Research Article

ANTIOXIDANT, ANTIMICROBIAL, ENZYME INHIBITORY AND CYTOTOXIC EFFECT OF *TAVERNIERA COUNEIFOLIA* PLANT MEDIATED SILVER NANOPARTICLES

Muhammad Nisar Ul Haq[1], Ghulam Mujtaba Shah[1], Rahmat Ali Khan[2]*, Wahid Ullah[2], Khursheed Ur Rahman[1], Zamarud Shah[3], Ammara Khan[4], Feroz Khan[5], Matiullah[6], Ihsan Ullah[7], Hajra Afeera Hamid[2]

[1]Department of Botany Hazara University Mansehra KP Pakistan, [2]Department of Biotechnology University of Science and Technology Bannu KP Pakistan, [3]Department of Biotechnology, Abdul Wali Khan University Mardan, [4]Department of Zoology, Wild life and Fisheries, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan, [5]Department of Zoology, University of Science and Technology Bannu KP Pakistan, [6]Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan, [7]Department of Botany, University of Science and Technology Bannu, KP, Pakistan.

Correspondence:
Rahmant Ali Khan, 2Department of Biotechnology University of Science and Technology Bannu KP Pakistan

Email: rahmatgul_81@yahoo.com

DOI:
10.38106/LMRJ.2024.6.4-06
Received: 16.09. 2024
Accepted: 09.12.2024
Published: 31.12.2024

ABSTRACT

This study was conducted to evaluate antioxidant, anticancer, enzyme inhibitory and antimicrobial potential of methanolic extract and AgNPs of *T. couneifolia*. The (DPPH) 1,1-diphenyl-2-picrylhydrazyl, (ABTS) 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), metal chelating, phosphomolybdenum, hydrogen peroxide scavenging and (FRAP) ferric reducing antioxidant power activities, antibacterial potential against *Klebsiella pneumonia*, *Escretia coli*, *Acetobacter*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, antifungal against *Aspergillus niger* and *Aspergillus flavus* and enzyme inhibitory including alpha amylase and acetyl cholinesterase were used to evaluate the methanolic extract and the synthesized AgNPs' antioxidant potential. T AgNPs displayed the highest enzyme and radical scavenging capabilities in comparison to the plant extract. Additionally, the BSL test was used to measure the cytotoxicity activity. Our findings unequivocally demonstrated that biosynthesized AgNPs had antimicrobial, antioxidant, enzyme inhibitory and cytotoxic action and reduced the growth capability.

Key words: Silver nanoparticles, antimicrobial, antioxidant, cytotoxicity, enzyme inhibition, *T couneifolia*.

INTRODUCTION

Reactive oxygen species (ROS) are activated oxygen molecules having free radicals such as super-oxide anion, hydroxyl radicals and non-radical species such as hydrogen peroxide and singlet oxygen (ROS) (1). The majority of oxidative disorders are brought on by oxidative stress through free radicals. (2-3). The ROS being produced on regular basis and need to neutralized in order to avoid any harmful effects, therefore a rebuts antioxidant defense system is required. A significant etiological element concerned in the increase of many chronic human diseases on of the key mechanism is the production of free radicals, these diseases include diabetes mellitus, cancer, atherosclerosis, arthritis, neurological diseases. The ageing process also involves generation of free radicals that results in oxidative damage. Antioxidant treatment has assumed an important role in the management and prevention of these conditions (4).

Although silver nanoparticles' anti-microbial impact has been extensively studied, their mechanism of action is still not entirely understood (5-6). Since it enhances membrane permeability and cell death to bacteria and fungi, the nanoparticles' tiny size and larger surface area offer chances for interactions with bacterial cells, which were the main causes of this activity. Silver nanoparticles can alter the permeability of the cell membrane and break down cells (7-8). Silver nanoparticle interactions with amino acids and enzymes resulting in bonding with amino acids (particularly to -SH group), formation of ROS, which then get attached to the cell membrane surface, penetrate in the bacteria and cause disruption of the cell function. Furthermore, it is explained by the fact that DNA, which is

primarily composed of the soft bases sulphur and phosphorus and which would undoubtedly cause cell death in the case of silver interacts with DNA (9-10).

Metal nanoparticles can function as both stabilizing and reducing agents in the synthesis of nanoparticles because of their distinct electrical, optical, magnetic, chemical, and mechanical characteristics. Numerous high-tech industries use these nanoparticles, such as medicine for imaging, rapid diagnostics, drug administration, tissue regeneration, cancer treatments, bactericidal and fungicidal agents, antioxidants, and the creation of new materials (11).

AgNPs production can be accomplished chemically (12), electrochemically (13), using X-rays (14), UV radiation treatment (15), or by photochemical reduction, ultrasonic aided, microwave, or laser ablation (16-17). Because the chemical method of producing nanoparticles was popular but could have negative environmental implications, researchers had to devise a green chemistry technique for nanoparticle synthesis (18). Utilizing bio resources (bacteria, fungi, yeasts, algae or plants) to create nanoparticles is a very practical, affordable and environmentally beneficial alternative (19). Therefore this study was conducted to evaluate antioxidant, anticancer, enzyme inhibitory and antimicrobial potential of methanolic extract and AgNPs of *Taverniera couneifolia*.

METHOD

Plant collection:

The plant *T. couneifolia* was collected from the area of Barganato District Bannu, Pakistan in its mature conditions identified by Prof. Dr. Ghulam Mujtaba Shah and a voucher specimen was submitted at Harbarium in the Department of Botany Hazara University Mansehra, Pakistan. The plant was collected and dried in a shed after three rounds of washing and after drying the plant was grind in to fine powder. 100g of plant powder was mixed with 300 mL of methanol for extraction. The filtrate was filtered and dried with a rotary evaporator after seven days (20).

Green synthesis and Characterization

The slandered technique was used to carry out the production of the silver nanoparticles. (20-21). FTIR, XRD, UV-Vis, EDX and SEM were among the commonly employed techniques used to characterize the synthesized AgNPs (20).

