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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MILLETS, ITS CHARACTERIZATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY

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ABSTRACT: The development of Nanotechnology has paved the way to study various nanomaterials. Nanoparticles are structures that measure the size from 1-100nm. Widely used nanoparticles are usually prepared using noble metals, that is, gold, silver, palladium, and platinum. At the same time, they find their way into a wide range of applications in medicine, electronics, energy saving, environment, textile, cosmetics, biomedical, *etc*. The natural reducing agents in plants, such as carbohydrates, flavonoids, *etc*., reduce the silver, generating potential silver nanoparticles. Silver nanoparticles are intended to be used for the present study generated through green synthesis using millets. Millets are small-seeded grasses that are hardy and grow well in dry zones as rain-fed crops under marginal conditions of soil fertility and moisture. The different varieties of millets intended to be used in the project are Barnyard millet, Finger millet, Foxtail millet, Kodo millet, little millet, Pearl millet, Prosomillet, Sorghum. The Silver nanoparticles synthesized through an aqueous extract of different varieties were checked for their quality using UV-VIS spectrophotometer and Scanning electron microscopy. The nanoparticles peaked in the range of 410nm-430nm, and the average diameter below 50nm confirmed the presence of silver nanoparticles. Thus, synthesized silver nanoparticles were checked for antimicrobial activity by exposing them to *E. coli, P. vulgaris, A. niger* and *T. viridae.* The silver nanoparticles were confirmed to have an effective antimicrobial activity against the mentioned microorganisms.

INTRODUCTION: In the recent past, silver nanoparticles (AgNps) have received enormous attention from researchers due to their extraordinary defense against a wide range of microorganisms and the appearance of drug resistance against commonly used antibiotics. The AgNPs have various applications in various fields like drug delivery, water treatment, agriculture etc. AgNps are widely used and applied in pastes, inks, adhesives, electronic devices, etc., due to high conductivity.

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AgNps have been synthesized using chemical reduction, gamma ray radiation, micro emulsion, electrochemical method, laser ablation, autoclave, microwave and photochemical reduction techniques. These methods have effective yields but are associated with limitations in using toxic chemicals and high operational costs and energy needs.

Considering the drawbacks of physio-chemical methods, cost-effective and energy-efficient alternatives for AgNP synthesis using microorganisms, plant extracts, and natural polymers as reducing and capping agents are emerging very fast. The synthesis of AgNP by biological entities is due to the presence of a large number of organic chemicals like carbohydrates, fat, proteins, enzymes & co-enzymes, phenols,

flavonoids, terpenoids, alkaloids, gum, etc, capable of donating electrons for the reduction of Ag+ ions to Ag0. The term Nanotechnology" was coined by Prof. Erie E. Drexler, the word "Nano- is derived from a Greek word for “dwarf” The horizon of Nanotechnology is broadened with its potential of conjugating with biomolecules for numerous applications such as drug delivery, biosensing *etc*. and outstanding optical properties about localized plasmon resonance. The size reduction of the particles gives the munique properties, unlike the bulk materials, such as the generation of new physical, chemical, and biological properties, flow characteristics, magnetic and thermodynamic properties, *etc*. Nanoparticles are broadly categorized as organic and inorganic nanoparticles. The organic division comprises carbon nanoparticles, whereas magnetic and noblemetal nanoparticles come under inorganic division. Noble metal nanoparticles like gold and silver nanoparticles are gaining increasing interest due to their versatile functionality. Nanoparticles exist in different forms, namely Nanopores, Quantum dots, Nanoshells, Nanotubes, Fullerenes, Nanospheres, Nanowires, Nanobelts, Nanorings, Liposomes, Nanocapsules, Nanorods, Dendrimers [1].

Silver Nanoparticles: Silver nanoparticles are synthesized from silver salts through a reduction reaction. Silver nanoparticles have gained popularity among researchers and have been a major research subject by their unique chemical and physical properties. It can be synthesized through physical, chemical, microemulsion, and biological methods [2].

Physical Methods: The para-amount physical approaches for synthesizing silver nanoparticles are laser ablation and evaporation-condensation. Examples include the use of tube furnaces at atmospheric pressure. Thus, generated silver nanoparticles are used for long-term inhalation toxicity studies [3].

Chemical Methods: The process of chemical reduction by inorganic and organic reducing agents is the most common approach for synthesizing silver nanoparticles. Reducing agents such as sodium citrate, N- N-dimethyl formamide (DMF), ascorbate, sodium borohydride (NaBH4), polyol process Tollen’s reagent, elemental hydrogen and are used for reduction of silver ions (Ag') in non-aqueous and aqueous agglomeration into oligomeric clusters is followed. Colloidal silver particles are formed eventually from these clusters.

Biological Methods: Similar to chemical reduction, biological methods involve the reducing agents from biological sources such as plant extracts, microbes, algae, etc. This is considered an eco-friendly and cost-efficient approach. Green synthesis refers to using plant extracts containing reducing agents such as phenols, nutrients, etc. Any plant part can be considered for the synthesis. With its slower reaction kinetics, the biological approach provides better control over crystal growth, manipulation, and stabilization [4].

The size and shape of synthesized silver nanoparticles depend on various physical and chemical parameters such as reaction temperature, pH ion concentration, and duration of the reaction contents in the biological extracts [5].

Millets: Millets are essentially small-seeded grasses that are hardy and grow well in dryzone under marginal soil fertility and moisture conditions. One of the oldest foods known to humans is millet. Millets are probably the first cereal grains to be used for domestic purposes. Semi-arid tropics of Asia and Africa have 97% of millet produced in developing countries. The crop is favored under dry, high-temperature conditions due to its short growing season and productivity. India is the largest producer of millets in the world, harvesting about 11 million tons per year which amounts to 40% of world's output. Millets possess 2% crude fibre. 60-70% carbohydrates, 15-5% fat and 7-11% proteins. They are excellent sources of vitamin B, antioxidants and magnesium. Millets are also a good source of other dietary minerals like manganese, iron and phosphorus. The different varieties of millets intended to be used in the project are finger millet proso millet, kodo millet, barnyard millet, little millet, pearl millet, foxtail millet, sorghum [3-4]. Their scientific names and appearance are shown in **Table 1.**

Foxtail Millet: The common name of *Setaria italica* is Foxtail millet, and is cultivated in Andhra Pradesh and Tamil Nadu. It is usually grown for its grains and also cultivated as a fodder plant. With

dense root system and thin adventitious roots, it grows up to 220m high. Studies on *Setaria italica* show that it has Antihyperglycemic hypolipidemic, Cytotoxic, antioxidant hypoglycemic, Anti-lipase Hepatoprotective, and Anti-inflammatory activities. It also is an appetite stimulant and is used for the treatment of sexual diseases [5].

