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Antioxidant and Antimicrobial Activity of Silver Nanoparticles Biosynthesized Using *Leptodenia Pyrotechnica*aerial Part

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Abstract: Nanotechnology is considered as a fast and constantly growing field in the biomedical and healthcare applications due to its relatively small size and vast advantages. Researches have proven that the silver nanoparticle synthesized from the green route has an excellent antimicrobial and antioxidant properties and is free of any adverse effects.In the present study the antimicrobial and antioxidant activity of *Leptodeniapyrotechnica* aerial part and silver nanoparticle (AgNPs) synthesized by *Leptodeniapyrotechnica* aerial part (LPA-AgNPs) have been investigated comparatively. The characterization of AgNPs was done by using scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy. The antimicrobial activity of LPA-AgNPs and methanol extract of *L. pyrotechnica* aerial part (LPAM) were tested against bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and fungal strains (*Candida albicans, Aspergillus niger*, *Trichoderma reesei*and *Penicilliumchrysogenum*) using the agar well diffusion method. And the catalase (CAT), peroxidase (POD) and ferric reducing antioxidant power (FRAP) assay also evaluated. The prominent transmission band in FTIR spectra was observed at 3443 cm^{-1} (hydroxy group, H-bonded OH stretch) and at 1638 cm^{-1} (alkenyl C=C stretch). In SEM characterization, the nanoparticles were in spherical shape with 23-86 nmrange of particle size. LPA-AgNPs showed highest antibacterial activity against *B. subtilis*, *P. aeruginosa*, *E. coli* and *S. aureus* with 33 mm, 17 mm, 14 mm and 20 mm inhibition zone, respectively in comparison with LPAM. Likewise antifungal properties of LPA-AgNPs were significant against *Trichoderma reesei* and *Aspergillus niger* with 12 mm inhibition diameter comparatively. The highest CAT (0.089µM H_2O_2 reduce/gm Fwt/sec), POD (0.657µM/L/gm dwt/sec) and FRAP (1103.21 µM) activity was also observed with green synthesized AgNPs. It can be concluded from the results that the silver nanoparticle synthesized from the *Leptodeniapyrotechnica* aerial part washighest antimicrobial and antioxidant activities as compared to *Leptodeniapyrotechnica* aerial part.

Keywords: Nanotechnology, *Leptodeniapyrotechnica*, Silver nanoparticle (AgNPs), FRAP, Antimicrobial activity, Antioxidant activity.

Introduction

Since ancient times, ayurveda has made tremendous advances in the field of healthcare. Various technologies have been evolved to efficiently address the problem of delivering medications with the ideal dose at the targeted site, hence increasing their bioactivity, at the

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same time as nanoscience has advanced (Naik *et al.,* 2020). The World Health Organization estimates that 80% of people consume herbal remedies directly as teas, decoctions, or extracts with readily available liquids like water, milk, or alcohol. Most plants exhibit antibacterial and anti-oxidative properties as a result of rising global trends in medicinal plant research (Kane *et al.,* 1950). Due to improper and frequent usage of existing antimicrobials, microbial resistance to antimicrobial agents is growing day by day. In order to combat the growing antimicrobial resistance, several researchers concentrated on essential phytoconstituents that were extracted from different medicinal plants like *Leptadeniapyrotechnica* (Pan *et al*., 2012).

Leptadeniapyrotechnica is an erect, ascending, shrub from family *Asclepiadaceae*. It is up to 1.5m-3m high with green stem and pale green alternating bushy branches with watery sap. In Pakistan it is commonly known as kheep or khip (Shetty and Singh, 1991). The genus *Leptadenia* consists of the following accepted species namely *Leptadeniaarborea, Leptadeniamadagascariensis, Leptadeniahastata, Leptadeniaabyssinica, Leptadeniapyrotechnica, Leptadenia reticulate* (Verma *et al.,* 2014). *L. pyrotechnica* have been reported to be rich in steroidal glycosides, alkaloids, flavonoids, triterpenes and polyoxypregnane derivatives (Sharma *et al.,* 2016).

Leptadeniapyrotechnica and its parts are traditionally used for different purposes. The fiber of *Leptadeniapyrotechnica* is used as antihistaminic and expectorant.Whole plant is used for the treatment of wound in Yemen folks and proved to have antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Fresh juice of the plant is used for abortion. Plant sap is applied to eczema and other skin disease and is also given in diabetes. The latex or the leaf paste is applied over the thorn injury for thorn removal. Whole plant infusion is mixed with buttermilk and given for uterine prolapsed and stomach disorders in sariska region of Rajasthan. It is used to cure constipation and is considered good for health in Bikaner region of Rajasthan. In the sudanodeccanian region of central Sahara it is traditionally used in fever, cough, kidney disorders, stones, urinary disease (Upadhyay *et al.,* 2011).

In this modern world, the synthesis and usage of materials with structural characteristics between those of atoms and bulk materials with at least one dimension in the nano range is known as nanotechnology (Brunner *et al.,* 2006). Nanotechnology is a significant area of scientific research that deals with the synthesis, development, and manipulation of particle structures with sizes ranging from 1 to 100 nm. Within this size range, both individual atoms/molecules and their associated bulk undergo fundamental changes in all of their properties like chemical, physical, and biological. Numerous industries, including those in the fields of medicine, cosmetics, biomedicine, food and feed, drug-gene delivery, environment, health, mechanics, and optics, as well as those in the fields of energy science, catalysis, light emitters, single electron transistors, nonlinear optical devices, and photo-electrochemical applications, are quickly adopting it (Korbekandi and Iravani, 2012).

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Green synthesis has been considered as another remedy in the field of medicine. Aside toxic chemical and physical method, biological method is considered using medicinal plants extract were used for the synthesis of nanoparticles. The surface and fraction of the atoms are responsible for the activity of the nanoparticles. This invention of green nanotechnology is considered ecofriendly and cost effective when compared to the others. The technology utilizes proteins as natural capping agents and its synthesis from plants utilize various secondary metabolites, enzymes, proteins and or other reducing agents which makes it suitable to use in various biomedical and clinical applications (Parthasarathy*et al.*,2019). Ayurvedic knowledge and nanotechnology could work together to address the complicated healthcare issues that are currently plaguing the world's healthcare system. Nanotechnology has thus far been used in a variety of disciplines, including energy production, the environment, catalysis, etc., and its positive impact on the healthcare system is particularly noteworthy (Naik *et al.*, 2020).