In vitro Antioxidant activity

DPPH radical scavenging activity

The capacity of synthetic stable DPPH radicals to be quenched into non-radical form was used to assess the silver nanoparticles' antioxidant properties (22). 2.7 ml of 2.5 mM DPPH was added to the reaction mixture that contained 0.3 mL of nanoparticles (25, 50, 100 µg/mL). The colour change in the reaction mixture was assessed spectrophotometrically at 517 nm after 45 minutes of incubation at 37 °C. The following formula was used to express the radical-scavenging activity (RSA) as a percentage of inhibition:

$$\%RSA = (ADPPH - AS)/ADPPH \times 100$$

ABTS scavenging activity

According to the method of Hatano et al (23), (ABTS) the 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulphonicacid) decolorization assay was used to measure the samples' overall antioxidant activity. The unit of total antioxidant activity is defined as the concentration of ascorbic acid with an equivalent antioxidant activity, expressed as mM ascorbic acid equivalent sample extract. (TAA).

Antioxidant power assay for ferric reduction

The Pulido et al. (24) technique was used to determine the antioxidant capabilities of various extracts from the material. In the ferric reducing antioxidant power (FRAP) experiment, equivalent concentration was defined as the quantity of antioxidant that increased absorbance. This amount was equivalent to the absorbance value of a solution containing 1 mmol/L of Fe (II).

Phosphomolybdenum assay

The green phosphomolybdenum complex production method developed by Prieto et al. (25) was used to assess the antioxidant activity of the samples. Ascorbic acid equivalents (mg) per gram of extract are the results that are provided as average values.

Assay for scavenging hydroxyl radicals

Using the approach proposed by Mahendran et al. (26), the hydroxyl radical inhibition of nanoparticles was measured. Using the Fenton reagent (Fe3+ascorbate-EDTA-H2O2), a hydroxyl radical was produced. The final reaction volume is 1 ml and contains varied quantities of nanoparticles (1-250 µg/ml), 2-deoxy-2-ribose (2.8 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), FeCl3 (100 mM), EDTA (100 mM), H_2O_2 (1.0 mM) and ascorbic acid (100 mM). At 37°C, the reaction mixture was incubated for one hour. By adding 1 ml of 2.8% TCA, reactions were stopped. The addition of 1 ml of 1% aqueous TBA and subsequent incubation at 90°C for 15 minutes produced the chromogenic adduct. At 532 nm, the intensity of the chromogen was measured in comparison to a suitable blank solution. As a positive control, catechin (a traditional OH scavenger) was used.

Invitro antimicrobial assays

Antibacterial activity

Antimicrobial potential was evaluated on solid (Agar-agar) medium on petri plates using the agar well diffusion technique followed (27). The activity involved the employment of two gram-positive bacteria (*Staphylococcus epidermidis* and *Staphylococcus aureus*) and three gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Acetobacter*). All of the bacterial strains were strellised for 24 hours in liquid broth.

After that media was prepared by adding 28g/L of nutrient agar in distilled water and then all of the media was autoclaved for 20 minutes at 121°C to sterilize it. Then media was poured in to patridishes. After solidification of the media bacterial colonies were spread on the media plats. After that wells were made and T AgNPs and T extract (100µg/mL) were injected into the wells through micropipettes. Moxifloxacin was used as positive control.

Antifungal activity study

Antimicotic potential was evaluated on saboured dextrose agar medium on petri plates using the agar well diffusion technique (27). Two fungal strains (*Aspergillus niger* and *Aspergillus flavous*) were used in the activity. All of the fungal strains were strellised for 72 hours in Saboured dextrose broth.

After that media was prepared by adding 68g/L of nutrient agar in distilled water afterward, all of the media was autoclaved for 20 minutes at 121°C to ensure its sterilization. Then media was poured in to patridishes. After solidification of the media fungal colonies were spread on the media plats. After that wells were made and T AgNPs and T extract (100µg/mL) were injected into the wells through micropipettes. Terbenafine was used as positive control.

Cytotoxic test using brine shrimp

By using a brine shrimp cytotoxicity assay, the toxicity of T couneifolia extract and AgNPs capped with T couneifolia was measured (28). 2.5 mL of sea water containing 10 larvae was mixed with solutions of 250, 500 and 1000 µg/mL of AgNPs and plant extract. Each sample vial received ten brine shrimp larvae that were carefully chosen and the final volume of each vial was adjusted with artificial sea water. Each vial contains 0.5 mL of plant extract, AgNPs, 4.5 mL of artificial seawater and 10 brine shrimp larvae. Each vial contains 5 mL of test solution in total. The tests were carried out three times. For the following 72 hours, all sample vials were stored at room temperature in an incubator, uncovered and near a light source. The quantity of alive shrimp was counted and meticulously recorded using a magnifying glass after 24, 48 and 72 hours.

In-vitro enzyme inhibitory assay

Acetyl cholinesterase assay

In this study, the Pd (II) complex's anti-AChE properties were confirmed using the accepted methodology (22). AChE hydrolysis rates (V) at various substrate AChE concentrations were computed in a 1 mL test mixture containing 10 mM DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)] and 50 mM phosphate buffer, pH 7.4, at 25°C (0.05–1 mM). 20 mL of diluted Bungarussindus venom were added to the reaction mixture indicated above.

The mixtures were then incubated at 37°C for 5 minutes. The reaction between the enzyme and substrate (AChE-ACh) got going quite quickly when the substrate (ACh) was added. AChE-ACh hydrolysis was measured using a UV-1602 double beam spectrophotometer from BMS Biotechnology Medical Service by measuring the formation of thiolate di-anion of DTNB every 15 seconds for a period of 90 seconds. The amount of yellow colour generated by the AChE-ACh reaction was used to measure the AChE activity. Three tests were performed on each sample.

Alpha-amylase Assay of AgNPs

AgNPs' in-vitro amylase enzyme inhibitory efficacy was evaluated using the Sher et al. (22) procedure. 0.1 g of potato starch was dissolved in 100 mL of a 16 mM C2H3NaO2 buffer to produce a starch solution (0.1 percent w/v). The enzyme was diluted by combining 700 µL of deionized water with 300 µL of stock amylase (250 units/mL). Sodium potassium tartrate and 3, 5 dinitro salicylic acid (96 mM) were combined to create a calorimetric reagent. At a concentration of 1 mg/mL, the standard (Metformin) and the stock solution of the extracts and AgNPs were prepared in deionized water and separated into 100, 200, 300, and 400 µg/mL. Each of the four strengths of plant extracts, AgNPs, or ordinary metformin was used in 250 µL of potato starch solution, 250 µL of amylase, and five minutes at room temperature (25 °C). 250 µL of sodium potassium tartrate and dinitro salicylic acid were added to each quantity. Water was used as a blank to monitor the reaction at 450 nm. As a positive control, metformin, a medication that is frequently prescribed, was employed. Two iterations of the experiment are required. The % inhibition was calculated using the following equation.

$$\% \ Inhibition = (Ac\text{-}As)/Ac100$$

The enzyme activity of plant extracts and AgNPs was measured by Ac, while the enzyme activity of conventional drugs is measured by As.