Kodomillet: *Paspalum scrobiculatum* is commonly known as Kodomillet. It is draught resistant and can be grown in any poor soil, majorly grown in Deccan Plateau. It is rich in high-quality protein and possesses high anti-oxidant activity compared to other millets. It is also rich in fiber and hence it may be beneficial for diabetic patients [6].

Barnyard Millet: This millet is classified into two species belonging to genus Echinochloa. *Echinochloa esculenta* is cultivated in Japan, north-eastern part of China and Korean Echinochloa jrumentacea is found in Pakistan, central Africa, India and Nepal. This millet grows on poor soil and has fast maturity and high storability. It does not contain glut an. Indian barnyard millet, usually grown in the Himalayan region, contains anti-feed ant sat concentrations higher than in rice [6].

Little Millet: *Panicum sumatrense* is commonly called as little millet and is cultivated in India, Srilanka, Myanmar, Pakistan, and other South East Asian countries. Little millet is rich inphenolic. acids, tannins, flavonoids, and phytate. It is pest, salt, and drought-tolerant [7].

Proso-Millet: The scientific name for Prosomillet is *Panicum miliaceum*. Initially domesticated in China, it has spread to European countries, Pakistan and India. Studies have shown that Prosomillet has antiproliferative properties against human breast cancer [8].

Pearl Millet: *Pennisetum glaucum*, commonly known as Bajraor Pearl millet, is cultivated primarily in Asia and Africa. It has abund antiron and zinc. It also contains high amount of antioxidants. Pearl millet has potential health benefit s in combating diseases and conditions like anemia, constipation, cancer and diabetes [9].

Finger Millet: Finger millet is scientifically named as *Eleuine coracana* and popularly knownas Ragi. It is extensively consumed in Karnataka and Andra Pradesh. It is one of the best sources of calcium and iron. Finger millet exhibits multi-microbial and antioxidant properties. In addition, studies have shown that it has phenolics that are inhibitors of aldose reductase and snake venom phospholipases. It also contains protein glycation inhibitors which help in the control of diabetes. Finger mill *et al.* so exhibit wound healing and cholesterol-lowering properties [10].

Jowar: Sorghum bicolour is commonly known as Jowar. It is one of the major cereal crops which is cultivated worldwide. Sorghum is also used for feed and animal fodder, building material, and fencing Sorghum has been studied for its high anti-inflammatory and anti-colon cancer activities. Sorghum is also utilized as a renewable energy source for biofuel production [11].

TABLE 1: DIFFERENT TYPES OF MILLETS WITH THEIR COMMON AND SCIENTIFIC NAMES

Common name	Scientific name	Image
Finger millet	*Eleusine coracana*	
Pros millet	*Panicum mifiaceum*	
Pearl millet	*Pennie twnglaucum*	

Foxtail millet	*Setaria italica*	
Jowar	*Sorghum bicolor*	
Little millet	*Panicum sumatrense*	
Kodomillet	*Pasalum scrobiculatum*	
Barnyard millet	*Echinochloa jrumenlacea*	

Millets are the forgotten foods that need to be brought back and cultivated in large numbers as it has the major advantage of very less consumption of water when it comes to cultivating it. Thus, millets have been in corporate as a medium to generate silver nanoparticles efficiently using their high phytochemical content. Not much work has been done on utilizing millets as reducing agents. Hence, a comparative study has been undertaken in the present study using eight varieties of millets available in India. The project aims to stand ardizeaprotocol for producing silver nanoparticles from any millet and using synthesized silver nanoparticles as ananti-microbial agent in various applications.

Silver nanoparticles find their applications as an antimicrobial agent in various commercial products such as air sanitizers, pillows, respirators, wet wipes, socks, detergents, soaps etc. The mechanism of action of silver nanoparticles on microbes that cause the antimicrobial effect is not known. Silver nanoparticles anchor the microbial cell wall, penetrate through it, and subsequently cause structural changes like modifications in the permeability of the cell membrane. This action ultimately causes cell death [12]. The generation of free radicals by the silver nanoparticles has been suggested as another mechanism of antimicrobial action where these free radicals make the cell membrane porous, leading to cell death [13]. The generation of silver ions by the silver nanoparticles interacts with many vital enzymes and inactivate them, invariably causing cell death [14]. Interference of silver nanoparticles with sulphur and phosphorus of DNA can result in complications in DNA replication process which in-tum leads to cell death [15].

Signal transduction inhibition in cert gram-negative bacteria is induced by silver nanoparticles through dephosphorylation of peptide substrate son tyrosine residues, thereby leading to stoppage of cell growth [16]. The present study aims at the green synthesis of nano from an aqueous extract of millets, characterization of synthesized nano by Uv-Vis spec and SEM, and evaluation of antibacterial and antifungal activity of synthesized nano through disc diffusion method.

MATERIALS AND METHODS: The millets were purchased from a local grocery store in Bangalore. The lab reagents were procured from Sigma Aldrich. Green Synthesis of AgNps 1 mM silver nitrate solution was prepared with double distilled water. 10% millet extract was prepared using double distilled water. The extract and silver nitrate solution was mixed in the ratio 1:5 and incubated for 24 hrs.

This was then centrifuged and air dried to obtain silver nanoparticles in powdered form. UV-Vis Spectrophotometric and SEM characterization UV-Vis spectrometer from Thermo is her Scientific was used and SEM was done at Centre for Nanosciences, IISC, Bangalore. Evaluation of Antimicrobial activity of AgNps.

The synthesized silver nanoparticles were checked for antimicrobial activity byexposing them to *E. coli*, *P. vulgaris*, *A. niger* and *T. viridae* using disc diffusion method. Nutrient Agar and Potato dextrose Agar were used for Antibacterial and Antifungal assays, respectively.