In the field of nanotechnology, silver nanoparticles stand out among the noble metals because of their exceptional qualities, including chemical stability, good conductivity, catalytic activity, and, most importantly, their antibacterial, antiviral, antifungal, and anti-inflammatory properties. These qualities allow them to be used in composite fibers, cryogenic superconducting materials, cosmetic products, the food industry, and electron beams (Ahmad *et al.*, 2003). Synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications. These silver nanoparticles are being successfully used in the cancer diagnosis and treatment as well (Popescu *et al.*, 2010; Baruwati*et al.*, 2009). For biomedical applications; being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions' as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities(Klaus-Joerger*et al.*, 2001). The antioxidant and antimicrobial activity of AgNPs synthesized by *Leptodeniapyrotechnica*aerial part has not been investigated yet with comparison from*L. pyrotechnica*aerial part. Here we present the antioxidant activity including catalase, peroxidase, FRAP and antibacterial and antifungal activities against pathogenic strains of AgNPs comparatively.

Material And Method

1. Collection Of Plant Materials

The experimental plant material of *L. pyrotechnica*aerial part was collected from near Albert Hall in front of Zoo, and adjoins area of Jaipur, Rajasthan.

2. Preparation Of Plant Extracts

The freshly harvested aerial part of *L. pyrotechnica*is properly cleaned with tap water before being let to air dry for around two to three weeks at room temperature (32 to 37°C). Using a homogenizer, the dried plant samples were reduced to powder. In a Soxhlet extractor, 50g of powdered plant material (50g/250ml) were consecutively extracted with methanol for 8 to 10

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hours. After obtaining the extracts, they were concentrated, and then dried to a consistent weight. Prior to conducting additional testing, dried extracts were stored at 20°C.

3. Preparation Of Silver Nanoparticles

Loo *et al*., 2012 method was used to create AgNPs by using aerial part of *L. pyrotechnica*. 100 ml of distilled water and 10 grams powder of *L. pyrotechnica*aerial part were added to a beaker, which was then boiled at 60°C for 10 minutes. After 10 minutes, a 0.45 µm Millipore membrane filter was used to filter the leaf extract, and then a 0.2 µm Millipore membrane filter was used. $AgNO_3$ (1 mM) was dissolved in 100 ml of Erlenmeyer flask filled with 12 ml of leaf extracts to create AgNPs. There were visible variations in the solution's colour. Characterization of silver nanoparticle was done by using Fourier Transform Infrared (FTIR) Spectroscopy and Scanning electron microscopy (SEM).

(a) **(b)**

Figure 1: Picture of *Leptodeniapyrotechnica*aerial part extract with AgNPs solution (a) before and (b) after the LPA-AgNPs

4. Characterization Of Silver Nano-Particles

Scanning electron microscopy (SEM)

Using a SEM [Carl ZEISS EVOR-18, Germany] operated at an extra high tension or accelerating voltage [EHT] of 20 kV, where WD was 8.5 mm, the real sizes of LPA-AgNPs were investigated. The sample discs were gradually loaded with a little quantity of the test ingredients. Before placing the materials on the specimen stage, the Quorum Q150RS rotary

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pumped sputter coater applied sputter coating (gold coating) to the materials for improved SEM image.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR Shimadzu Spectrometer [IR Affinity-1; class-1 laser product, Japan] diffuse reflectance operating mode with a resolution of 4 cm1 was used to examine the sample. This device needs a tiny quantity of dried, KBr pellet-ground material. This activity was carried out to learn about the functional groups that are spread over the material surfaces.

5. ANTIMICROBIAL ACTIVITY

Microbial Strains, culture medium and inoculum preparation:

Clinical laboratory bacterial strains of *Bacillus subtilis* (MTCC 10619), *Pseudomonas aeruginosa* (MTCC 0424), *Escherichia coli* (MTCC 443) and *Staphylococcus aureus* (MTCC 3381) and fungal strain viz. *Candida albicans* (MTCC 183) , *Aspergillus niger*(MTCC 872), *Trichoderma reesei*(MTCC 164) and *Penicilliumchrysogenum*(MTCC 5108) were collected from the standard cultures of Microbiology Laboratory, SMS Medical College Jaipur, Rajasthan.

Determination of Antibacterial Assay

The agar well diffusion technique was used to inspect the LPA-AgNPs and LPAM *in vitro* anti-bacterial efficacy in contrast to bacterial strains (Gram negative and Gram positive) (Perez *et al*., 1990). The bacteriological medium was Mueller Hinton agar No. 2 (Hi Media, India). In 100% Dimethylsulphoxide (DMSO), the methanol extract was diluted to a concentration of 10 mg/ml. To create a solid plate, Mueller Hinton agar was liquefied, chilled, and then put onto sterile petri plates. It was made using a standardized inoculum (1.5108 CFU/ml, 0.5 McFarland) and sterile 0.9% saline water. The seeded agar plates were prepared with 6 mm wells. The test substance was added to the well in increments of 20, 40, 60, and 80 µl. At 37°C, the plates were incubated overnight. Zone diameters surrounding each well were used to assess the extract's antibacterial spectrum for each type of bacterial species. The agent's zone of inhibition diameters were compared to those of the commercially available control antibiotic, Ciprofloxacin (40 µl). In order to measure the resultant zone diameter using an antibiotic zone reader to the closest mm, the antibacterial drug was deducted from the test zones. To reduce error, the experiment was run three times; the mean data are shown.

Determination of Antifungal Assay

Well diffusion method was used to study the LPA-AgNPs and LPAM anti-fungal activity (Bonjar*et al.,* 2005). The fungi were revive onto SDA (Merck, Germany) and incubated for 24h at 37°C and 25°C for 2 to 5 days, respectively. The concentration of the fungal spore suspensions in sterile phosphate buffer saline (PBS) was set at 106cells/ml rolling on the agar medium's surface after dipping a sterile brush into the fungus solution. The plates were dried for 15 min. at RT. Using a decontaminated glass tube, 6 mm-diameter holes were made in the

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culture medium. For each well, 20, 40, 60, and 80 µl of fresh extracts were given until full. At 37°C, plates were incubated. Bio-activities were measured by measuring the width of the inhibitory zone after 24-hour incubation (in mm). As an antifungal positive control, ketoconazole (40 µl) was utilized. The means were computed for each experiment, which was carried out in triplicate.

6. Antioxidant Activity

In vitro antioxidant activity of LPA-AgNPs and *L. pyrotechnica*aerial part were determined by using catalase activity, peroxidase activity (enzymatic) and FRAP (non-enzymatic) assay.

Catalase (CAT) activity

With slight modifications, Teranishi*et al.,* 1974 technique's was used to measure catalase activity. A 1 gm sample was homogenized in chilled/ice cold phosphate buffer (50 mM; pH 7.0), and the supernatant was used as an enzyme extract after centrifuging at 10,000 rpm for 10 min at 4°C. The reaction mixture (3 ml) is made up of 0.1 ml of supernatant and 2.7 ml of PO_4 buffer (50 mM; pH 7.0). H_2O_2 (20 mM) was added in 0.2 ml to begin the reaction. For three minutes, at 410nm there absorbance was found to be decreased. The CAT activity was given as gm fw/sec/M H_2O_2 decrease.

