Biogenic Production of Silver Nanoparticles Utilizing an Arid Weed (*Saccharum munja* Roxb.) and Evaluation of its Antioxidant and Antimicrobial Activities

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Abstract

Biomimetic methods of achieving production of silver nanoparticles have been developed in the present study under benign conditions of normal temperature and pressure. The methods are distinguished by the fact that they need very low energy inputs and generate no hazardous waste. Moreover,this method is based on the utilization of arid 'weed', which have no competing use. Hence the method developed opens an avenue for gainful utilization of this arid weed. The synthesis involves reacting metal stock solution with the plant extract in different stoichiometric ratio. By employing different proportions of metal solution and plant extracts, nanoparticles of unique shapes and sizes can be fabricated. Nanoparticles synthesized were spherical plate like structure and size ranging 40-50nm. The plant extracts serve as reducing-cum-stabilizing agents. Yet another mentionable aspect of the present work is that the synthesized nanoparticles has significant antioxidant potential and antimicrobial efficacy against pathogenic microbial strains. In summary, the distinguishing feature of the present work is that it enables the synthesis of silver nanoparticles by facile, non-hazardous, energy-frugal, and inexpensive methods; while gainfully utilizing the arid weed *Saccharum munja* (Roxb.).

Keywords: *Saccharum munja;* nanotechnology; silver nanoparticles; antioxidant activity; antimicrobial activity.

1.0 INTRODUCTION

Modern industrialization and urbanization shifting the environmental conditions by releasing various gases, chemicals, perilous substances which should be controlled by the products secreted by the nature favors the research on nanoparticle synthesis. The term "nanotechnology" was introduced by Norio Taniguchi at the International Conference on Industrial Production in 1974 in order to describe the super thin process of materials with nanometer and its mechanisms. Nanotechnology refers to the field of creation and utilization of metabolites existing in nature and manipulating at a nanoscale. Nanoparticles are the smaller tiny nanosized particle which usually ranges from 1nm-100nm(Abbasi *et al*., 2009; Dubchak*et al*., 2010).

Synthesis of nanoparticles in the field of biotechnology is getting tremendous attention due to its diverse application like drug delivery, biomedicine, cancer treatment, molecular based diagnosis, bio-imaging *etc*. Biological process for the nanoparticle synthesis should be cost effective, clean, non-toxic, non-lethal, eco-friendly (Abbasi *et al*., 2011; 2012a; 2012b; Zulfiqar*et al*.,2019; Rathoure *et al*., 2022). Nanoparticle are of great interest now-a-days due to its varying properties like size, distribution, morphology from which one can easily study the biomedical outcomes like catalytic activity, optical properties, antibacterial, and electrical as well as magnetic properties in other fields(Awwad *et al*., 2013; Banerjee *et al*., 2014). Nanoparticle are synthesized using various different technique such as vapor deposition, microwave, electrochemical reduction, radiolysis reduction, thermal decomposition, chemical reduction of metal salts and room temperature synthesis using hydrazine hydrate (Hyungsoo*et al*, 2001; Huang *et al*, 2006). Green biosynthesis approach is unique where the reaction occurring

may be oxidation or reduction whereas in chemical approach leads to toxicity due to the presence of chemicals absorbed (Parasharu, 2009).

Bio-nanotechnology employ biological principles and physical, chemical methods to produce nanosized particles which have specific functions. For the production of stable novel nanosized particles which uses the green biosynthesis method researchers mainly focuses on the plant-based extracts having medicinal or therapeutic properties (Khan *et al.*, 2018). The applications of NPs are highly suitable for biological molecules, because of their variant properties. The process for the biological molecules to synthesis metal nanoparticles are highly controlled which was found to be reliable and ecofriendly (Harekrishna*et al.*, 2009; Anuradha, 2013). Metallic nanoparticles have huge application in the field of industry due to high availability, compatible, functional and capacity to target drugs.

Over the years, the silver nanoparticles are gaining tremendous attention as it is been proved effective in antimicrobial efficacy, diagnosis and treatment of various diseases (Khan *et al.*, 2014). In medical and industrial processes, metallic silver particles inhibit the microorganism and majorly used as skin ointment, prevent skin burn, medical devices (Jiang *et al.*, 2004).Due to the effective physiochemical property the silver nanoparticle is highly used in biomedical imaging like SERS and in molecular labeling using surface plasmon resonance andscattering cross-section of each nanoparticle (Mody*et al*., 2010). Silver nanoparticles inhibit the microbial pathogens and are widely included in air purifier, vacuum cleaner coating, respirators, food storage packaging, wound dressing, coating of refrigerators etc. Silver metal has broad range for antimicrobial activity which shows mechanism of cell toxicity by the release of reactive oxygen species ROS (Nel*et al*., 2009). It penetrates the bacterial cells resulting in cell membrane damage.

Another synthesis process of silver nanoparticles by using the Aloe vera extract was carried at 24 h of incubation (Chandran*et al*., 2006). Similarly, another study was carried out by Krishnaraj*et al*. (2010) using leaf extracts of *Acalyphaindica*, the silver nanoparticles were synthesized rapidly within 30 min of incubation period. The aqueous silver nitrate solution turned in to brown color within 30 min, with the addition of leaf extract. Amount of brown colorincreased in direct proportion to the incubation period (Krishnaraj*et al*., 2010).A component Phyllanthin from*Phyllanthusamarus*was reported as capping ligands in the synthesis of silver nanoparticle (Kasthuri*et al*., 2009).