Source of Plant Material: The millets chosen for the study were of eight varieties brought from grocery stores in Bangalore. The varieties. Included *Paspalum scrobiculatum* (Kudomillet), *Panicum miliaceum* (Prosomillet), *Penniselum glaucum* (fingermillet), *Echinochloa frumenlacea* (Barnyard millet), *Pamcum sumalrense* (Little millet), *Eleusme coracana* (Fingermillet), *Sorghum bicolour* (Jowar), *Eraria italica* (Foxtail millet).

Source of Microorganisms: The test microorganisms used were bacteria: Bacillus Subtilis, Proteus vulgaris and fungi: Aspergillus niger, Trichoderma viride. These bacteria and fungi were obtained from the Indian Institute of Science (Bangalore, India).

The culture of bacteria and fungi was maintained on nutrient agar and potato dextrose agar slants at 4°C throughout the study and used as stock culture.

Source of Chemicals and Media: Silver nitrate and Luria broth were procured from Hi-Media Laboratories Pvt. Ltd. Mumbai, India. Agar Agar, Folin Ciocalteu's phenol reagent, and Sodium carbonate were procured from sdfine-chem Ltd, Mumbai, India., Sisco Research Laboratories Pvt. Ltd., Mumbai, India, Fisher Scientific, Mumbai, India, respectively.

Instruments and apparatus used: The instruments and apparatus used in the lab for the study are: Electronic balance measuring cylinders, pipettes, beakers, conical flask, spatula, glass rods, autoclave, hotairoven, refrigerator, pH meter, laminar air flow, boiling water bath, UV• VIS Spectrophotometer, Scanning Electron Microscope (at Indian Institute of Science).

Preparation of Aqueous Extract of Millets: To prepare the aqueous extract, 10g of each millet type was added to 100 ml of double distilled water and heated to boilina. Microwave oven. This was then cooled and centrifuged for10 min at 5000 rpm at room temperature. The supernatant as collected and filtered and used.

Green Synthesis of Silver Nanoparticles: The process was done with two variables: concentration of silver nitrate solution and the ratio of volumes of aqueous solutions of chosen millets to silver nitrate solution. The concentrations of silver nitrate in aqueous solutions were lmM, 3mM and 5mM.

To each working concentration of silver nitrate solution prepared, volumes of aqueous extracts of chosen millets to silver nitrate solution were mixed in the 1:1, l:2, and l:5 ratio. The incubation time for the process was chosen from literature which was 24 hrs.

After 24 hrs, the solutions were centrifuged for 30min at 8000 rpm in 4°C. The supernatant was discarded, and the pellet was dried for 24 hrs at 50 °C in hot air oven. Thus obtained pellet was ground in motor pestle to a fine powder and used for characterization and antimicrobial screening.

Characterization of Synthesized Silver Nanoparticles: The Silver nanoparticles were characterized using a UV-VIS spectrophotometer (from Thermo Fisher Scientific Pvt. Ltd) and Scanning Electron Microscopy (Indian Institute of Science, Bangalore). The expected peaking of silver nanoparticles in the spectrometric graph between 410nm and 430nm was checked using a UV-VIS spectrophotometer. The Scanning Electron Microscopy determined the size of the silver

nanoparticles. The expected size of then a noparticle was below 60nm.

Estimation of Total Phenol Content in Aqueous Extract of Millets: The total phenol content in the aqueous extract of millets was determined by spectrophotometric method. The reaction mixture was prepared by mixing I ml of aqueous extract of millet, 0.5 ml of in Folin-Ciocalteu's reagent and lml of 5% Sodium carbonate solution. This was then incubated in dark at room temperature for 5min and the volume was made-up to l0ml using double distilled water. The absorbance was measured using a spectrophotometer at 725nm. The above procedure was repeated for the standard solution of 10% Gallic acid, and the calibration graph was plotted. The concentration of total phenols in the aqueous extract of millets was measured. Using 10% Gallic acid as a standard graph and expressed in terms of Gallic acid equivalents.

Determination of Antimicrobial Activity of the Synthesized Silver Nanoparticles: The bactericidal activity of the silver nanoparticles was determined using disc diffusion method against bacteria (*Proteus vulgaris*, *Bacillus subtilis*) and fungi (*Aspergillus niger*, Trichodermaviride). The culture medium is prepared per the manufacturer's protocol and autoclaved before use. The pure cultures of bacteria and fungi were swabbed on nutrient agar and potatodextrose agar plates; respectively. The synthesized silver nanoparticles were smeared in discs prepared u sing Whatman filter paper. Niacin 50mcg and Nystalin 50mcg antibiotic discs were used as a positive control for bacterial and fungal culture plates, respectively. The bacterial culture plates were incubated at 3°C for 24 hrs, and the fungal culture plates were incubated at room temperature for 5 days. The culture plates were carefully observed for the clear zone of inhibition.

RESULTS AND DISCUSSION:

Green Synthesis of Silver Nanoparticles: Aqueous extracts of chosen millets were prepared as required and arc shown in **Fig. 8**. The silver nanoparticles were synthesized according to the said protocol with different ratios of Silver Nitrite to aqueous extract of millets and at different concentrations of Silver Nitrate. The color change from yellow to reddish dark brown, as noticed in all the reaction mixture, indicates silver nanoparticle production. The silver nanoparticles were formed at all the chosen conditions which are shown in **Fig. 9** and 10. The Silver Nitrate concentration of 1mM and 1:2 ratio of aqueous extract of millets to Silver Nitrate gave the best spectrometric graphs for all the millet types. Hence it could be concluded that these two above conditions could be used for generating silver nanoparticles, utilizing aqueous extracts of any type of millet.

Characterization of Silver Nanoparticles:

UV-Vis Spectroscopy: The silver nanoparticles generated under the different explained conditions were initially characterized by UV-Vis spectroscopy in the range 350 mn to 700mn. The resulting spectrometric graphs **Fig. 2, Fig. 3, Fig. 4, Fig. 5**, **Fig. 6** and **Fig. 7** indicated peaking between 410nm and 440nm due to the characteristic surface plasmon resonance caused by silver nanoparticles.