Peroxidase activity

With the adjustments listed below, the Chance and Maehly, 1955 technique was used to measure the peroxidase activity. After homogenizing the 0.2gm sample with 10ml of phosphate buffer for 20 minutes, it was centrifuged at 10,000 rpm. The enzyme extract was obtained from the supernatant. Pyrogallol, H_2O_2, and 2.2 ml of phosphate buffer were also added. After adding 0.2 ml of enzyme extract, at 420 nm the absorbance was measured to assess how much purpurogallin had generated.

FRAP assay

Using the Pulido *et al.,* 2000 technique, ferric reducing antioxidant power (FRAP) tests were carried out. After adding 50 ml of methanol, the 5 gm sample was incubated for 24h at 37 °C. Sample from the incubator was filtered and dried in a petri dish. In accordance with 1 mg/ml, the dried material was dissolved in methanol. For FRAP estimate, the sample size range of 10 µl to 100 µl was chosen. Up to 1 ml of volume was made up with methanol. Prior to use, the FRAP reagent was incubated for 30 minutes at 37°C in 10 ml of acetate buffer (0.2 M; pH 3.6), TPTZ (10 mM) in 1 ml of HCl (40 mM), and 1 ml of $FeCl_3$ (20 mM). Each tube received 1 ml of FRAP reagent, which was carefully vortexed before being tentatively incubated for 30 minutes at 37°C. At 593 nm, absorbance was observed. The blank was made without extracting any material. Micro molars are used to express the results.

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Results And Discussion

1) Characterization Of Silver Nanoparticles

Scanning electron microscopy (SEM)

The morphology of LPA-AgNPs has been characterized by using SEM. SEM images of AgNPs shown in Figure 2 obtained by the reduction of $AgNO_3$ by aqueous extract of *Leptodeniapyrotechnica*aerialpart. The color of LPA-AgNPs was change from yellow to olive-green after reduction visible in Figure 1. The shape of AgNPs was spherical with the sizes between 23-86 nm. Likewise, the SEM results of Umaru *et al.,* (2020) showed the synthesized nanoparticles were in different magnification ranges which clearly demonstrated the presence of spherical shaped nanoparticle with mean average diameter of 70 nm.In the study of Rotimi *et al.,* (2018), the SEM analysis of the silver nanoparticle synthesized from aerial parts of *Callistemon citrinus*demonstrated triangular shaped materials. Also, Roy and Bharadvaja (2019) confirmed the shape (spherical) and size (55 nm) of the silver nanoparticle through SEM synthesized from *in vitro*-grown *Plumbagozeylanica*.In2016, Ali, *et al.* synthesized silver nanoparticles (AgNPs) using apple extract as a reducing agent and aqueous silver nitrate as the precursor. The characterization was done using Field emission scanning electron microscopy (FE-SEM). The FE-SEM image showed morphological structure of the AgNPs. The DLS assesses the average sizes of the AgNPs to be 30.25 ± 5.26 nm. Overall, the synthesized AgNPs were spherical in shape and exhibit aggregation.

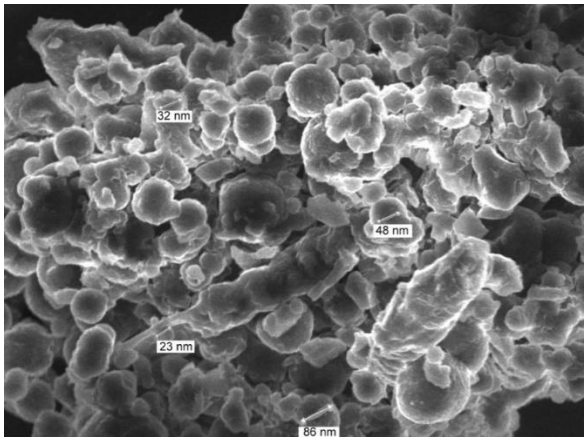

Figure 2: Scanning electron micrograph (SEM) of LPA-AgNPs

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR analysis of synthesized LPA-AgNPs was carried out to identify the functional groups of the active biomolecules playing roles of reducing as well as capping agents in the synthesis of nanoparticles. In the FTIR analysis a broad band emergence was observed at 3443 cm^{-1} (hydroxy group, H-bonded OH stretch) and at 1638 cm^{-1} (alkenyl C=C stretch). Various absorption peaks were observed at 1088 cm^{-1} (secondary alcohol, C-O stretch vibration in alcohol group), at 606 cm^{-1} (alcohol, OH out-of-plane bend) and between 1200-1400 cm^{-1} at 1389 cm^{-1} (phenol group or tertiary alcohol, OH bend). Similarly, Umaru *et al.*, in 2020 synthesized zinc oxide nanoparticles using *Leptadeniahastata* leaf extracts where the FTIR studies illustrated peak at 3363.66 cm^{-1} (functional group (O-H) bond), 2970.49 cm^{-1}

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(C-H bond) which showed the presence of methyl carbon in the chemical structure, 1772.35 cm-1 (C=C stretching), a peak at 1502.35 (C=C stretch in aromatic ring) and 1409.81 (C=O stretch in polyphenols and C-N stretch of Amide-I in protein).Sharifi-Rad and Pohl (2020) synthesized the silver nanoparticle from *Pulicaria vulgaris* Gaertn. aerial part extract. The FTIR study revealed several peaks at 639 cm^{-1} (C–Cl stretching vibrations), 1610 cm^{-1} (C=C and carbonyl (C=O) stretching vibrations of amide groups (amide I/II)), 2930 cm^{-1} (C–H stretching vibrations) and 3432 cm^{-1} (OH functional groups in phenolic components and alcohols with strong hydrogen bonds). In the study of Rotimi *et al.*, (2018), the FTIR study of the silver nanoparticle synthesized from aerial parts of *Callistemon citrinus*gave different absorption bands at about 1700 cm^{-1} in all spectra's establishing the C=O stretching owing to amide bond, another remarkable peak at 3400 cm^{-1} was seen in the crude extract which was ascribed to the O-H stretching from water as a result of the aqueous nature of the plant extracts used.In2016, Ali, *et al.*synthesized silver nanoparticles (AgNPs) using apple extract as a reducing agent and aqueous silver nitrate as the precursor. The FTIR spectrum of the AgNPs were recorded in order to identify the functional groups of the extract involved in the reduction of the synthesized AgNPs. The medium-intensity bands at 2364.89 cm^{-1} and 2342.38 cm^{-1} in the IR spectrum of the AgNPs indicate the presence of ethylene groups in the material bound to the AgNPs.