RESULT

DPPH Radicle scavenging Activity

It is used to examine the in vitro scavenging abilities of medicinal plants since DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) is a free radical capable of acquiring an electron from an antioxidant. The extract of *Taverniera couneifolia* and Synthesized AgNPs for DPPH show free radical scavenging action in Figure 1. Plant extracts and

AgNPs were employed at quantities ranging from 25 to 100 µg/mL. *T. couneifolia* has an 83.93% scavenging value, and T AgNPs have a 91 % scavenging value.

Assay of ABTS radical cation:

The 2, 2-Azobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation-removing capacities of extracts, AgNPs and a reference substance, ascorbic acid, were examined. Plant extracts and AgNPs were employed on the concentration of 25 to 100 µg/mL. The scavenging abilities of both plant extracts against ABTS radical cations were found to be different. *T.couneifolia* extract (72%) and T AgNPs (81.5%) exhibited scavenging activity of the ABTS radical, Figure 2 showed the greatest ABTS radical scavenging activity It was found that *T.couneifolia* extract and T AgNPs, which had strong activity near the reference compound, had ABTS radical scavenging capacity of 94%.

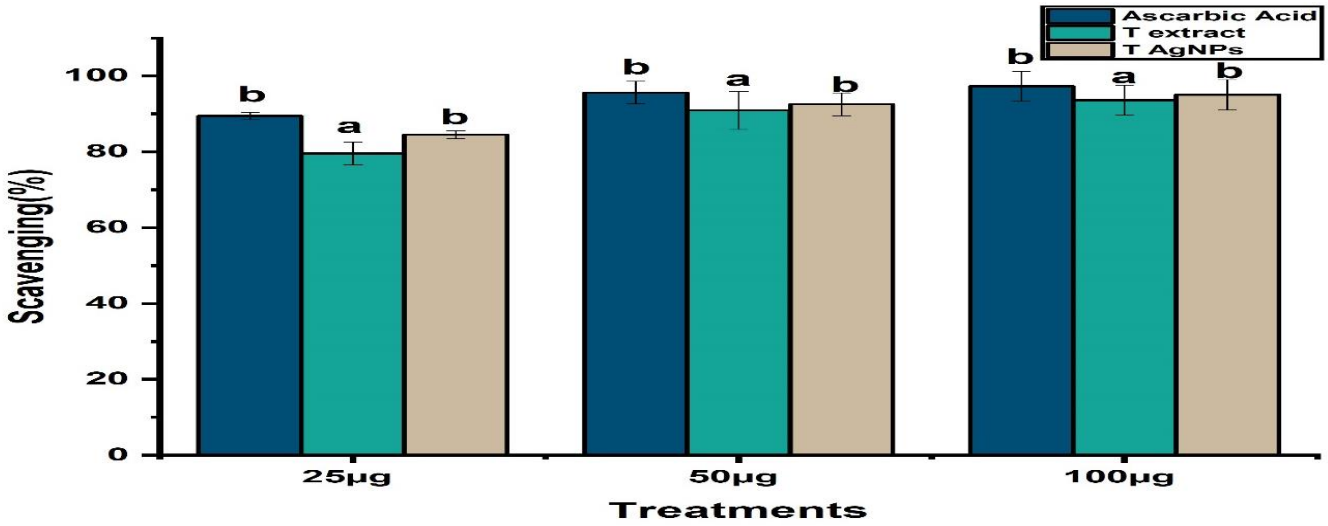

Figure 1. DPPH scavenging potential of Plant methanolic extract and AgNPs.

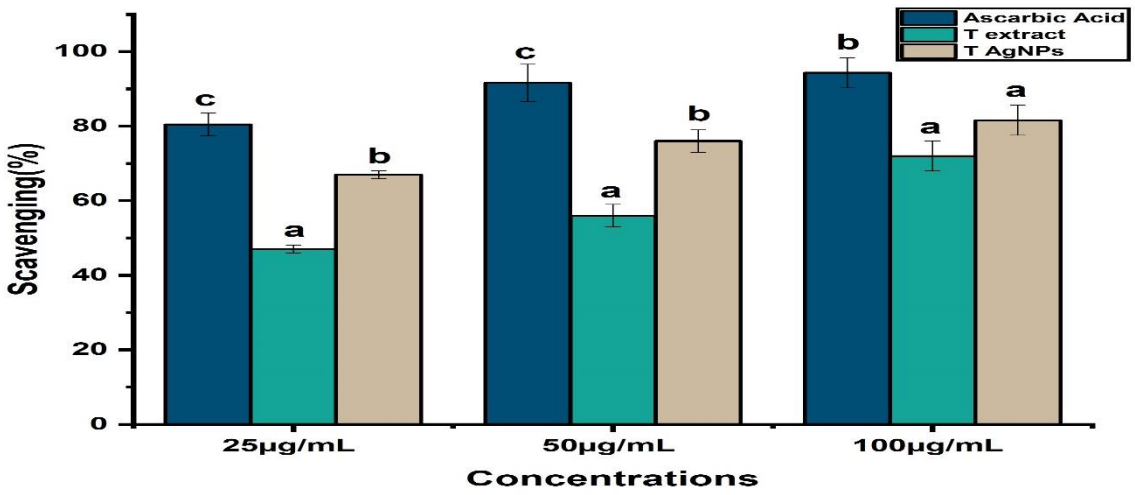

Figure 2. ABTS scavenging potential of Plant methanolic extract and AgNPs.

Hydrogen peroxide scavenging activity

Because it is transformed into hydroxyl free radical in living cells, hydrogen peroxide may be hazardous to them. At particular locations in the cell, hydroxyl radicals may readily penetrate the cell membrane, and the majority of biomolecules react with them, resulting in severe tissue damage and eventual cell death. The removal of hydroxyl

radicals is critical to the health of the biological system. Figure 3 shows the values of scavenging effect of hydroxyl radical by *T couneifolia* extracts and Synthesized AgNPs along with ascorbic acid.