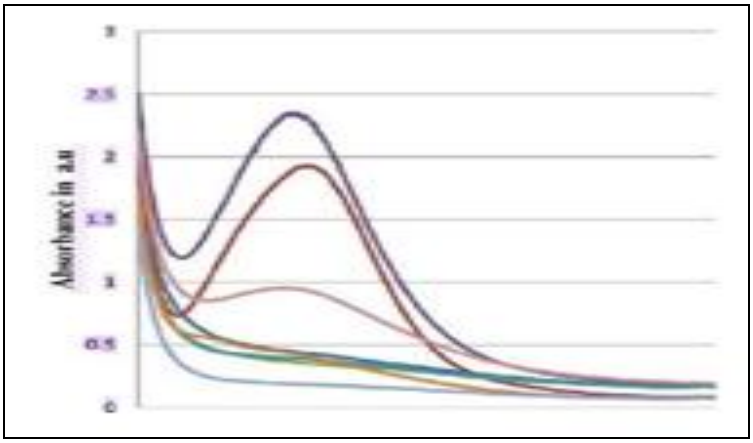

FIG. 1: UV-VIS SPECTRUM OF GENERATED SILVER NANOPARTICLES USING LMM SILVER NITRATE SOLUTION

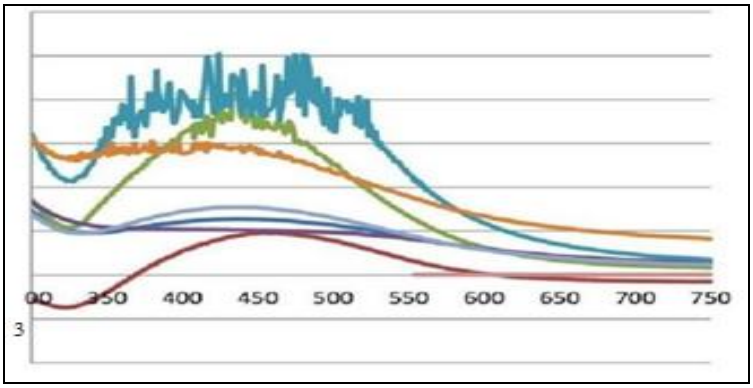

FIG. 2: UV-VIS SPECTRUM OF GENERATED SILVER NANOPARTICLES EMPLOYING 3MM SILVER NITRATE SOLUTION

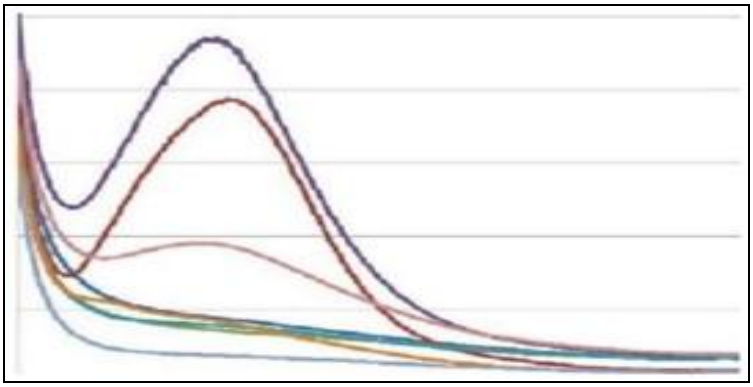

FIG. 3: UV-VIS SPECTRUM OF GENERATED SILVER NANOPARTICLES EMPLOYING 5MM SILVER NITRATE SOLUTION

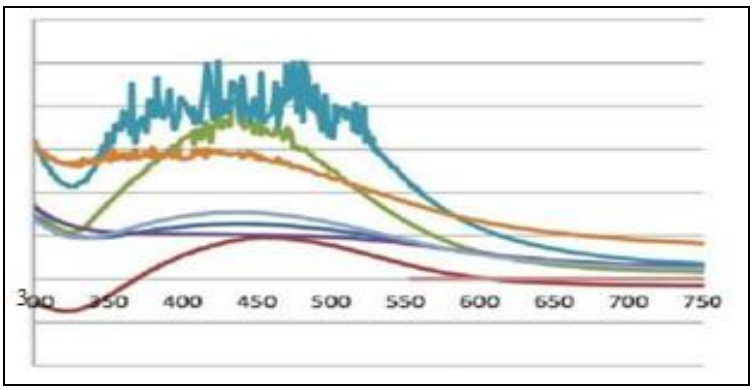

FIG. 4: UV VIS SPECTRUM OF SILVER NANOPARTICLES USING 1:1 VOLUMES OF MILLET EXTRACT TO SILVER NITRATE SOLUTION RESPECTIVELY

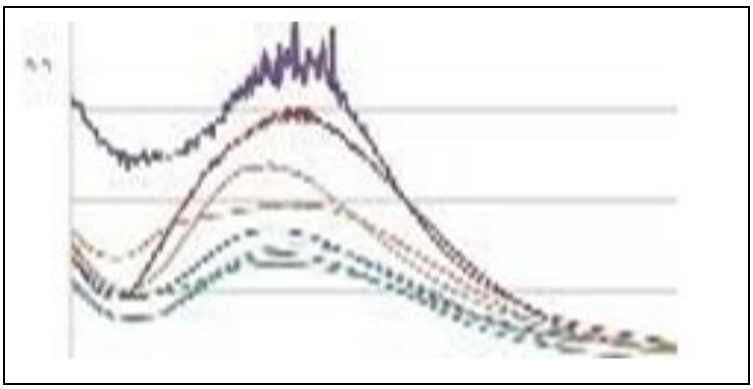

FIG. 5: UV-VIS SPECTRUM OF SYNTHESIZED SILVER NANOPARTICLES WITH 1:2 VOLUMES OF MILLET EXTRACT TO SILVER NITRATE SOLUTION RESPECTIVELY