Sutradhar*et al*. (2014) reported the synthesis of copper oxide nanoparticles using tea leaf and coffeepowder extracts using microwave irradiations at 540 W within 7–8 min. Gondal and others applied laser ablation process for the production of Nano metal oxides like ZnO, ZnO_2, SnO_2, Bi_2O_3, NiO and MnO_2 (Gondal, 2011).Silver biocomposites have been reported usingchemical reduction process and $AgNO_3$, $NaBH_4$ were used as silver substance a solid support, natural polymeric stabilizer and chemical reduction agent(Shameli*et al*., 2011).

The simplest and eco-friendly method for nanoparticle synthesis is using plant source. The plant extract contains natural substances like phenol, flavonoids, alkaloids which has great reducing tendency and promotes the nanomaterial synthesis. The biological approach involves natural phenomenon which take place in biological systems and created a lot of interest in the term "green nanotechnology". Green synthesis has different process parameters and reaction conditions such as temperature, pH, solvent medium, time and reducing agents *etc*, for greater stability, high yield and controlled size and shape (Chandran*et al.*, 2006; Abbasi *et al.*, 2016a; 2016b). Considering the fact of green biosynthesis method, the plant extract of *Saccharum munja* an arid grass belongs to the family Gramineae (MHFW. 2005) is utilized for AgNPs. The extract of *S. munja* has a unique bioreducing property. The present study aims at biogenic production of silver nanoparticles and evaluation of its antioxidant and antimicrobial potential.

2.0 MATERIALS AND METHODS

2.1Plant Description

Saccharum munja (Roxb.) belongs to the family Gramineae found along river banks. The common name for this plant is Kana, sarkanda, Moonja and which is distributed from North West India to Pakistan. The plant is large grass which has various medicinal values and help to treat disease. The extract of *S. munja* has potential research effect in biological, medicinal and nanotechnology fields. (Tenzin *et al.,* 2017).*S. munja* is a perennial wild grass and grows up to 2 meters in height. Its white flowers are of ornamental value. It forms patches and long root network that binds the soils and form thick biomass tufts. The plant contains various nutritional properties and the stem of munja is sweet, cooling useful in burning sensations, thirst, urinary complaints, eye diseases (Sandeep, 2010).

2.2 Preparation of aqueous extracts of the*Saccharum munja*.

Fresh, mature and diseased free leaf blade portion of*Saccharum munja*plant was collected from the local region adjacent to Nims University Rajasthan campus. Further, the leaf was completely washed in running water and sterilized using deionized water and kept for drying at room temperature.For the aqueous extract preparation, a known amount of leaf sample was taken and chopped into small uniform size and wastransferred into flask for boiling with 100mL of distilled water in waterbathfor about 10-15 min. Then the aqueous extract was cooled to room temperature and the supernatant was filtered through Whatman No.1 filter paper.

2.3 Silver Nanoparticle synthesis and Characterization

The synthesis of silver nanoparticle was done by preparing 1 mM stock solution of silver nitrate ($AgNO_3$)dissolved in 100mL of deionized water andstored in amber color bottle which iscovered inblack plastic sheet tokeep away from light. A stoichiometric ratio of 1:9of aqueous extract of leaf was mixed with silver nitrate stock solution. The synthesis of nanoparticle began immediately with the appearance of brown color. Then this mixture was incubated at room temperature. The synthesized silver nanoparticles were characterized using UV-Visible spectrophotometer, Hr-SEM, EDAX Electron microscopic analysis and Fourier Transform infra-red spectroscopic analysis.

2.4 *In vitro* antioxidant assays

2.4.1 DPPH˙ radical scavenging assay

The antioxidant activity of aqueous extractof *Saccharum munja* leafblade extract and Ag nanoparticlesweredetermined on the basis of the DPPH free radical scavenging activity (1, 1-diphenyl 2-picrylhydrazyl - DPPH). One mL of 0.1 mM DPPH solution dispersed in methanol was added with 1 ml of different concentrations ranging from 20-120 µg/mL of leaf bladeextract and nanoparticle. The mixture was then allowed to stand for 30 min incubation in dark. Distilled water was used as the reference standard. One mL methanol and 1 mL DPPH solution were used as the control (Blois, 1958). The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517nm. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{of DPPH}^{\cdot}\ \text{radical inhibition} = \left[\frac{\text{Control} - \text{Sample} * 100}{\text{Control}}\right]$$

2.4.2 Phosphomolybdenum reduction assay

The antioxidant capacity of the aqueous extractof *Saccharum munja* leaf bladeextract and AgNPs assayed as described by Prieto *et al.* (1999). The leafbladeextract and AgNPs with concentrations ranging from 20 to 120 µg/mL was mixed with reagent solution containing 4 mM (ammonium molybdate), 28 mM (Sodium phosphate) and 600 mM (Sulphuric acid). The reaction mixture was incubated in water bath at 90ºC for 90 min. The absorbance of the colored complex was measured at 695 nm. Distilled water was used as standard reference.The percentage of inhibition was calculated using the following formula:

$$\%\ \text{of Phosphomolybdenum radical inhibition} = \left[\frac{\text{Sample} - \text{Control} * 100}{\text{Sample}}\right]$$

2.5 Antibacterial activity assay

The antibacterial activity of leafblade extract and synthesized AgNPs were examined againstvarious Gram-negative strains such as *Escherichia coli*, *Proteus vulgaris* as well as Gram-positive strains such as *Staphylococcus aureus*, *Bacillus subtilis* were used for the evaluation of antibacterial activity.