Plant extract and AgNPs were employed at quantities ranging from 25 to 100 µg/mL. *T couneifolia* extract and T AgNPs showed pronounced scavenging activity (78 %) and (91 %) which indicates that both T extract and T AgNPs have strong scavenging capacity as compare to control (97 %).

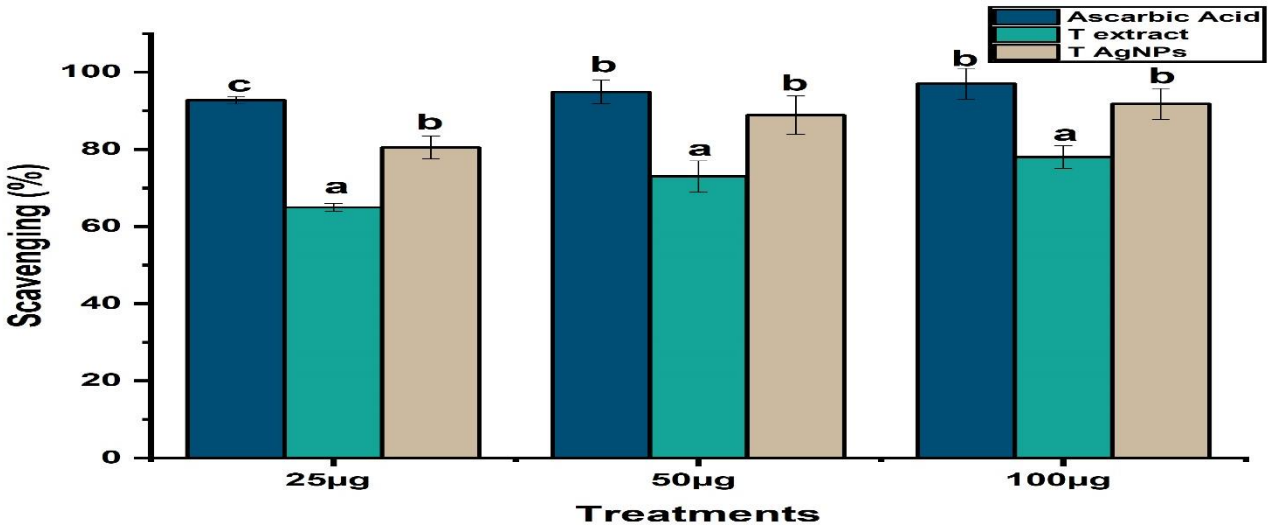

Figure 3. H2O2 scavenging potential of Plant methanolic extract and AgNPs.

Reducing ferric ion Power Assay

In FRAP test, the ability *T couneifolia* extracts and synthesized AgNPs together with ascorbic acid were investigated. Plant extracts and AgNPs were employed at quantities ranging from 25 to 100 µg/mL. Silver nanoparticles has demonstrated lowering power of Fe ions as comparison to control the higher concentrations of extract and synthesized AgNPs ranges from Ascorbic Acid 94.83 %, T extract 70.26 % and T AgNPs 86 % (Figure 4). Both the extract and AgNPs has good reducing properties. This might be caused by the phenolic content and flavonoids found in the extracts of, *T couneifolia* and synthesized AgNPs.

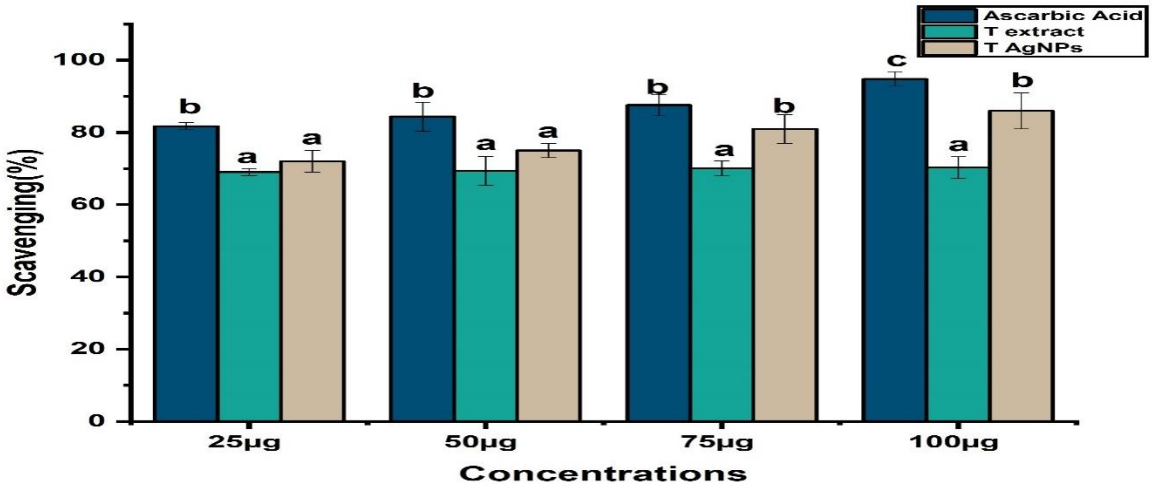

Figure 4. Ferric Chloride scavenging potential of Plant methanolic extract and AgNPs.

Phosphomolybdate or Total antioxidant activity

Ascorbic acid was used as a reference along with Phagnalon niveum, Taverniera couneifolia extracts, and Synthesized AgNPs to measure the Phosphomolybdate or total antioxidant activity. Ascorbic acid showed 94%, T extract 84 % and T AgNPs 89% both extracts and plant mediated AgNPs have shown similar results. This could be as a result of the high phenolic contents and the presence of flavonoids, as depicted in Figure 5.

