaces were confirmed by using UV-Vis spectroscopy and SEM-EDAX analysis.

Biogenic synthesized FB-AgNPs showed excellent antibiofilm potential against predominant UTI infection causing *Proteus mirabilis*. Inspection of SEM images of pristine (glass and catheter) and functionalized surfaces showed that pristine surfaces had luxuriant growth of rod-shaped bacteria with well desired morphology whereas scanty growth and deformed bacterial cells found on the functionalized surfaces. Present study demonstrated a facile approach for synthesis of biogenic AgNPs and further utilization of biogenic AgNPs for functionalization of surfaces (glass and catheter surface with FB-AgNPs) in order to fabricate the antibiofilm surfaces. Functionalized surfaces exhibited impressive results against one of the prominent biofilm former *Proteus mirabilis*. This study could be used in future for further *in vivo* investigation and further scaling up to commercialization.

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