Tetracycline was chosen as the standard reference for bacteria. The controls consist of solidifying agar onto which was solvent, and the test compounds were soluble in it.

2.5.1 Nutrient broth agar medium analysis

Nutrient broth agar medium was preparedaccording to the standard methods and was suspended in 200 mL of distilled water in a 500 mL conical flask, stirred, boiled to dissolve and then autoclaved at 121ºC for 15 minutes. The hot medium was poured in sterile petriplates which were kept in the aseptic laminar chamber and medium was allowed to solidify for 15 min (John *et al.*, 2017).Determination of antibacterial potential of the leafbladeextract was carried out using the agar well diffusion method. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The control, extract and standard were then poured into each well of desirable concentrations. Tetracycline was used as the standard with the concentration of 20 µg. All the plates containing sample loaded wells were incubated for 24 h at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of extract and standard were measured by calculating the diameter of zone of inhibition.

2.6 Antifungal Activity assay

The antifungal activity of leafblade extract and synthesized AgNPs was examined against fungal culture *Aspergillusniger*using agar well diffusion method with Potato Dextrose Agar medium (Potato 2gm and Dextrose 3gm). Fluconazole was used as the standard reference for fungal culture.

2.6.1 Potato Dextrose Agar medium analysis

Potato Dextrose Agar medium was prepared using standard procedure and suspended in 100mL distilled water. The sterilization was done by autoclaving for 20 minutes and hot medium was poured in sterile petriplates which were kept in the aseptic laminar chamber and medium was allowed to solidify.Determination of antifungal potential of the leaf blade extract was carried out using the agar well diffusion method against fluconazole and zone of inhibition was observed after incubation for 24-48hrs.

3.0 RESULTS AND DISCUSSION

3.1 UV-Vis Spectrophotometer

In this study, the bio-reduction of silver ions in the aqueous solution of silver nitrate reaction with the *Saccharum munja*leafbladeextract has been characterized by UV-Vis spectroscopy ranging from 200 to 600 nm. The onset for the formation of silver nanoparticle can be visually demonstrated by the appearance of reddish brown colour during the reaction of metallic solution with plant extract. The UV-Visible spectrum forsilver nanoparticle clearly shows the presence of peak at various nanometers. The maximumabsorption for AgNPswas obtained at 450 nm(Fig: 1).

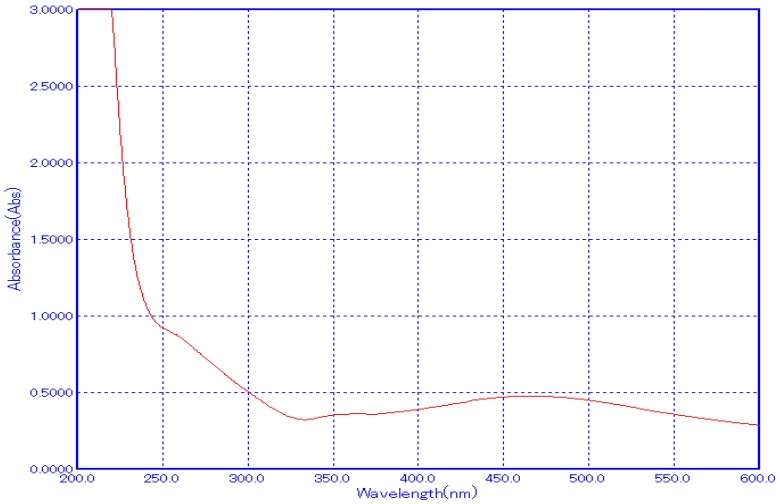

Fig 1: UV-Vis absorption spectrum for synthesized AgNPs.

3.2 Hr-SEM and EDAX Electron microscopic studies

The Hr-SEM images of AgNPs obtained from reactantmixtures, revealed the presence of Ag peak at EDAX spectra, showed that the particles were spherical plate like structure size ranging 40-50nm. (Fig: 2,3)

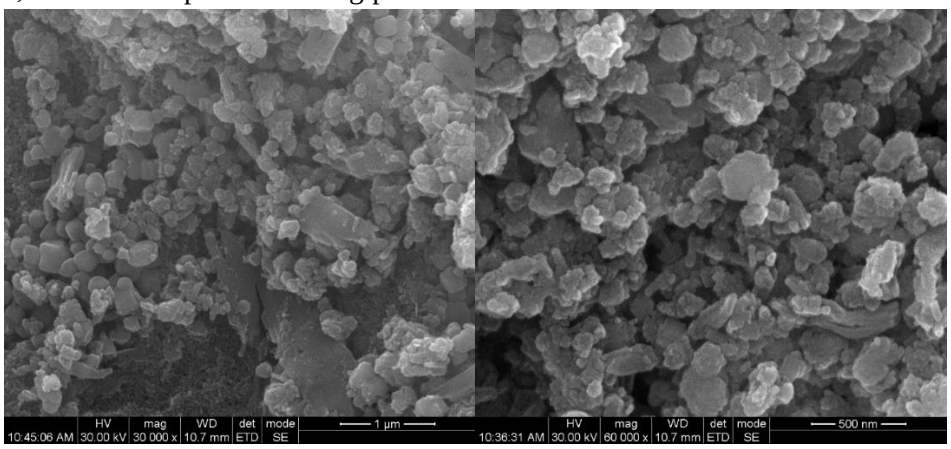

Fig 2: Hr-SEM micrograph of AgNPs synthesized at different magnification.