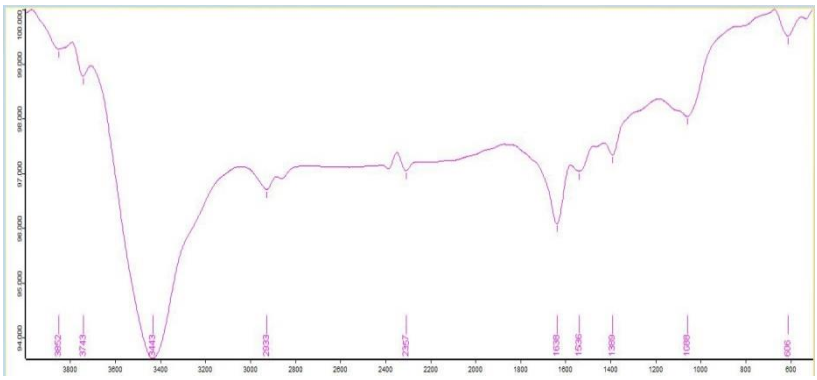

Figure 3: FTIR spectrum of LPA-AgNPs

2) Antimicrobial Activity

Antibacterial activity

The synthesized silver nanoparticles have possessed a good antimicrobial activity. Khatun *et al.* (2015) described that the antioxidant, anthelmintic, antimicrobial and other therapeutic potential of *L. pyrotechnica*aerial part extracts was due to the presence of alkaloids, flavonoids, reducing sugar, saponins, steroids, tannins compounds. In the present study the antibacterial activity of LPA-AgNPs and LPAM were evaluated against with four bacterial strains as gram positive *Staphylococcus aureus* (MTCC 3381), *Bacillus subtilis* (MTCC 10619) bacterial colonies, gram negative *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 0424) bacterial colonies as shown in Table 1 to 4. When the methanolic extract of the *L. pyrotechnica*aerial part and synthesized silver nanoparticles were compared for the antibacterial activity then maximum zone of inhibition against *S. aureus* recorded in

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plant-AgNPs 20mm at (80µl) and in case of methanolic aerial part extract zone of inhibition of 9 mm was recorded. AgNPs showed antibacterial activity against *Bacillus subtilis* at all concentration and highest inhibitory activity (33 mm) in 80µlwhile in methanolic extract activity was found in 80µl with 11 mm zone of inhibition. As shown in Table 4, plant-AgNPswas more effective against *E. coli* as compared with synthesized Ag nanoparticles. Antibacterial activity against *Pseudomonas aeruginosa*found better in both methanolic and silver NPs extract but more inhibited by synthesized silver nanoparticle plant extract (17 mm) as compared with *L. pyrotechnica*aerial part methanolic extract (9 mm). In the study of Mishra *et al.,* 2017, the ethanolic extract of *L. pyrotechinica* showed the highest activity against *P. aeruginosa* (21.00 ± 0.50 mm) and *C. parapsilosis* (14.00 ± 0.57 mm) and lowest against *S. aureus* (10.00 ± 0.49 mm) suggesting its efficacy in pneumonia, bacteremia, candidiasis, and urinary tract infections. The lowest activity was exhibited by residual portion against *S. typhi* (10.00 ± 0.11 mm). Likewise, in the study conducted by Ghaneisan*et al.,* in 2015 where they evaluated the antimicrobial activity of *L. pyrotechnica*against *E. coli, C. albicans,* and *A. niger* (62.5 µg/ml), and against *S. aureus* (>1000 µg/ml). The root and fruit methanolic extract of *L. pyrotechnica*generated the best results against *S. aureus* and *S. epidermidis* (Munazir*et al.,* 2012). The antimicrobial activities of methanol extract of *Leptadenia hastate* studied by Eze *et al.,*2022 showed that it was only sensitive against *Bacillus cereus* as compared to *Staphylococcus aureus, Pseudomonas aeruginosa,* and *E. coli* while aqueous extract does not show activity against any organism tested. The maximum zone of inhibition with the *B. subtilis* (24.3 ± 1.3 mm), followed *by E. coli* (20.0 ± 0.4 mm), was noticed at 150 µg/ml concentration of methanol leaves extract of *L. reticulata* biosynthesized AgNPs (Kumara *et al.,* 2015).

Antifungal activity

Silver nanoparticles are well-known as the most universal antimicrobial substances due to their strong biocidal effect against microorganisms, which has been used for over the past decades to prevent and treat various diseases (Oei*et al.,* 2012). AgNPs are also widely used as anti-fungal (Kim *et al.,* 2009), anti-inflammatory (Nadworny*et al.,* 2010). In this study LPAM and silver nanoparticles produced from this plant were displayed significant antifungal activity against with four fungal isolates such as *C. albicans, A. niger, T.reesei* and *P. chrysogenum*. A good antifungal activity has been recognized only in synthesized silver nanoparticles of *L. pyrotechnica*aerial part extract against *Trichoderma reesei*and *Aspergillus niger* of 12 mm at 80 µl concentration while the rest of show little or no zone of inhibition. The results were depicted in Table- 5-8. Similarly, Abdallah and Ali in 2022 investigated the therapeutic potential of green synthesized gold nanoparticles using ethanolic leaf extract of *Leptadeniahastata* (LH-AuNPs) where LH-AuNPs excreted antifungal activity against *Aspergillus fumigatus* with MIC 64 µg/ml and inhibited the radial growth of *A. fumigatus* by 30% compared to the control. LH-AuNPs caused distortion and collapse of fungal hyphae and deterioration of cell walls. The antifungal potential of hexane stem bark extract of *L. hastata* showed high inhibition rate of 3.22±0.04, 3.47±0.05, 3.55±0.05, 3.00±0.09 (*Aspergillus Flavus*) and 3.08±0.04, 3.27±0.05, 3.32±0.08, 2.82±0.08 (*Aspergillus*

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niger) respectively at day five over the other pathogens (Umaru *et al*., 2018). Similarly, in a study conducted by Ij*et al.,* in 2018 *L. hastata* showed significant inhibition with diameter rate with 3.38±0.08 to 5.00±0.09 days five for 25ppm to 250ppm. The antimicrobial activities of methanol extract of *Leptadenia hastate* studied by Eze *et al.*,2022 showed that it was sensitive against *Candida albicans* while aqueous extract does not show activity against the organism tested.

3) Antioxidant Activity

Enzymatic antioxidant activity

CAT is an antioxidant enzyme present in all cells responsible for the protection of cells from highly reactive hydroxyl radicals ($OH^{\cdot}$) by breaking it into non harmful products of H_2O and O_2 during the metabolic process. In the present study, silver nanoparticle of *Leptodeniapyrotechnica* aerial part showed 0.089 µM H_2O_2 reduce/gm Fwt/sec. highest CAT properties as compared to methanolic extracts (0.04 µM H_2O_2 reduce/gm Fwt/sec.). Soon and Tan also conducted a study for the evaluation of anti-oxidant activity of ethanolic extract of *Morinda officinalis* in streptozotocin-induced diabetic rats where they observed an increment in the CAT activity as compared to normal rats which indicate that the extract can reduce the reactive oxygen free radicals and improve the activities of the hepatic anti-oxidant enzymes. In 2016 Jothy*et al.,* studied the effect of *P. longifolia* leaf extract radioprotective activity on spleen by X-ray. The mice treated with *P. longifolia* leaf extract showed a significant decrease in catalase activity as compared to the control group where the catalase activity was seen to be highest.In 2015, Karami*et al.,* conducted an experiment to study the effects of silver nanoparticles (AgNPs) on the contents of free amino acids, protein, lipid peroxidation (MDA) and antioxidant enzymes activity, viz., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) in tomato plants. Catalase activity increased significantly both in shoots and the roots at all AgNP concentrations compared with the control.