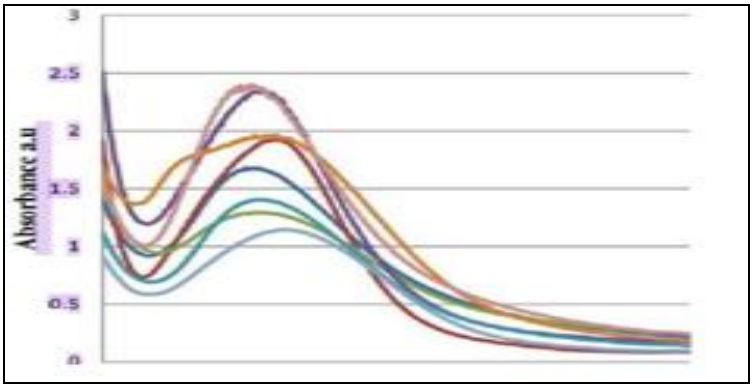

FIG. 6: UV-VIS SPECTRUM OF SYNTHESIS ZED SILVER NANOPARTICLES WITH 1:5 VOLUMES OF MILLET EXTRACT TO SILVER NITRATE SOLUTION, RESPECTIVELY

The curves obtained in the spectrometric graphs were due to the surface plasmon resonance. Perfect peaking between 410nm and 440nm concerning silver nanoparticles is dependent on various factors such as the concentration of silver nitrate solution and reducing agents in the extracts. time taken for the formation of silver nanoparticles, temperature etc. Each condition change would lead to either disturbance in the peaks or no peaks. It may also lead to shift in the peak which is read as red shift.

The ratio of aqueous extract of millets to silver nitrate solution was kept constant with varying concentrations of silver nitrate solution. With 1mM silver nitrate solution, *Eleusine coracana* (Finger millet), *Paspalum scrobiculatum* (Kodomillet) and sorghum bicolour (Jowar) gave good peaking at around 430nm. In contrast, the rest of the millets did not show significant peak, but there was no disturbance observed. With 3mM and 5mM silver nitrate solution, Echinochloafru mentacea (Barnyard millet), *Paspalum crobicularum* (Kodo millet). *Panicum miliaceum* (Prosomillet) gave a light peak in gataround 450nm, thereby showing redshift; the rest of the millets did not show peaking at all. The silver nanoparticles synthesized using l mM silver nitrate solution with the volume ratio of aqueous extract of millets to silver nitrate solution being 1:2, were dried to obtain black powder **Fig. 8**. The yield obtained was between 3mg to 7mg **Table 3.**

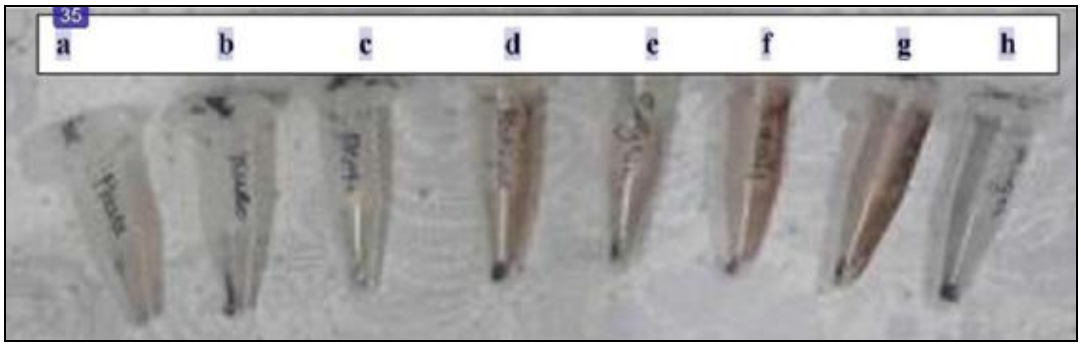

FIG. 7: DRIED POWDERED SILVER NANOPARTICLES. A) *PENNISETUM GLAUCUM* B) *PASPALUM SCROICULATUM*C) *PANICUM MILIACEUM* D) *ECHINOCHLOA FRUMENTACEA*E) *SORGHUM BICOLOUR* F) *SETARIA ITALICA*G) *PANICUM SUMATRENSE* H) *ELEUSINE CORACANA*

TABLE 2: AMOUNT OF SILVER NANOPARTICLES OBTAINED IN POWDERED FORM

Millet type	Weight of Eppendorf tube in (g)	Eppendorf tube silver Nanoparticles in (g)	Actual weight of obtained silver nanoparticles
Paspalum crobiculatum	1.096	1.103	7
Pa11icum miliaceum	1.067	L.070	3
Pennisetum glaucum	1.059	1.062	3
Echinochloa	1.078	1.082	4
Eleusine coracana	1.098	l.103	5
Panicum sumatrense	1.075	l.08l	6
Selaria ilalica	1.067	l.072	s
Sorghum bicolour	1.091	1.098	7

Scanning Electron Microscope (SEM): Characterization of silver nanoparticles was done at the Center for Nanosciences, Indian Institute of Science, Bangalore.

The image serves to lead the morphology of the synthesized silver nanoparticles. The nanoparticles generated from all the eight chosen millets showed spherical shape and with sizes between 20mn to 44nm. The SEM images obtained is shown in **Fig. 9**. The sizes of the silver nanoparticles are tabulated in **Table 4.**

TABLE 3: AVERAGE SIZE OF SYNTHESIZED SILVER NANOPARTICLES

Millet	Average size of silver nanoparticles obtained in nm
Eleusine coracana	43.89
Panicum sumatrense	24.67
Setaria italica	27.84