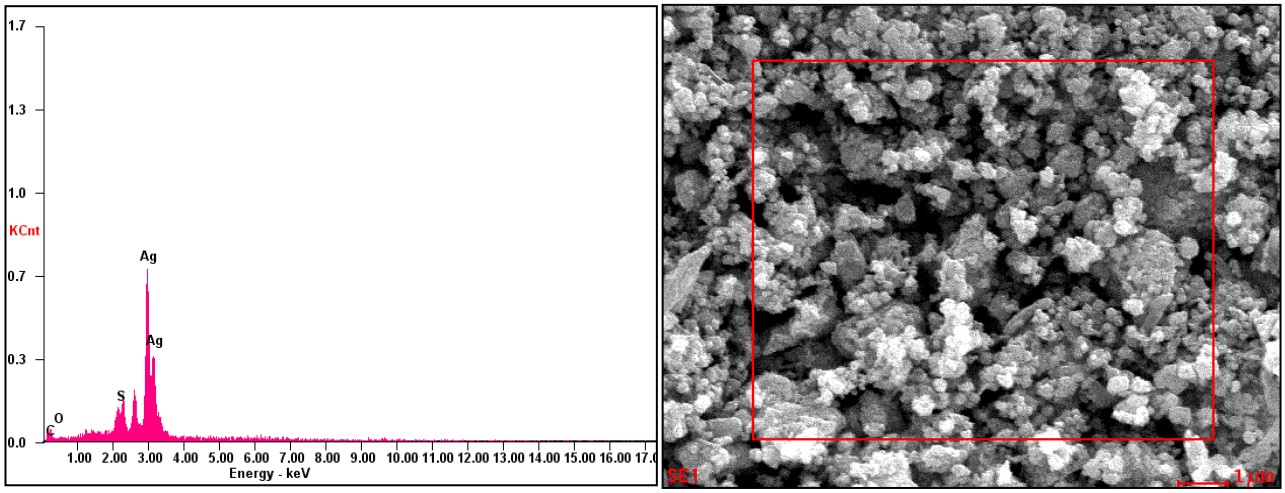

Fig 3: EDAX of synthesized AgNPs.

3.3 Fourier Transform infra-red spectroscopic studies

The FT-IR spectra give information about the molecular environment of the organic molecules on the nanoparticle surface.FT-IR examination were used to identify the presence of potential biomolecules found in S. *munja*extract that could have played a role in the reduction of synthesized and measures the subsequent stabilization-capping agent of nanoparticle synthesized by leaf blade extract were identified using FT-IR. FT-IR is used to analyze the chemical composition of organic chemicals, polymers, biological samples, inorganics, and minerals and give both qualitative (identification) and quantitative (amount) analysis.

The results of AgNPs FTIR analysis shows different stretches of bonds at different peaks; 3428- N–H stretch, 2917, 2849, 1631, 1241 etc. were observed. Thereis presence of strong absorption bands at 1650–1550 $Cm^{-1}$and 1090–1020 Cm^{-1} region and weaker signals in the 1550–1350 Cm^{-1}region. (Fig: 4)

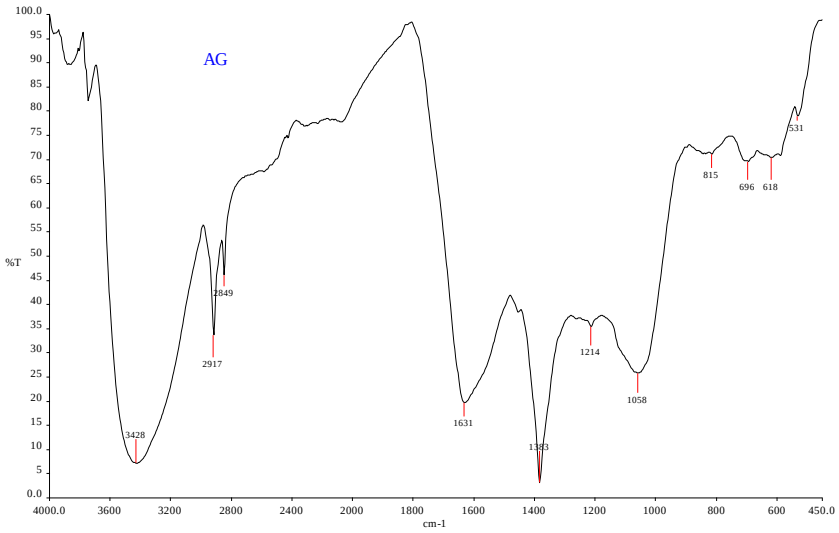

Fig 4: FTIR spectra of AgNPssynthesized by *Saccharum munja* leaf blade extract.