The enzyme peroxidase is an oxidoreductase, which means it catalyzes the oxidation-reduction reaction by using the free radicals that turn various substances into oxidized form. The activity of peroxidase involves electron donation to ferricyanides and ascorbic acid to break them into harmless components (Albuquerque and Rocha, 2019). Peroxidases are the key enzyme of defense related pathways in plants (El-Sayed and Verpoorte, 2004) and play core role in response to wide range of pathogens (Van *et al.,* 2006). In this study, 0.263µM/L/gm dwt/sec. peroxidase activity was recognized with methanolic *L. pyrotechnica*aerial part extract and 0.657 µM/L/gm dwt/sec. with silver nanoparticle *L. pyrotechnica*aerial part extract. Accordingly, AgNPs showed maximum peroxidase activity in comparison to methanolic extracts. However, Nair *et al.,* (2018) observed the peroxidase activity of *L. pyrotechnica*at pH 6 to be the highest (72%) and lowest (8.5%) at pH 3, respectively.In Banerjee, *et al.,* 2014study investigated an efficient and sustainable route of AgNPs preparation from 1 mM aqueous $AgNO_3$ using leaf extracts of three plants, *Musa balbisiana* (banana), *Azadirachtaindica* (neem) and *Ocimumtenuiflorum* (black tulsi). A

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toxicity evaluation of these AgNPs containing solutions was carried out on seeds of Moong Bean (*Vigna radiata*) and Chickpea (*Cicer arietinum*). Spectrophotometric analysis revealed that POD activity was significantly higher ($p < 0.01$) in AgNPs treated chickpea samples in comparison to the control treated ones. POD activity increased both in a time-dependent and dose-dependent manner. Sharma, *et al.,* 2019study reports the optimization of various parameters for green synthesis of silver nanoparticles using aqueous leaf extract of *Ocimumgratissimum*. Peroxidase activity of the moong bean extracts was estimated. POD activity increased in moong bean seeds treated with different concentrations of AgNPs synthesized using *O. gratissimum* leaf extract. In 2015, Karami*et al.,* conducted an experiment to study the effects of silver nanoparticles (AgNPs) on the contents of free amino acids, protein, lipid peroxidation (MDA) and antioxidant enzymes activity, viz., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) in tomato plants. AgNPs stress resulted in increase in the POD activity in the leaves and roots of tomato plants. At 75 and 100 mg/l concentrations POD activity in the leaves increased by approximately 103 and 80%, respectively, and in the roots at 75 mg/l by 87% compared with the control.

Non-enzymatic antioxidant activity

Ferric ion reducing antioxidant power (FRAP) Assay

It is well recognized that free radicals play a significant part in a wide range of clinical manifestations. Due to their scavenging reactive oxygen species, they protect us from a variety of diseases and protect the antioxidant defense mechanism (Umamaheswari and Chatterjee, 2008). The highest Fe^{+3}ion reducing activity was observed with synthesized silver nanoparticle of *L.pyrotechnica*aerial part extract. The antioxidant activity measured by FRAP method is shown in Table 11, 12. The free radical scavenging activity of the synthesized nanoparticle and methanolic aerial part *L. pyrotechnica* extract was found to increase with increasing concentration (Figure 6-7). At 10 µl concentration of methanolic aerial part extract of *L. pyrotechnica*61.07 µM and at 100 µl 911.07 µM concentration of ferrous ion was recognized. While LPA-AgNPs exhibited 143.21 µM ferrous ions at 10 µl and 1103.21 µM at 100 µl that was maximum with respect to methanolic aerial part extract of activity. Khasawneh*et al.* (2011) investigated antioxidant, antiliPODygenase and cytotoxic activity of ethyl acetate, n-butanol and water partitioning fractions of aerial parts of the *Leptadeniapyrotechnica* by using FRAP, ABTS, DPPH and β-carotene bleaching assay for antioxidant activity and MCF-7 Human breast cancer cell line or cytotoxic activity which showed good antioxidant, anti-lipoxygenase and cytotoxic potentials.In2018, Bharathi and Bhuvaneshwari study reports an eco-friendly phyto-synthesis of silver nanoparticles (AgNPs) using aqueous flower extract of *Cassia angustifolia*. The reducing ability of the synthesized AgNPs and plant extracts was determined using FRAP assay. The percentage of antioxidant activity was increased with increasing concentration of AgNPs. FRAP-IC_{50} value for AgNPs was estimated to be 63.21 ± 0.75µg/ml. In addition, antioxidant activity of AgNPs was potentially equal to plant extract. Ferric ion-reducing activity of the AgNPs was estimated from their ability to reduce oxidized Fe^{3+} (color less) to Fe^{2+} (blue colour). Abdellatif*et al.,* 2022developed a green and cost-effective method for the synthesis of silver nanoparticles (AgNPs) using *Thymus vulgaris, Mentha piperita,* and *Zingiber officinale* extracts. In their

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work, the FRAP values for free extracts, $AgNO_3$, and AgNPs extract were estimated. All types of AgNPs showed significantly ($p < 0.05$) antioxidant activities higher than either the $AgNO_3$ or free plant extracts, indicating, indicating that incorporation of free extract with AgNPs possesses a higher reducing capacity than their free form due to the synergistic activities. Konappa*et al.*, 2021 also study aimed to synthesize silver nanoparticles using *T. harzianum* cell filtrate and investigate different bioactive metabolites based on LC-MS/MS analysis. The ferric reducing antioxidant power (FRAP) of AgNPs (0.2–1.0 mg/ml) and culture filtrate increased with increasing concentration and reached a peak of 66.4% and 73.98% at 1 mg/ml, respectively. The standard, ascorbic acid, exhibited maximum reducing power, with 97.49% at 1 mg/ml.