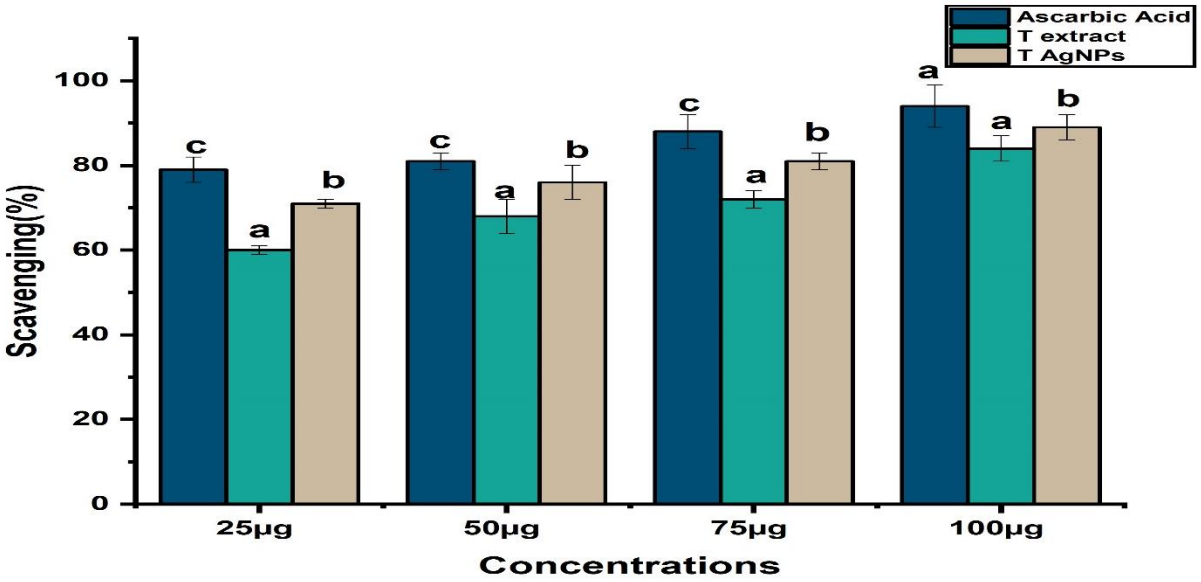

Figure 5. Phosphomolybdate scavenging potential of Plant methanolic extract and AgNPs.

Antibacterial potential of plant extracts and AgNPs

It was found that the antibacterial potential of T. couneifolia and T. AgNP extracts is nearly 10 mm or greater. Moxifloxacin was utilised as the control. Five distinct bacterial strains, three of which were Gram negative (*Klebsiella pneumonia, Escretia coli, Acetobacter*) and two of which were Gram positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*). As a comparison to plant extracts, synthesised AgNPs shows the highest zone of inhibition. *T. couneifolia* extract and T AgNPs the highest zone of inhibition was reported in *Klebsiella pneumonia* 17mm by T AgNPs as shown in Figure 6.

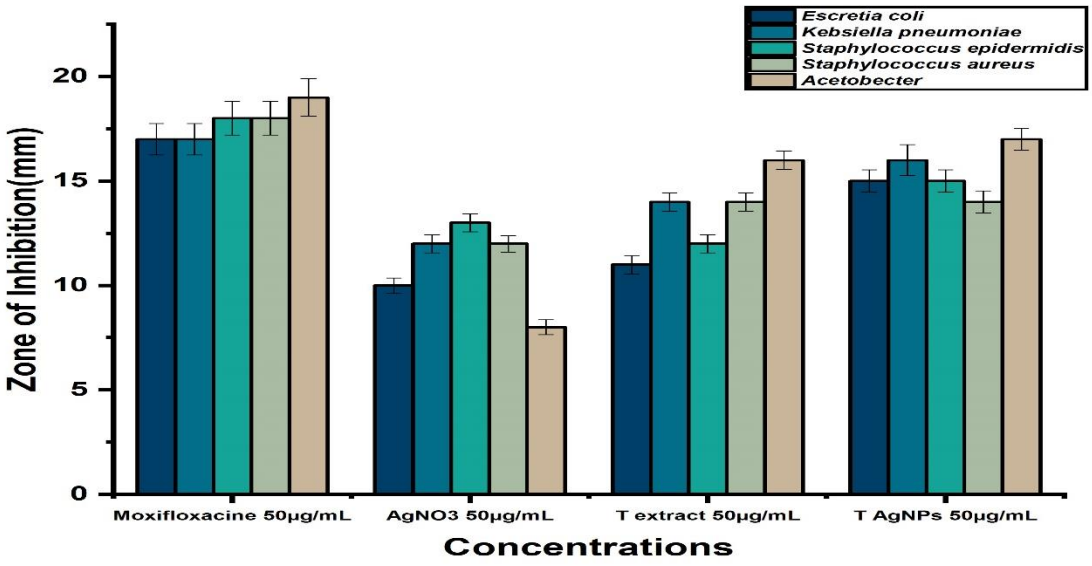

Figure 6. Antibacterial effect of T couneifolia methanolic Extract and TAgNPs

Antifungal effect of plant extracts and AgNPs

Aspergillus flavus and *Aspergillus niger* were utilised as test fungi, and Terbenafine was used as a positive control in this work to assess the antifungal capability of both plant extracts and manufactured Silver nano particles. As compare to control it was shown that the maximal zone of inhibition was 15mm for both the fungal strains by TAgNPs which is greater than that of plant extract Figure 7.

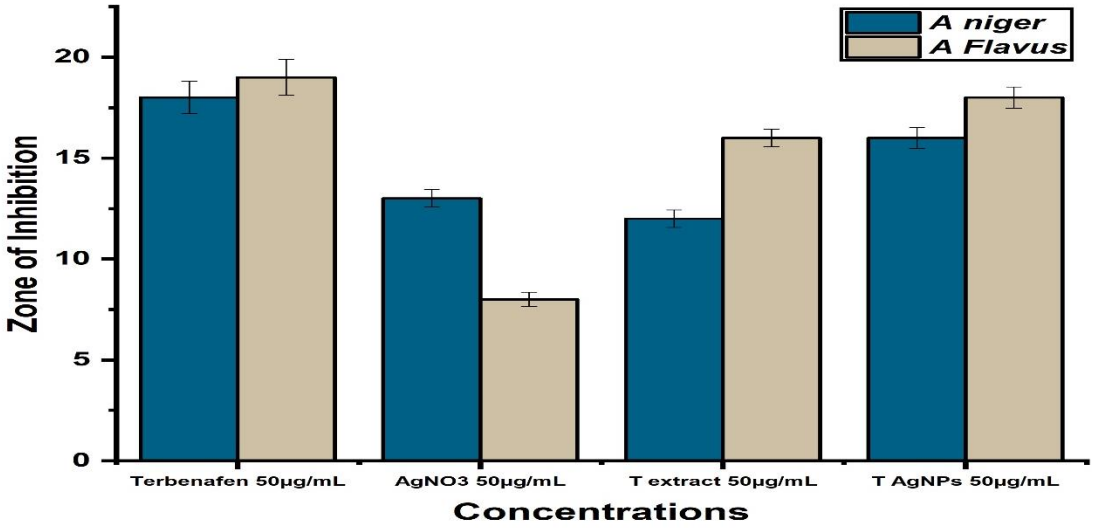

Figure 7. Antifungal effect of T couneifolia methanolic Extract and TAgNPs.