3.4 DPPH˙ radical scavenging assay

Aqueous extract of *Saccharum munja* leaf blade extract and Ag nanoparticles demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the leaf blade extract.It was observed that the highest DPPH˙ radical scavenging activity of leaf blade extract as 63.21% and AgNPsas 47.63% at 120 µg/mL concentration.The IC_{50} value were found to be 78.26µg/mL and 125.97 µg/mL concentration for leaf blade extract and AgNPs respectively, when compared with standard (IC_{50} = 11.98 µg/mL concentration) ascorbic acid solution .

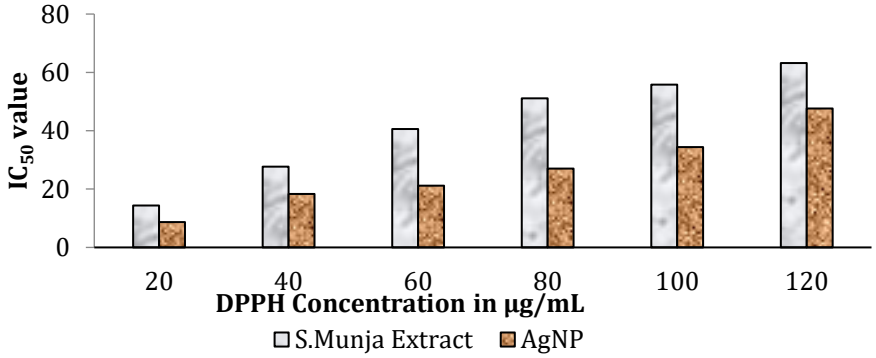

Fig 5: DPPH radical scavenging activity of aqueous extract of *S. munja* leaf blade extract and AgNPs

3.5 Phosphomolybdenum reduction assay activity

The total antioxidant activity of aqueous extract of *Saccharum munja* leaf blade extract and Ag nanoparticleswas measured by Phosphomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo(V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum Phosphomolybdenum reduction was 89.96% for leaf blade extract and87.37% for AgNPsat 120 µg/mL concentration. It was compared with the standard ascorbic acid.

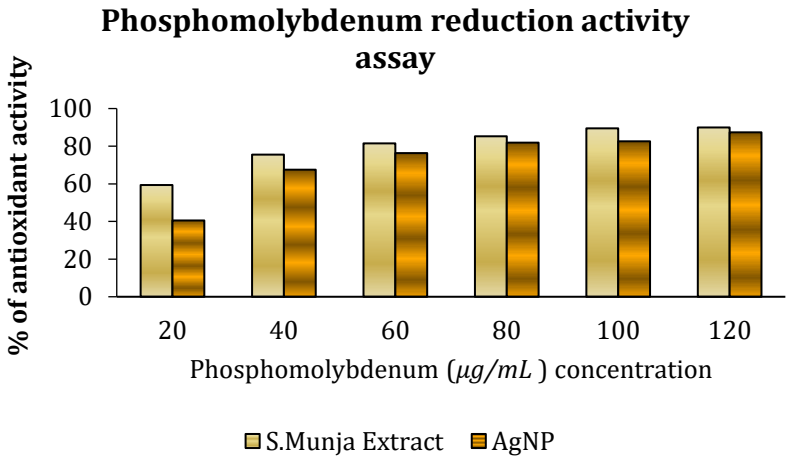

Fig 6: Phosphomolybdenum reduction activity of aqueous extract of *S. munja* leaf blade extract and AgNPs.

3.6 Antibacterial Analysis

The aqueous extract of *Saccharum munja* leaf blade extract and AgNPs was investigated for antibacterial activity against microorganism including Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli*,*Proteus vulgaris*). The antibacterial sensitivity of the crude leaf blade extract and nanoparticles and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in petriplates.The antibacterial activity of these extracts could be correlated as due to the presence of secondary metabolites such as flavonoids, phenolic compounds, terpenoids, tannin and alkaloids that adversely affect the growth and metabolism of microbes

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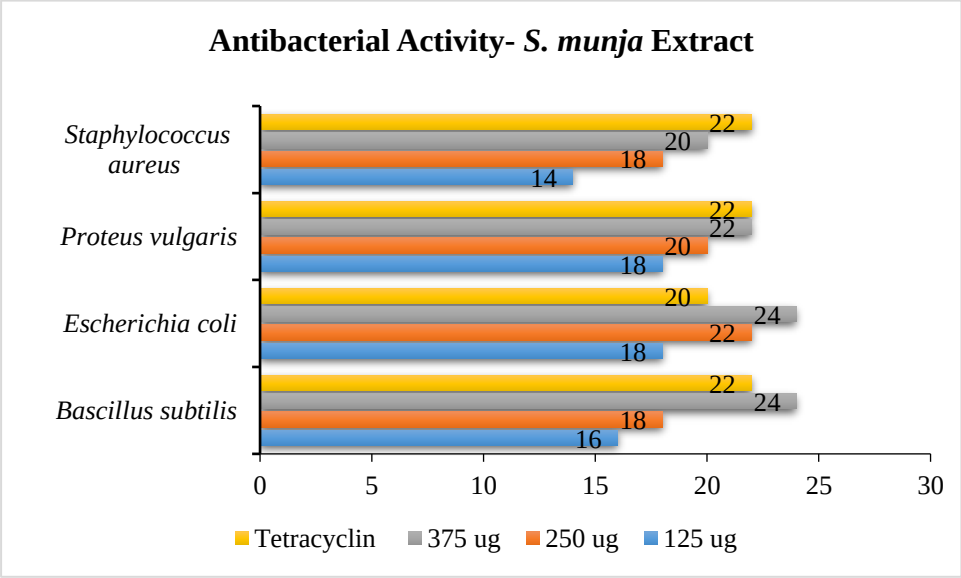