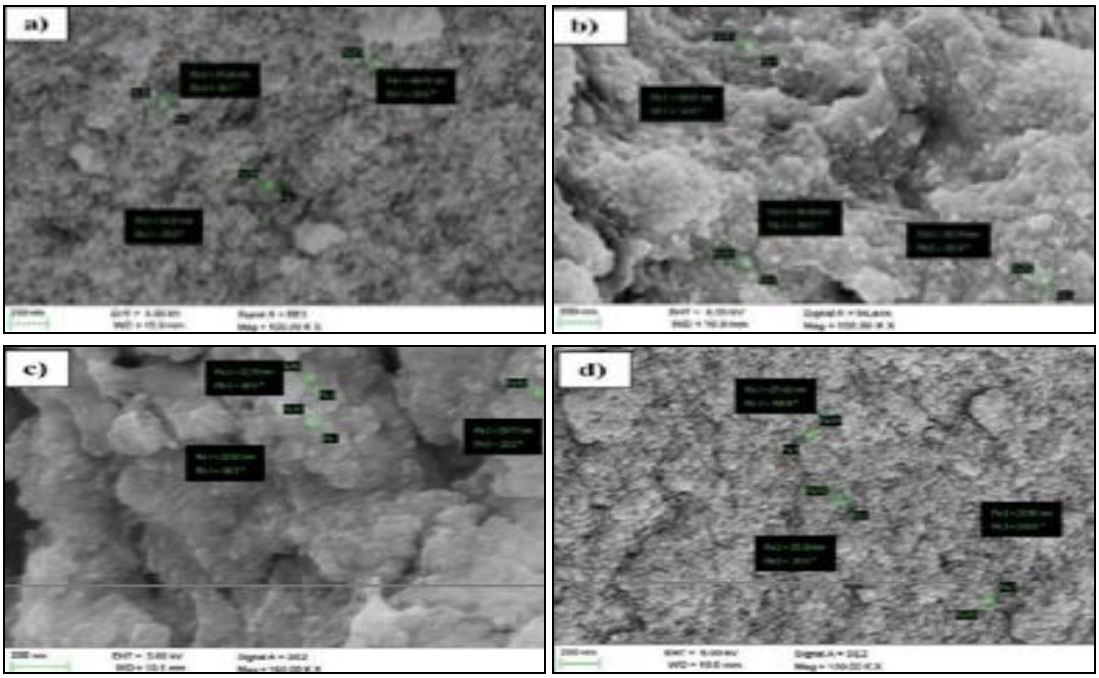

FIG. 8: SCANNING ELECTRON MICROSCOPY IMAGES OF SILVER NANOPARTICLES SYNTHESIZED FROM *ELEIISINE CORACANA* B) *PANICUM SUMATRENSE*C) *SETARIA ITALICA* D) *SORGHUM BICOLOUR* SEM IMAGES OF *ELEUSINE CORACANA*

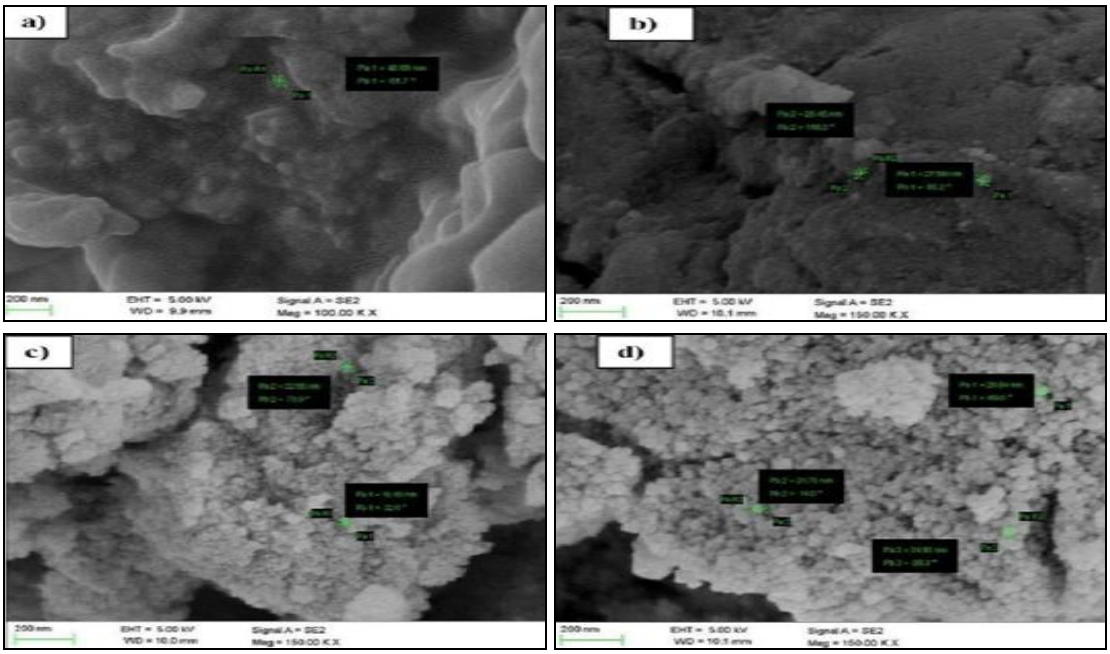

FIG. 9: SCANNING ELECTRON MICROSCOPY IMAGES OF SILVER NANOPARTICLES SYNTHESIZED FROM) *ECHINOCHLOA. FRUMENTACEA B*) *PANICUMMI FIACEUMC*) *PASPAIUM SCROBACULATUM* D) *PENNISETUM GLAUCUM*

In the second set of experiments, the concentration of silver nitrate solution was kept constant at is1mM and the ratio of volumes of aqueous extract to silver nitrate solution was varied. l mM silver nitrate solution was chosen because it showed smooth spectrometric graphs without disturbance. Among the three ratios chosen, 1:2 ratios of volumes of aqueous extract to silver nitrate solution was the best for generating silver nanoparticles from all eight millets as the spectrometric graphs were smooth and peaked at around 430nm. The silver nanoparticles in the solution were centrifuged to obtain a pellet that was dried and powdered. This powdered form, shown in **Fig. 9** was used for further characterization. *Sorghum bicolour* and *Penniselum glaucum* were very clear and spherical.

Total Phenol Content Assay for Aqueous Extract of Millets: Phenolics are a major reducing agent in plants. A total phenol content assay was performed to estimate the phenolics present in aqueous extracts of chosen millet types which were regarded as one of the major reducing agents for the synthesis of silver nanoparticles, shown by the formation of blue complex during the reaction. The phenolic were estimated using the standard gallic acid curve shown in **Fig. 11**. The absorbance data and the estimated amount of phenolics in each of the aqueous extracts of millets were tabulated in **Table 5.**

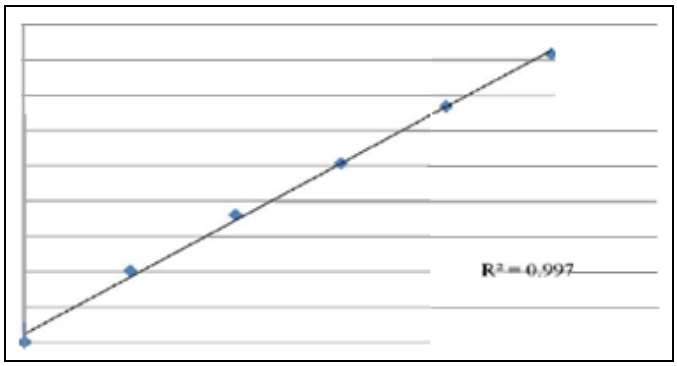

FIG. 10: STANDARD GRAPH FOR TOTAL PHENOLIC CONTENT ASSAY USING STANDARD GALLIC ACID

Among the aqueous extracts of millets, *Paspalums crobiculatum* contained the highest amount of phenolics, and *Panicum miliaceum* contained the least amount of phenolics (*Paspalums crobiculatum* had the highest amount of phenolics it aided the fast reduction of silver ions and generating the silver nanoparticles. Thus, it can be concluded that the more phenolics present in the extracts, the less time is required for the generation of silver nanoparticles, and their action goes for completion in as hort span of time.