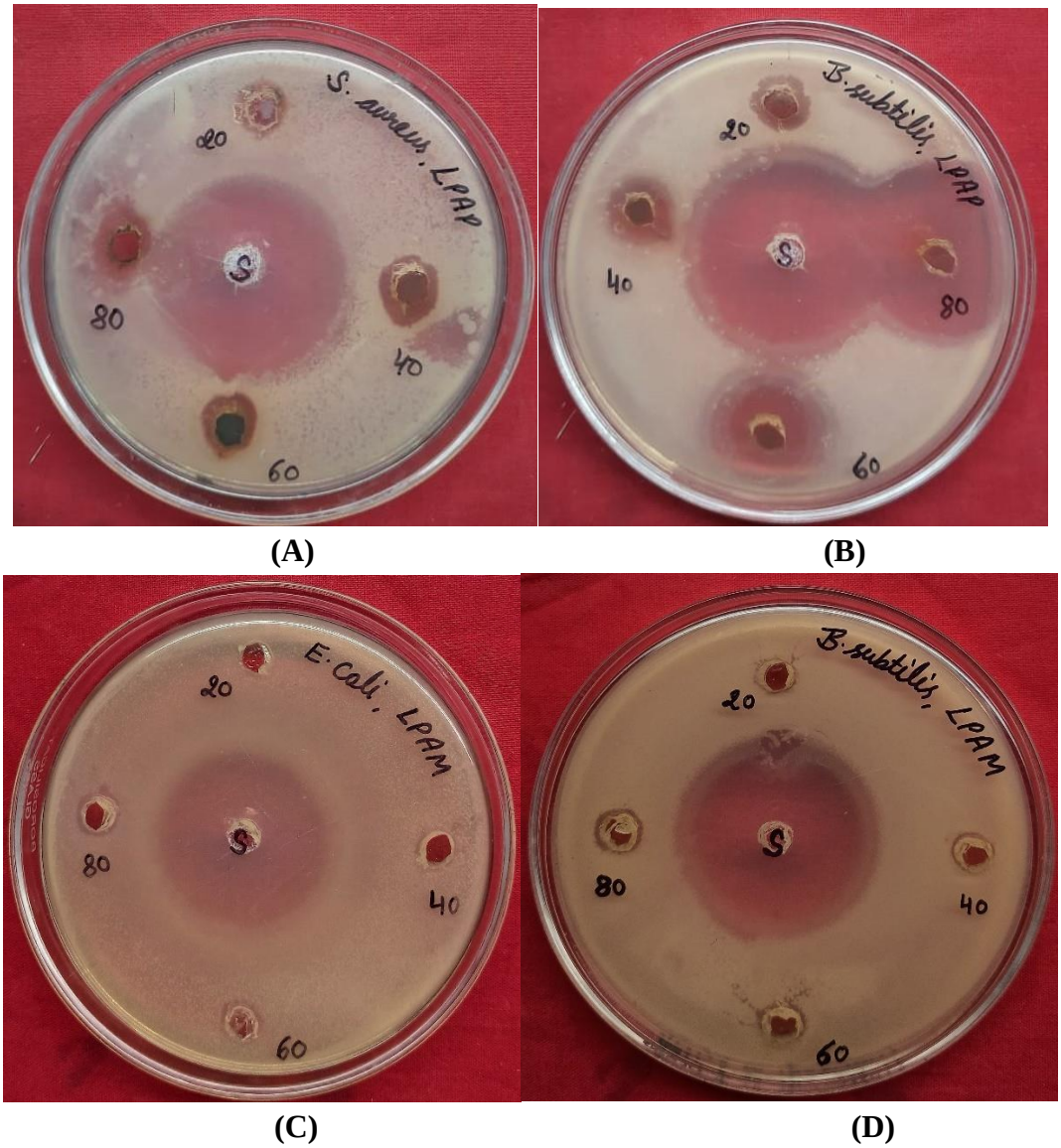

Figure 4: Maximum antibacterial activity of synthesized silver nanoparticle (LPA-AgNPs) and *L. pyrotechnica*aerial part methanolic (LPAM) extract against (A) *Staphylococcus aureus* (B) *Bacillus subtilis* (C) *Escherichia coli* and (D) *Bacillus subtilis*

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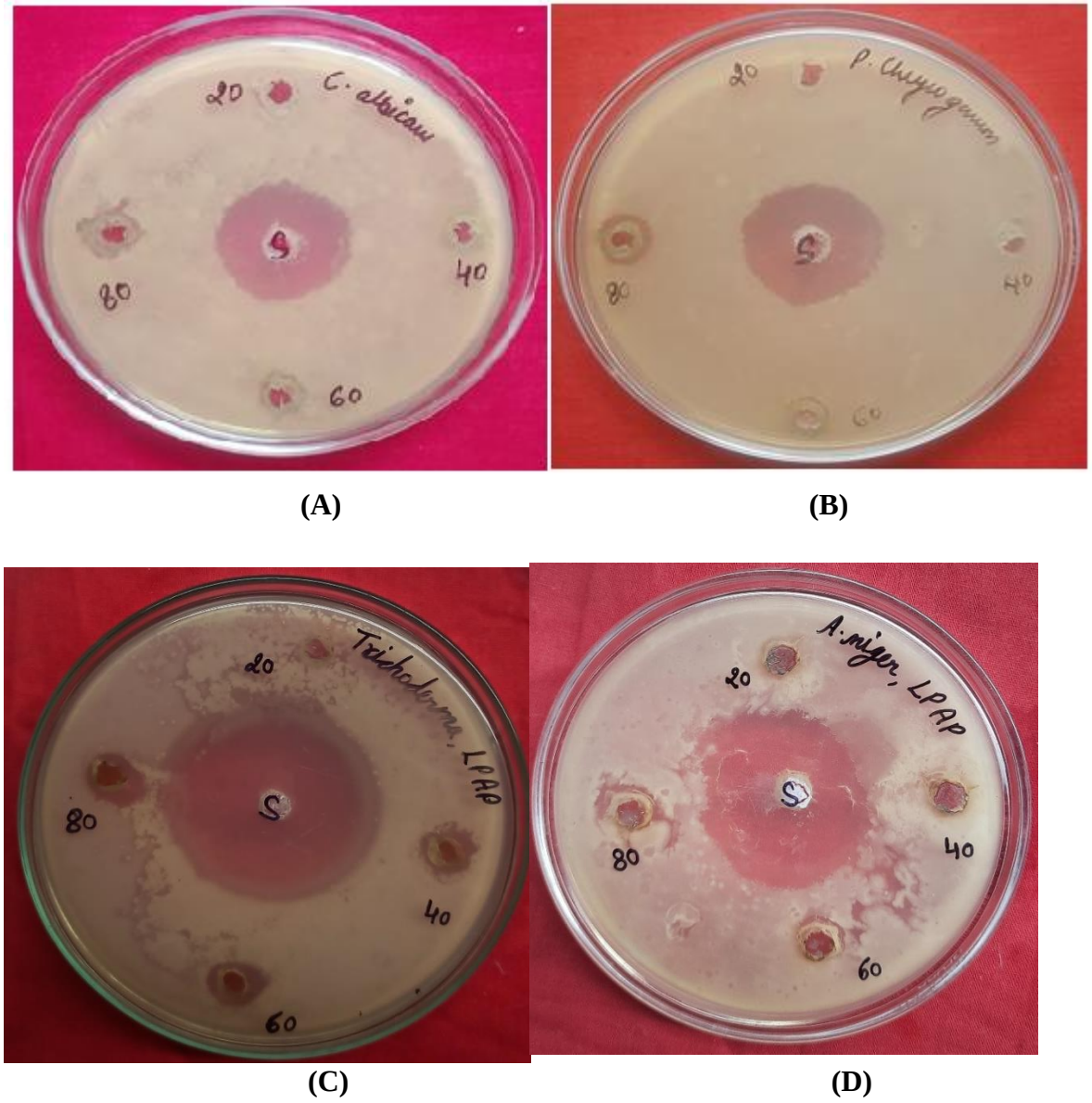