Fig 7: Antibacterial activity of plant extract *w.r.t* standard.

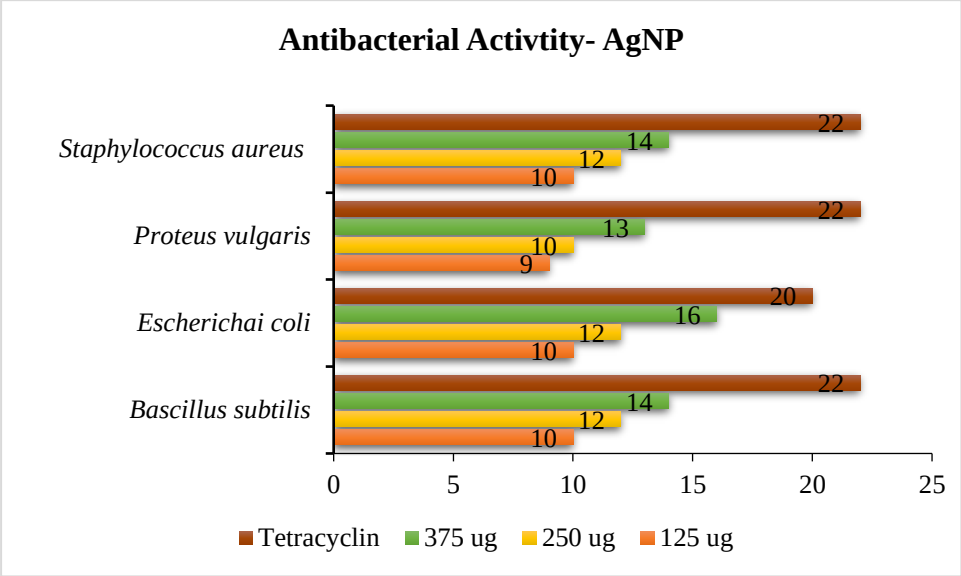

Fig 8: Antibacterial activity of synthesized AgNPs *w.r.t* Standard.

3.7 Antifungal Analysis

The aqueous extract of *Saccharum munja* leaf blade extract and Ag nanoparticles was investigated for antifungal activity against *Aspergillusniger*microorganism. The antifungal sensitivity of the standard (125*µg/ml*), crude plant extract (250*µg/ml*) and nanoparticles (250*µg/ml*) and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in petriplates.

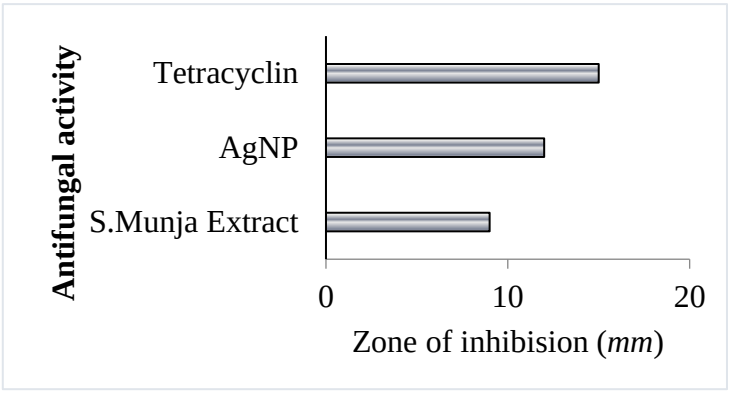

Fig 9: Antifungal activities of plant extract and synthesized AgNPs.

4.0 SUMMARY AND CONCLUSION

The present study reports the first ever attempt to gainfully utilize the arid weed (*Saccharum munja*) in generating silver nanoparticles. The weed studied by us give rise to enormous quantities of biomass which has little existing economic value.We have demonstrated, with the experiments described that the weed extract (*Saccharum munja*) can be used for silver nanoparticles synthesis and their antioxidant and antimicrobial activities were determined. Equally significant is the fact thatthe process developed by us utilized the weedfor silver nanoparticles synthesis provides us with substrates whichhave no competing use. Even more significantly thisapproach opens an avenue for periodic harvesting of the weed thereby reducing the harm they cause to theenvironment. Yet, another mentionable aspect of thepresent work is that the synthesized nanoparticles has significant antioxidant potential and antimicrobial efficacy against pathogenic microbial strains.In summary,

the distinguishing feature of the present work is that it enables the synthesis of silver nanoparticles by facile, non-hazardous, energy-frugal, and inexpensive methods; while gainfully utilizing the arid weed *Saccharum munja*.Future research perspectives include the use of silver nanoparticles in biomedical imaging and molecular labeling due to the facet with unique surface plasmon resonance and scattering cross-section of each nanoparticle

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

[1]. Dubchak S., Ogar A., Mietelski J.W. and Turnau K., Influence of silver and tianium nanoparticles on arbuscular mycorhiza colonization and acumulation of radiocaesium in Helianthus anus, Span. J. Agric. Res., 8(1), 103-108, (2010).