Cutotoxic potential of plant extracts and AgNPs

This study examined the cytotoxic potential of both biosynthesized T AgNPs and the crude methanolic extract of T couneifolia. Various concentrations of plant extract and synthesized AgNPs i.e. 250µg/mL, 500 µg/mL and 1000 µg/mL were used. At different time periods i.e. 24hrs, 48hrs and 72hrs the lethality was calculated. As compared to the extract the AgNPs has shown more cytotoxic effect. As the concentration is increased, it leads to the improvement in cytotoxic effect.

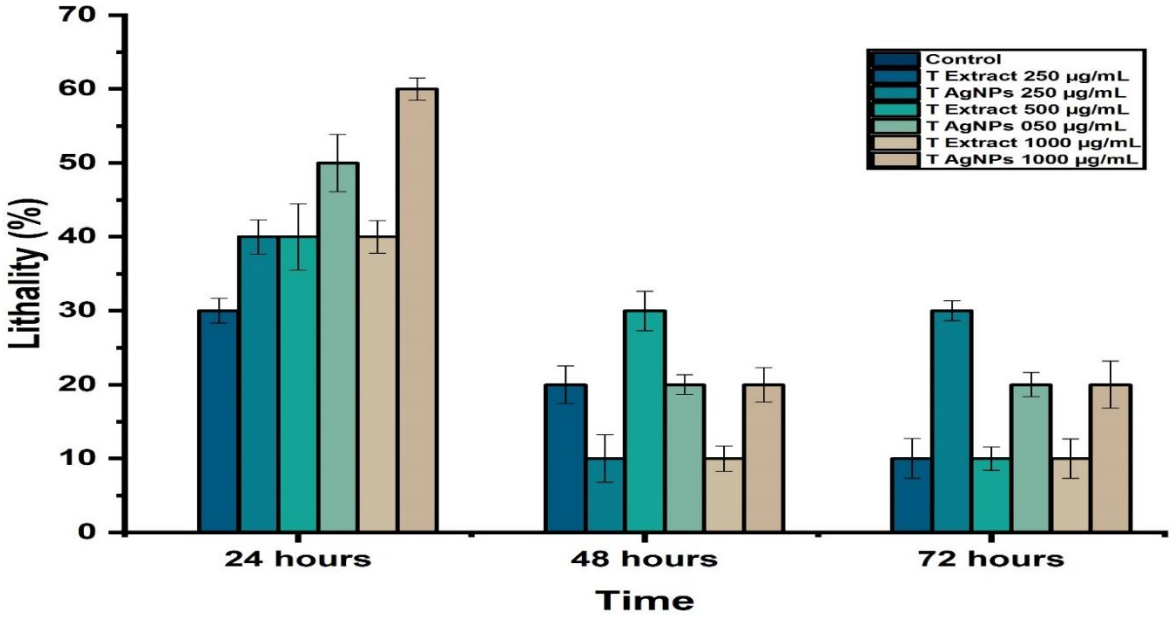

Figure 8. Cytotoxic effect of T couneifolia methanolic Extract and TAgNPs

Acetyl cholinesterase assay

An enzyme inhibitory potential of anti-Alzimer potential of T couneifolia plant extracts, as well as synthetic T AgNPs, has been investigated in this work. When compared to plant extracts, synthesized T AgNPs had the comparable inhibitory effect because concentration increases lead to greater inhibition. T extract had a 65%, and T AgNPs had a 66% (76 %) as shown in Figure 9. Higher concentrations of plant extracts and AgNPs inhibited acetyl cholinesterase (T extract 65 % and 76 % TAgNPs).

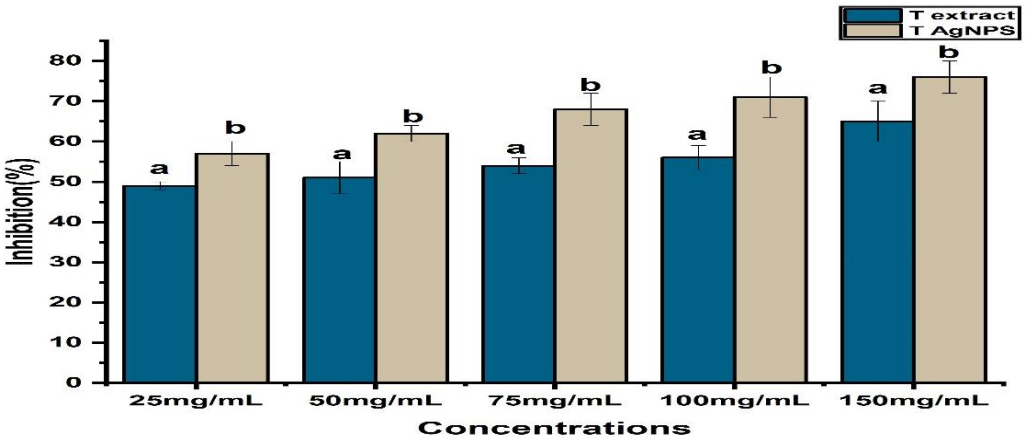

Figure 9. Anti-acetylcolintrase effect of T couneifolia methanolic Extract and TAgNPs.

Alpha- Amylase enzyme inhibitory assay

Both plant extracts and AgNPs showed Alpha-amylase activity. Plant extracts were available in four distinct concentrations and AgNPs utilized (25, 50, 75,100 µg/mL). As compared to metformin, demonstrated inhibition of 85%maximum inhibition for *T. couneifolia* extract and T AgNPs were 61% and 71%, respectively at 100 µg /mL (Figure 10).