Figure 5: Maximum antifungal activity of synthesized silver nanoparticle (LPA-AgNPs) and *L. pyrotechnica* aerial part methanolic (LPAM) extract against (A) *Candida albicans* (B)*Penicilliumchrysogenum* (C) *Trichoderma reesei*and (D) *Aspergillus niger*

Table 1: Antibacterial activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and synthesized nanoparticles (AgNPs) against *Staphylococcus aureus*

Plant samples	Inhibition zone (mm)				
	Standard	20µl	40µl	60µl	80µl
LPAM	34	Nil	Nil	8	9
LPA-AgNPs	34	10	13	14	20

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Table 2: Antibacterial activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and synthesized nanoparticles (AgNPs) against *Bacillus subtilis*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
LPAM	34	8	9	10	11
LPA-AgNPs	34	11	13	20	33

Table 3: Antibacterial activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and synthesized nanoparticles (AgNPs) against *Pseudomonas aeruginosa*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
LPAM	34	Nil	Nil	8	9
LPA-AgNPs	34	13	14	15	17

Table 4: Antibacterial activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and synthesized nanoparticles (AgNPs) against *Escherichia coli*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
LPAM	34	Nil	8	9	11
LPA-AgNPs	34	10	12	13	14

Table 5: Antifungal activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and silver nanoparticles (AgNPs) against *Candida albicans*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
LPAM	25	Nil	Nil	7	9
LPA-AgNPs	25	Nil	Nil	Nil	Nil

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Table 6: Antifungal activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and silver nanoparticles (AgNPs) against *Aspergillus niger*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
LPAM	25	Nil	Nil	Nil	Nil
LPA-AgNPs	25	Nil	Nil	9	12

Table 7: Antifungal activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and silver nanoparticles (AgNPs) against *Trichoderma reesei*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
LPAM	25	Nil	Nil	7	9
LPA-AgNPs	25	Nil	10	11	12

Table 8: Antifungal activity of *Leptodeniapyrotechnica*aerial part methanolic (LPAM) extract and silver nanoparticles (AgNPs) against *Penicilliumchrysogenum*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
LPAM	25	Nil	Nil	7	10
LPA-AgNPs	25	Nil	Nil	Nil	Nil

Table 9: Catalase activity of *Leptodeniapyrotechnica*and synthesized nanoparticles

Plant Samples	Catalase activity (µM H_2O_2 reduce/gm Fwt/sec.)
*L. pyrotechnica*aerial part	0.04±0.02
LPA-AgNPs	0.089±0.011

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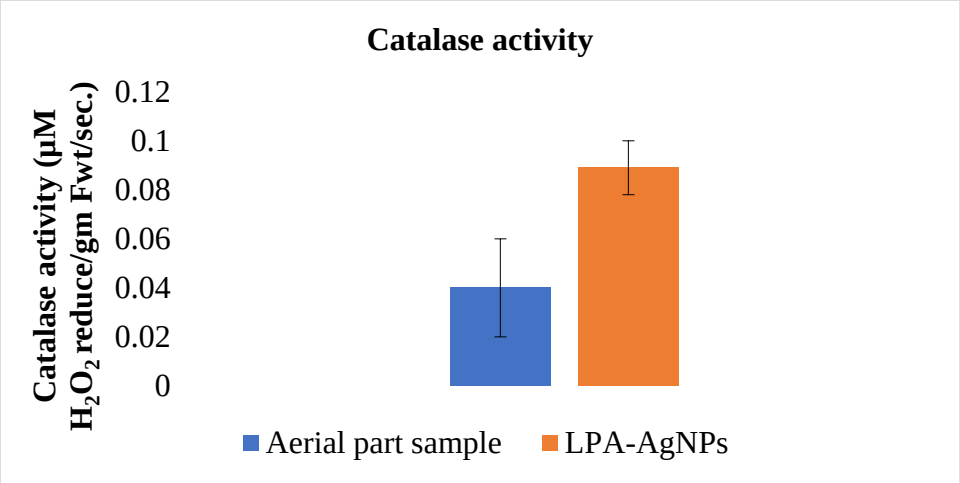

Figure 5: Catalase activity of *Leptodeniapyrotechnica*aerial part extract and silver nanoparticle

Table 10: Peroxidase activity in of *Leptodeniapyrotechnica*and synthesized nanoparticles

Plant Samples	Peroxidase activity (µM/L/gm dwt/sec.)
*L. pyrotechnica*aerial part	0.263±0.003
LPA-AgNPs	0.657±0.028

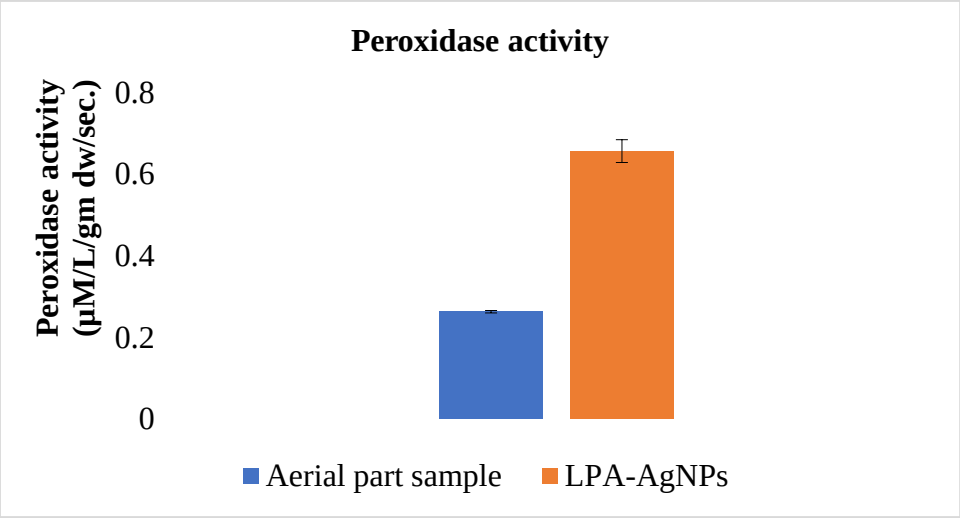

Figure 6: Peroxidase activity *Leptodeniapyrotechnica*aerial part extract and silver nanoparticle