[2]. H. Zulfiqar, Z. Ayesha, N. Rasheed, Z. Ali, K. Mehmood, A. Mazher, M. Hasan and N. Mahmood, Nanoscale Adv., 2019

[3]. Awwad, A.M.; Salem, N.M.; and Abdeen, A.O. (2013). Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. International Journal of Industrial Chemistry, 4(1), 1-6.

[4]. Banerjee, P.; Satapathy, M.; Mukhopahayay, A.; and Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing, 1(1), 1-10.

[5]. Navaladian, S.; Viswanathan, B.; Viswanath, R.P.; and Varadarajan, T.K. (2007). Thermal decomposition as route for silver nanoparticles. Nanoscale research letters, 2(1), 44-48.

[6]. Rajasekharreddy, P.; Rani, P.U.; and Sreedhar, B. (2010). Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach. Journal of Nanoparticle Research, 12(5), 1711-1721.

[7]. Hyungsoo C., Sung-Ho P., Seedless Growth of Free- Standing Copper Nanowires by Chemical Vapor Deposition J. Am. Chem. Soc. (2004) 126(20), 6248– 6249.

[8]. Huang L., Jiang H., Zhang J., Zhang Z., Zhang P., Synthesis of copper nanoparticles containing diamondlike carbon films by electrochemical method Electro. Comm., (2006) 8(2), 262–266.

[9]. Hyungsoo C., Sung-Ho P., Seedless Growth of Free- Standing Copper Nanowires by Chemical Vapor Deposition J. Am. Chem. Soc. (2004) 126(20), 6248– 6249

[10]. M. Z. H. Khan*, F. K. Tareq, M. A. Hossen, M. N. A. M. Roki., Green Synthesis and Characterization of Silver Nanoparticles Using CoriandrumSativum Leaf Extract, Journal of Engineering Science and Technology, Vol. 13, No. 1 (2018) 158 – 166

[11]. Harekrishna Bar, D.K.B., Gobindasahoo P, priyankaSarkar, Sankar PD., "Green synthesis of silvernanoparticles using latex of Jatrophacurcas.". Colliod surface A, 2009. 39(3): p. 134-139.

[12]. Khan, A.; Rashid, R.; Murtaza, G.; Zahra, A. Gold nanoparticles: Synthesis and applications in drug delivery. Trop. J. Pharm. Res. 2014, 13, 1169–1177.

[13]. Jiang H, M.S., Wong ACL, Denes FS, "Plasma enhanced deposition of silver nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics.". J ApplPolymSci, 2004. 93: p. 1411-1422.

[14]. Shobha G, Vinutha M and Ananda S: Biological synthesis of copper nanoparticles and its impact - a review. Int J PharmaceutSci Invent 3: 28-38, 2014.

[15]. Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M. (2006) Synthesis of gold

nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnol.Prog., 22: 577–583.

[16]. MHFW (2005) The Ayurvedic pharmacopoeia of India. Ministry of Health and Family Welfare, Department of ISM and Health, India 3: 188-189

[17]. Paul JA Borm, David Robbins, Stephan Haubold, Thomas Kuhlbusch, Heinz Fissan, Ken Donaldson, RoelSchins, Vicki Stone, Wolfgang Kreyling, JurgenLademann, Jean Krutmann, David Warheit, Eva Oberdorster,. The potential risks of nanomaterials: a review carried out for ECETOC, Particle and Fibre Toxicology, 2006, Volume 3

[18]. Parasharu K, S.a.S.A., "Bioinspired Synthesis of Silver Nanoparticles". Digest Journal of Nanomaterials and Biostructures, 2009. 4: p. 159-166.

[19]. Mody VV, Siwale R, Singh A, Mody HR. Introduction to metallic nanoparticles. J Pharm Bioallied Sci. 2010;2(4):282-9.

[20]. Nel, A., Xia, T., Madler, L. & Li, N. Toxic potential of materials at the nanolevel. Science 311, 622–627 (2006).

[21]. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and biosystems engineering. 2009 Jan 1;32(1):79-84.

[22]. C.Krishnaraj, E.G.Jagan, S.Rajasekar, P.Selvakumar, P.T.Kalaichelvan, N.Mohan., Synthesis of silver nanoparticles using Acalyphaindica leaf extracts and its antibacterial activity against water borne pathogens, Colloids and Surfaces B: Biointerfaces Volume 76, Issue 1, 1 March 2010, Pages 50-56

[23]. Kasthuri J., Kathiravan K., Rajendiran N. (2009). Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: a novel biological approach. J Nanopart Res, 11(5), 1075–1085.