TABLE 4: PHENOLIC CONTENTS IN µG/ML OF AQUEOUS EXTRACTS OF CHOSEN MILLET TYPES

Millet type	Concentration of phenolics per ml of extract
Paspalums crobiculatum	137.31
Panicum miliaceum	19.44
Penniselum Jilaucum	32.44
Echinochloarum entacea	24.44
Panicum sumatrense	30.19
Eleusine coracana	33.56
Sorhum bicolour	28.19
Selaria ifalica	28.81

Antimicrobial Screening: Antimicrobial activity of the silver nanoparticles was tested against bacteria and fungi. Gram-negative and gram-positive bacteria were chosen. Disc diffusion method was followed for the testing. This test was performed to check if the synthesized silver nanoparticles could retain its property of exhibiting antimicrobial action.

Bacillus subtilis is a catalase-positive, gram-positive, rod-shaped bacterium "which is [4-10] micrometers long and 0.25-1.0 µm in diameter. It is commonly found in the upper layers of soils [18]. *Proteus vulgar* is belonged to category of gramnegative bacterium and is also catalase positive, commonly found in intestinal tract of animals and humans. It is also known to cause urinary tract infections and wound infections [19]. *Aspergillus niger* is a fungus that causes black mold disease in fruits and vegetables. It is less likely to cause any diseases in humans and finds its use in industries such as those which produce citric acid and gluconic acid [20]. Trichoderma virideis most commonly found in soil and is known to produce green mold. The zone of inhibition was seen in all the culture plates indicating that the synthesized silver nanoparticles can act as an antimicrobial agent. Thus it can be deduced that the synthesized nanoparticles can be used as an antimicrobial agent against gram-positive and gram-negative bacteria, as well as against fungi. The zone of inhibition in the culture plates is shown in **Fig.12, Fig. 13,** and **Fig. 14.**

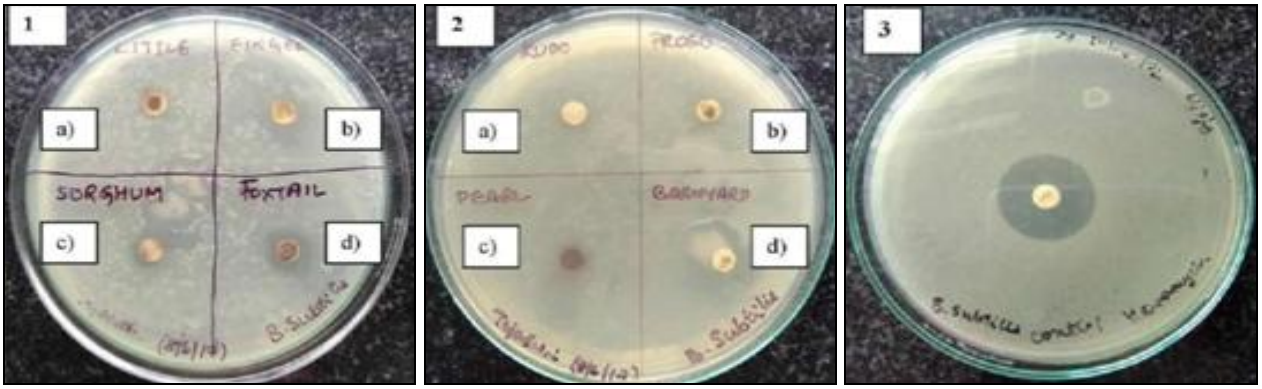

FIG. 11: CULTURE PLATES INDICATING ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES GENERATED FROM AQUEOUS EXTRACTS OF MILLETS AGAINST *BACILLUS SUBTILIS*. 1A, 1B, 1C 1D-ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED USING DIFFERENT MILLETS. 2A, 2B. 2C. 2D -ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED USING DIFFERENT MILLETS. 3) ZONE OF INHIBITION SEEN USING ANTIBIOTIC KANAMYCIN (POSITIVE CONTROL) 30 MCG

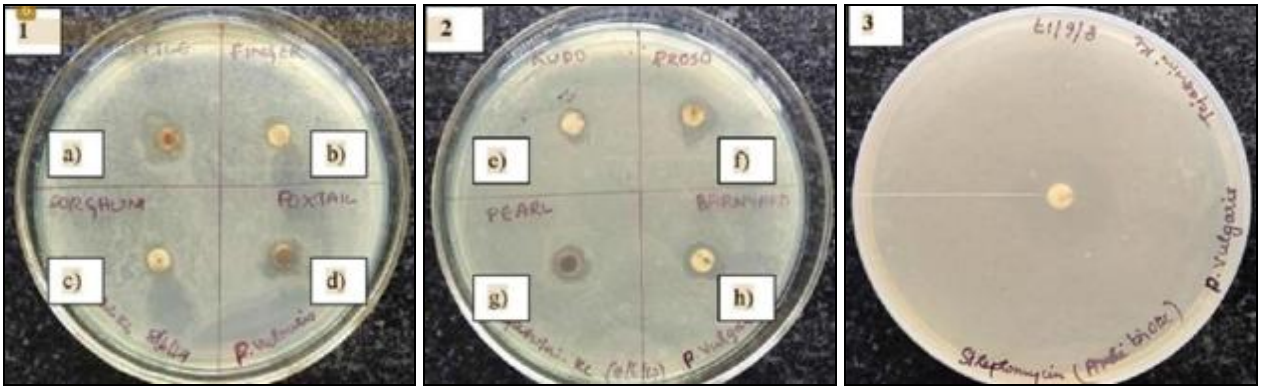

FIG. 12: CULTURE PLATES INDICATING ANTI-BACTERIAL ACTIVITY OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACTS OF MILLETS AGAINST *PROTEUS VULGARIS*. LA, LB, LC, LD-ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED USING DIFFERENT MILLETS. 2A 2B, 2C, 2D- ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED USING DIFFERENT MILLETS. 3) ZONE OF INHIBITION SEEN USING ANTIBIOTIC STREPTOMYCIN (POSITIVE CONTROL) 30 MCG