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Table 11: FRAP activity of *Leptodeniapyrotechnica*aerial part

***L. pyrotechnica*aerial partsample**	**Concentration (µM)**
10 µl	61.07±2.474295
20 µl	192.5±3.243809
30 µl	221.07±1.24
40 µl	431.07±3.23
50 µl	542.5±3.97
60 µl	645.35±3.00
70 µl	658.92±4.06
80 µl	640.35±2.22
90 µl	720.35±2.72
100 µl	911.07±3.86

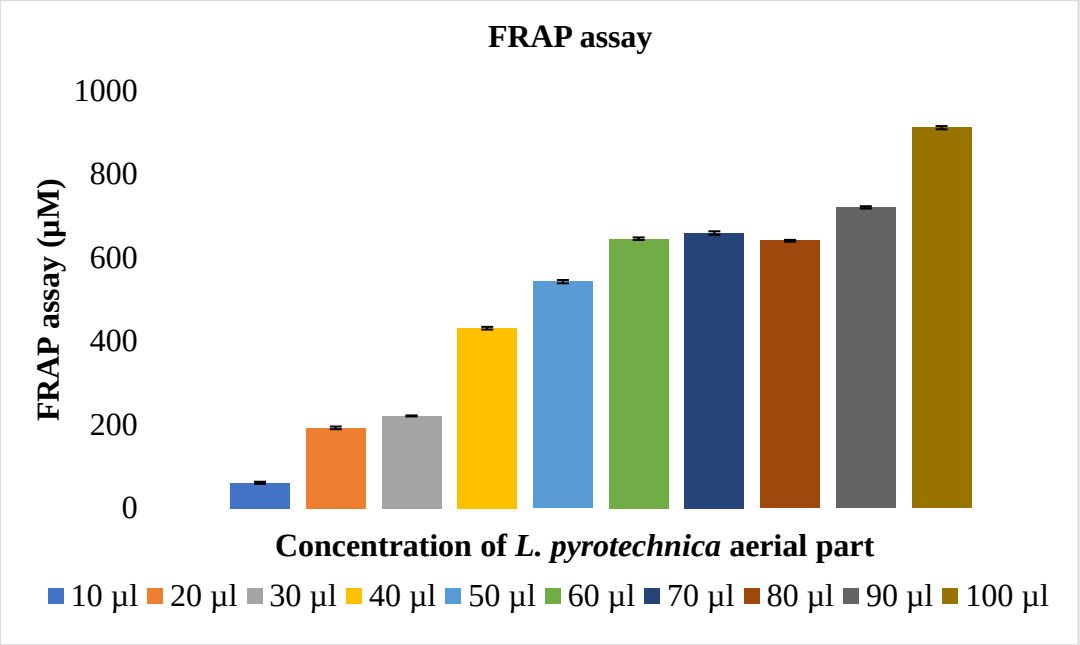

Figure 6: FRAP assay *Leptodeniapyrotechnica*aerial part

Table 11: FRAP activity of LPA-AgNPs

LPA-AgNPs	**Concentration (µM)**
10 µl	143.21±3.84
20 µl	323.92±2.61
30 µl	398.2±3.31
40 µl	456.78±3.71

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50 µl	548.21±4.17
60 µl	682.5±3.63
70 µl	736.07±4.15
80 µl	871.78±2.30
90 µl	994.6±2.48
100 µl	1103.21±1.53

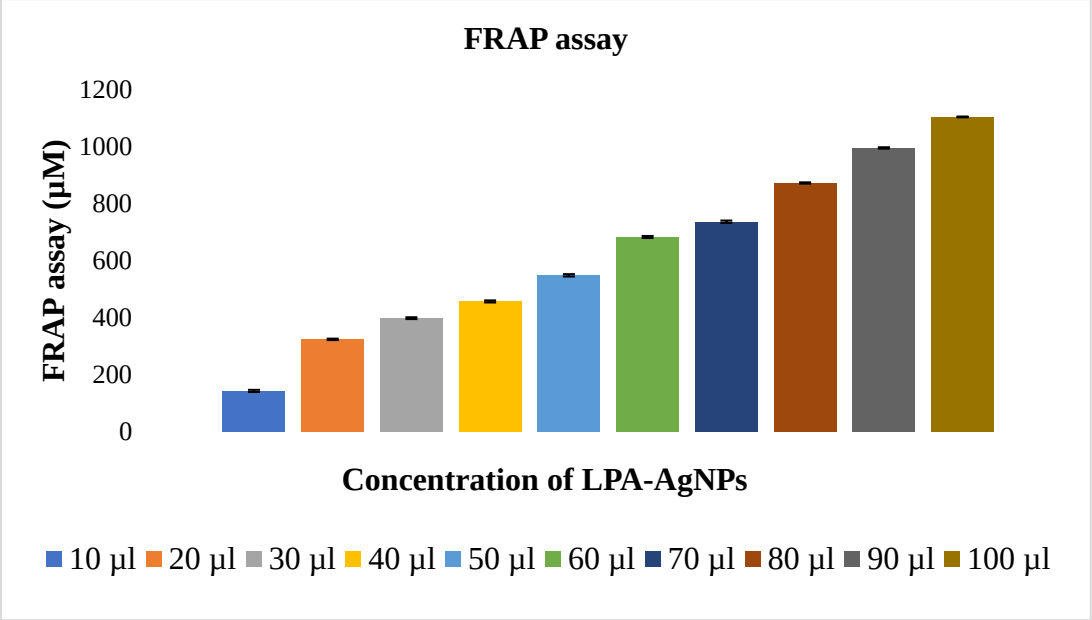

Figure 7: FRAP assay of LPA-AgNPs

Conclusion

Medicinal plants are the local heritage with the global importance. World is endowed with a rich wealth of medicinal plants. Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. One such plant is *Leptodeniapyrotechnica*and its parts are traditionally used for different purposes. The results of the present study indicate that the silver nanoparticle synthesized from the aerial part of *Leptodeniapyrotechnica*exhibit good antioxidant property as they expressed their ability to scavenge H_2O_2 free radicals and also reduced oxidized Fe^{3+} (color less) to Fe^{2+} (blue colour) compared to the methanolic extracts of *L. pyrotechinca.* The LPA-AgNPs also exhibited a better antimicrobial activity when compared to the methanolic extract of *L. pyrotechnica* aerial part. The nanoparticles were spherical in shape with varying sizes ranging from 23 to 86 nm. This is a simple, eco-friendly process and has potent applications in biomedical and pharmaceutical applications. It can be concluded that the aerial part of *L. pyrotechinca*can be used efficiently to produce AgNPs and hence can be explored as a new source of alternative medicine for treating many human ailments and provide a convincing support to its future clinical use in modern medicine.

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