[24]. Sutradhar, P., Saha, M., and Maiti, D. 2014. Microwave synthesis of copper oxide nanoparticles using tea leaf and coffee powder extracts and its antibacterial activity. Journal of Nanostructure in Chemistry, 4(1), 86

[25]. Gondal MA, Seddigi ZS, Nasr MM, Gondal B. Spectroscopic detection of health hazardous contaminants in lipstick using Laser Induced Breakdown Spectroscopy. J Hazard Mater.2010; 175:726–732.

[26]. KamyarShameli, Mansor Bin Ahmad, Ali Zamanian, ParvanhSangpour, ParvanehShabanzadeh, YadollahAbdollahi, Mohsen Zargar., Green biosynthesis of silver nanoparticles using Curcuma longa tuber powder, Int J Nanomedicine. 2012; 7: 5603–5610.

[27]. Tenzin C, Jeyanthi P, Kumar A, Sujesh S, Ramalingam C., (2017) Estimation of Bioactive Compounds from Saccharum munja Extract for theEvaluation of Anti-oxidants and Anti-bacterial Activities. J Food Process Technol 8: 671.

[28]. Sandeep, Rahar; NagpalNavneet; Swami Gaurav; AroraManisha; Bansal Suraj; Bansal Suraj; SinglaShwali; SinglaShwali; SinglaShwali (2010). "Medicinal Aspects of Saccharum munja".Research Journal of Pharmacy and Technology. 3 (3): 636–639.

[29]. Varsha Nigam1 & J. S. Sodhi.,Some Medicinal Plants with Antioxidant Activity- A Review, International Journal of Pharmacy and Biological Sciences, Volume 4| Issue 1 |JAN-MAR|2014|173-178

[30]. Moszer, I., Jones, L.M., Moreira, S., Fabry, C., and Danchin, A. (2002) SubtiList: the reference database for the Bacillus subtilisgenome. Nucleic Acids Res 30: 62–65.

[31]. Katz, E., and Demain, A.L. (1977) The peptide antibiotics of Bacillus: chemistry, biogenesis, and possible functions.Bacteriol Rev 41: 449–474.

[32]. Lowy, F.D. (1998) Staphylococcus aureus infections. N Engl J Med 339: 520–532.

[33].Matthew A. Croxen, Robyn J. Law, Roland Scholz, Kristie M. Keeney, Marta Wlodarska, B. Brett Finlay., Recent Advances in Understanding Enteric Pathogenic Escherichia coli., Clinical Microbiology Reviews Oct 2013, 26 (4) 822-880

[35].DominikaDrzewiecka., Significance and Roles of Proteus spp. Bacteria in Natural Environments, Microb Ecol. 2016; 72(4): 741–758.
[36]. Pitt, J.I. and A.D. Hocking, 1997. Fungi and Food Spoilage. 2nd Edn., Springer, New York, ISBN-10: 0834213060, pp: 593.
[37]. Blois MS., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 29: 1199-1200.
[38]. Prieto,.P,Pineda,.M and Anguilar,.M.,1999. Spectrophotometric quantisation of antioxidant capacitythrough the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal. Biochem, 269: 337-341.
[39]. Sheila John, Sarah Jane Monica, Priyadarshini S, Sivaraj C , Arumugam P: Antioxidant and Antimicrobial Efficacy of Lemon (Citrus limonum L.) Peel.Int. J. Pharm. Sci. Rev. Res., 46(1), 2017; 115-118
[40]. Abbasi, T., Anuradha, J., and Abbasi, S. A. (2009). Nanotechnology and its potential in revolutionizing the pollution control scenario. J. Institution of Public Health Engineers, India, 10, 5–12.
[41]. Abbasi SA, Abbasi T, Anuradha J, Neghi N, Prathiba S, Ganaie SU. Gainful utilization of four otherwise worthless and problematic weeds for silver nanoparticle synthesis.Offl J Patent Off. 2011 Jul;11869.

[42]. Abbasi, T., Anuradha, J., & Abbasi, S. A. (2016a). Clean and rapid biomimetic synthesis of gold nanoparticles using all parts of the aquatic weed water hyacinth (Eichhorniacrassipes). Journal of the Indian Chemical Society, 93, 851–865.

[43]. Abbasi SA, Abbasi T, Anuradha J. A process for synthesis of metal nanoparticles from aquatic weeds.Offl J Patent Off. 2012a;6184.

[44]. Abbasi, T.; Anuradha, J.; Abbasi, S.A. Utilization of the pernicious aquatic weed salvinia (Salviniamolesta D S Mitchell) in generating gold nanoparticles. Indian J. Biotechnol. 2016b, 15, 382–391.

[45]. Anuradha J. Use of some aquatic and terrestrial weeds in the ‘green’ synthesis of gold nanoparticles (Doctoral dissertation, Centre for Pollution Control and Environmental Engineering, Pondicherry University Pondicherry University).

[46]. Abbasi SA, Abbasi T, Anuradha J. A process for synthesis of bimetallic silver gold nanoparticles from weeds.Official Journal of the Patent Office. 2012b;3:944.

[47]. Rathoure AK, Anuradha J, Sanjeevi R, Chauhan DS, Tripathi S. Effective Removal of Heavy Metals From Aqueous Solution by Nano-Composites: Bio Remediation Using Nano Technology. Research Anthology on Emerging Techniques in Environmental Remediation, Pg. 560-568.