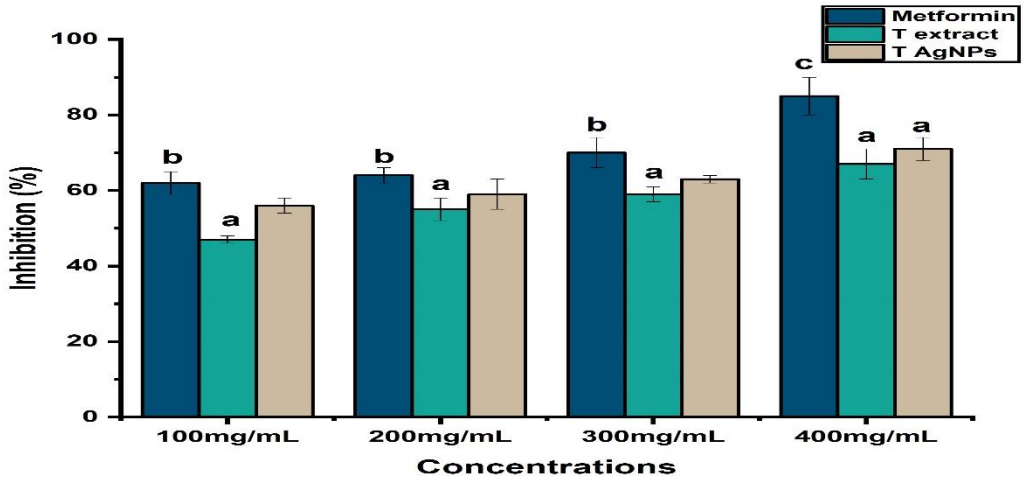

Figure 10. Alpha-Amylase inhibitory effect of T couneifolia methanolic Extract and TAgNPs

DISCUSSION

The study explored *T. couneifolia* methanolic extract and T AgNPs and showed many positive activities which are qualifying for their medicinal potential. It has shown significant antioxidant Activity. Oxidation is a critical step in the energy generation process, however during normal metabolism, numerous reactive oxygen species, including hydroxyl radicals, hydrogen peroxides and superoxide radicals, are created when oxygen is consumed. Reactive oxygen species, at small concentrations, help regulate development and transmit signals (29).

Oxidative stress is caused by an excessive number of reactive oxygen species, which destroy proteins and lipids molecules. It was shown that both plant and synthesized AgNPs extracts had high levels of antioxidant activity. T couneifolia was found to be more efficient in scavenging ABTS (2, 2, azinobis-3-ethylbenzothiazoline-6-sulphonic acid) and DPPH (1, 1, Diphenyl-picryl hydrazyl) Figures 1 and 2. As an example of a highly reactive compound that may cause significant damage to nearby biomolecules, phosphomolybdate Figure 5, ferric ions radical Figure 4, and hydrogen peroxide Figure 3, extracts from *T. couneifolia* successfully scavenge these reactive species. Bioactive polyphenolic and phenolic compounds in plant provide antioxidant capacity by reducing oxidative stress-inducing free radicals.

Using a variety of in vitro systems, including DPPH, ABTS, H2O2, phosphomolybdate and ferric reducing antioxidant power (FRAP) activities, fruit extract and AgNPs have demonstrated antioxidant capacity. The outcomes were comparable to those that had previously been published (26, 30-31).

AgNPs have been found to have antibacterial efficacy against both Gram-positive and Gram-negative pathogens. However, it had better antibacterial action against Gramnegative bacteria like *E. coli* and *K. pnemonei* than it did against Grampositive bacteria like *S. aureus* (Gram-positive) (32-33), This may be a result of the gramme negative bacteria's single or double layer peptidoglycan, single layer cell wall. The many layers of peptidoglycan that make up the cell wall of Gram-positive bacteria generate a more stiff structure, which complicates the diffusion and penetration of the AgNPs (34). Undoubtedly, the silver cations produced from AgNPs are what cause the bactericidal activity in relation to changes in the structure of microorganisms' membranes, which ultimately result in increased permeability of the bacteria's membrane and cell death (35). The outcomes are consistent with previously published studies.

AgNPs has significant antifungal activity and demonstrated 32mm zone of inhibition against Aspergillus spp a reported by [36]. A zone of inhibition was shown to surround AgNPs in a different study for all yeast, mould and A. flavus species with the exception of *A. oryzae* and *Chrysosporium* spp. *P. glomerata* (25.7 ± 0.6 mm) produced the largest zone of inhibition in the mould category, but *C. kruzei* (19.3 ± 1.5 mm) produced the largest zone of inhibition in the yeast category (37). The obtained results are in line with that of the previous work done.

According to the current work, Kumar et al. found that AgNPs produced by *Sargassum ilicifolium* at a concentration of 100 nM caused 100% mortality of A. salina. As a result, the biosynthesized AgNPs can be utilised as an eco-friendly anti-biofilm agent against marine microorganisms that produce biofilms as well as to stop the succession of existing biofilms according to previous studies AgNPs synthesized from *Milletti apinnata* have enhanced cytotoxic effect as compare to plant extracts alone [39].Anwar et al has reported 35% mortality of brin shrimp at 271µg/mL of *Justicia diffusa* AgNPs which was higher than plant extract (28).

Additionally, AgNPs inhibited acetyl cholinesterase more potently than plant extracts. Adsorption or contact of the nanoparticles with the acetyl cholinesterase protein is the primary cause of nanoparticle inhibition. Nanoparticles' interaction with cholinesterase proteins is yet unclear. Due to their lipophilicity and the hydrophobicity of the enzyme's environment, nanoparticles exhibit a strong affinity for cholinesterase. The obtained results were comparable with previous studies (40-41).

Bauhinia variegate flowers extract and AgNPs showed strong α -Amylase inhibitory action at 40 µg/mL 70 and 85 % in previous studies (42). It has been shown that Halymenia poryphyroides and AgNPs has inhibited α -Amylase 91 % at 1mg doses in another study (43).

CONCLUSION

According to the findings of the current study, silver nanoparticles mediated by plants demonstrated excellent antioxidant qualities when compared to plant extracts, suggesting that these metal nanoparticles could be employed to treat a variety of ailments brought on by oxidative stress. The *T. couneifolia* plant extract was used to create the silver nanoparticles, which demonstrated remarkable antibacterial properties against clinically isolated multidrug resistant human infections. Further, the above AgNPs revealed to possess an effective antifungal property against, *A.niger* and *A. flavous*. It is further observed that TAgNPs has shown anti-acetylcolintrase, invitro anti-diabetic as well as cytotoxic effect which concludes that it may be used in larger scale in the drug formulation.