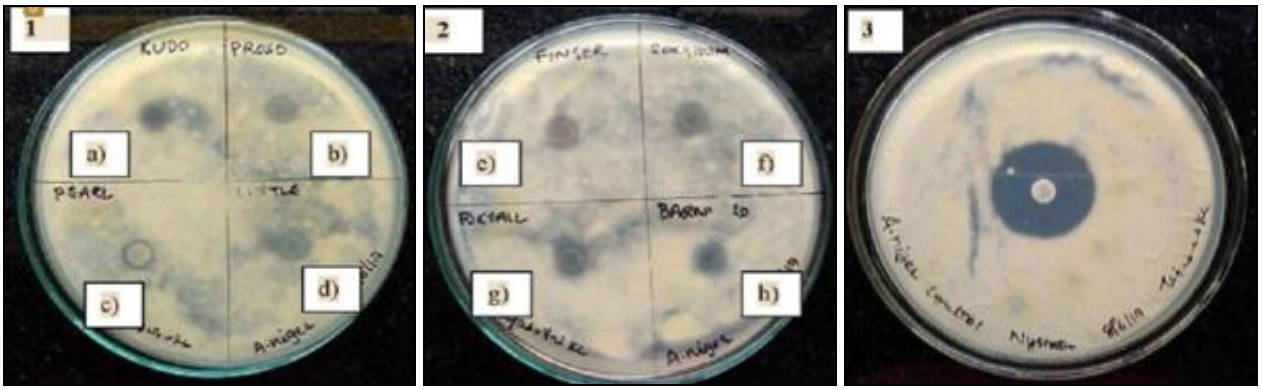

FIG. 13: CULTURE PLATES INDICATING ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM AQUEOUS EXTRACTS OF MILLETS AGAINST *ASPERGILLUS NIGER*. LA, 1B, 1C, 1D-ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED USING DIFFERENT MILLETS.2A,2B,2C,2D-ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED USING DIFFERENT MILLETS. 3) ZONE OF INHIBITION SEEN USING ANTIBIOTIC NYSTATIN (POSITIVE CONTROL) 50MCG

Antimicrobial screening was performed using the silver nanoparticles as an antimicrobial agent against bacteria and fungi. This was done to check the functionality of the synthesized silver nanoparticles. The microorganisms considered for the study were *Bacillus subtilis*, *Proteus vulgaris*.

Aspergillus niger and *Trichoderma viride*. The zone of inhibition was seen in all the culture plates indicating that the synthesized nanoparticles were fully functional and could be used for antimicrobial applications.

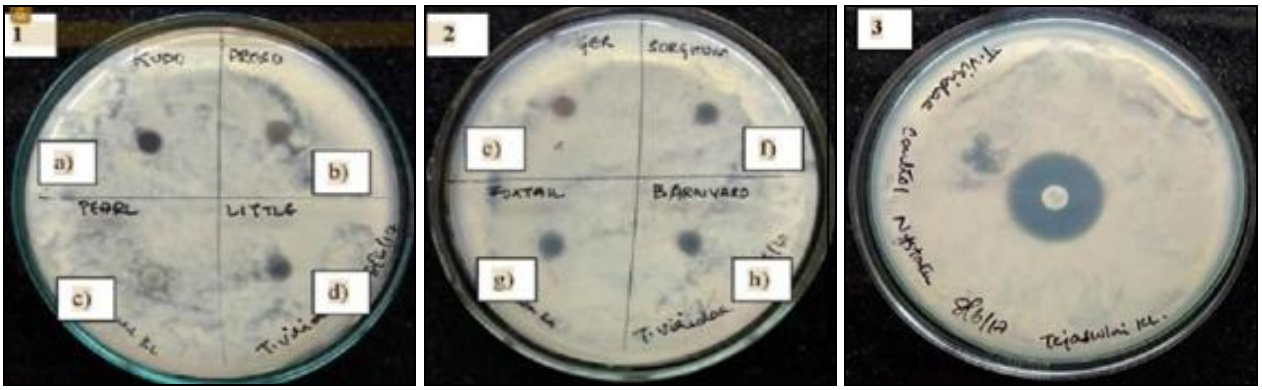

FIG. 14: CULTURE PLATES INDICATING ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM AQUEOUS EXTRACTS OF MILLETS AGAINST *TRICHODERMA VIRIDE*. 1A, 1B, 1C, 1D- ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED FROM DIFFERENT MILLETS. 2A, 2B, 2C, 2D- ZONE OF INHIBITION SEEN USING SILVER NANOPARTICLES SYNTHESIZED USING DIFFERENT MILLETS. 3) ZONE OF INHIBITION SEEN USING ANTIBIOTIC NYSTATIN (POSITIVE CONTROL) 50MCG

Future Scope: The present study dealt with the standardization of variables like the concentration of silver nitrate, the ratio of silver nitrate to aqueous extracts of millet and incubation time. Further, other parameters, such as temperature, agitation etc., can be standardized. The synthesis of silver nanoparticles can be done using ethanol or any other solvent extracts of millet.

Further characterization of synthesized silver nanoparticles through Fourier Transform Infrared Spectroscopy (FTIR), Transmission electron microscopy (TEM), X• Ray diffraction studies, etc., can be done for a deeper understanding of the features of silver nanoparticles. Phytochemical assays of the aqueous extracts, such as flavonoid content, antioxidant assay, etc., can be performed to compare the reducing agents present, which are responsible for synthesizing silver nanoparticles. The silver nanoparticles' Minimum Inhibitory Concentration (MIC) can be determined and used for the antimicrobial formulations. The obtained silver nanoparticles can be used for any available applications.

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