Conflict of Interest:

Authors declared no conflict of interest.

Ethical Approval: The study was approved by the Institutional review board/Ethical review board

REFERENCES

1. Gutteridge, J.M.C.; Halliwell, B. Invited Review Free Radicals in Disease Processes: A Compilation of Cause and Consequence. Free Radic. Res. Commun. 1993, 19, 141–158.
2. Halliwell, B.; Gutteridge, J.M.C. [1] Role of Free Radicals and Catalytic Metal Ions in Human Disease: An Overview. Methods Enzymol. 1990, 186, 1–85.
3. Finkel, T.; Holbrook, N.J. Oxidants, Oxidative Stress and the Biology of Ageing. Nature 2000, 408, 239–247.
4. Lobo, V.; Patil, A.; Phatak, A.; Chandra, N. Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. Pharmacogn. Rev. 2010, 4, 118.
5. Qureshi, A.A.; Tariq, S.; Din, K.U.; Manzoor, S.; Calligaris, C.; Waheed, A. Evaluation of Excessive Lifetime Cancer Risk Due to Natural Radioactivity in the Rivers Sediments of Northern Pakistan. J. Radiat. Res. Appl. Sci. 2014, 7, 438–447.
6. Mohamed, N.H.; Ismail, M.A.; Abdel-Mageed, W.M.; Shoreit, A.A.M. Antimicrobial Activity of Latex Silver Nanoparticles Using Calotropis Procera. Asian Pac. J. Trop. Biomed. 2014, 4, 876–883.
7. Marslin, G.; Selvakesavan, R.K.; Franklin, G.; Sarmento, B.; Dias, A.C.P. Antimicrobial Activity of Cream Incorporated with Silver Nanoparticles Biosynthesized from Withania Somnifera. Int. J. Nanomedicine 2015, 10, 5955.
8. Zulfiqar, H.; Zafar, A.; Rasheed, M.N.; Ali, Z.; Mehmood, K.; Mazher, A.; Hasan, M.; Mahmood, N. Synthesis of Silver Nanoparticles Using Fagonia Cretica and Their Antimicrobial Activities. Nanoscale Adv. 2019, 1, 1707–1713.
9. Vickers, N.J. Animal Communication: When i'm Calling You, Will You Answer Too? Curr. Biol. 2017, 27, R713--R715.
10. Gupta, A.; Bonde, S.R.; Gaikwad, S.; Ingle, A.; Gade, A.K.; Rai, M. Lawsonia Inermis-Mediated Synthesis of Silver Nanoparticles: Activity against Human Pathogenic Fungi and Bacteria with Special Reference to Formulation of an Antimicrobial Nanogel. IET nanobiotechnology 2014, 8, 172–178.
11. Mody, V. V; Siwale, R.; Singh, A.; Mody, H.R. Introduction to Metallic Nanoparticles. J. Pharm. Bioallied Sci. 2010, 2, 282.
12. Shameli, K.; Ahmad, M. Bin; Yunus, W.Z.W.; Ibrahim, N.A.; Darroudi, M. Synthesis and Characterization of Silver/Talc Nanocomposites Using the Wet Chemical Reduction Method. Int. J. Nanomedicine 2010, 5, 743.
13. Yin, B.; Ma, H.; Wang, S.; Chen, S. Electrochemical Synthesis of Silver Nanoparticles under Protection of Poly (N-Vinylpyrrolidone). J. Phys. Chem. B 2003, 107, 8898–8904.

14. Darroudi, M.; Ahmad, M.B.; Zak, A.K.; Zamiri, R.; Hakimi, M. Fabrication and Characterization of Gelatin Stabilized Silver Nanoparticles under UV-Light. Int. J. Mol. Sci. 2011, 12, 6346–6356.
15. Kutsenko, A.; Granchak, V. Photochemical Synthesis of Silver Nanoparticles in Polyvinyl Alcohol Matrices. Theor. \& Exp. Chem. 2009, 45.
16. Darroudi, M.; Ahmad, M.B.; Zamiri, R.; Abdullah, A.H.; Ibrahim, N.A.; Shameli, K.; Husin, M.S. Preparation and Characterization of Gelatin Mediated Silver Nanoparticles by Laser Ablation. J. Alloys Compd. 2011, 509, 1301–1304.
17. Kahrilas, G.A.; Haggren, W.; Read, R.L.; Wally, L.M.; Fredrick, S.J.; Hiskey, M.; Prieto, A.L.; Owens, J.E. Investigation of Antibacterial Activity by Silver Nanoparticles Prepared by Microwave-Assisted Green Syntheses with Soluble Starch, Dextrose, and Arabinose. ACS Sustain. Chem. \& Eng. 2014, 2, 590–598.
18. Rajkuberan, C.; Sudha, K.; Sathishkumar, G.; Sivaramakrishnan, S. Antibacterial and Cytotoxic Potential of Silver Nanoparticles Synthesized Using Latex of Calotropis Gigantea L. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2015, 136, 924–930.
19. Edison, T.J.I.; Sethuraman, M.G. Instant Green Synthesis of Silver Nanoparticles Using Terminalia Chebula Fruit Extract and Evaluation of Their Catalytic Activity on Reduction of Methylene Blue. Process Biochem. 2012, 47, 1351–1357.
20. Ul Haq, M.N.; Shah, G.M.; Menaa, F.; Khan, R.A.; Althobaiti, N.A.; Albalawi, A.E.; Alkreathy, H.M. Green Silver Nanoparticles Synthesized from Taverniera Couneifolia Elicits Effective Anti-Diabetic Effect in Alloxan-Induced Diabetic Wistar Rats. Nanomaterials 2022, 12, 1035.
21. Khan, N.A.; Niaz, A.; Zaman, M.I.; Khan, F.A.; Nisar-ul-haq, M.; Tariq, M. Sensitive and Selective Colorimetric Detection of Pb2+ by Silver Nanoparticles Synthesized from Aconitum Violaceum Plant Leaf Extract. Mater. Res. Bull. 2018, 102, 330–336, doi:10.1016/j.materresbull.2018.02.050.
22. Sher, N.; Ahmed, M.; Mushtaq, N.; Khan, R.A. Calligonum Polygonoides Reduced Nanosilver: A New Generation of Nanoproduct for Medical Applications. Eur. J. Integr. Med. 2020, 33, 101042, doi:10.1016/j.eujim.2019.101042.
23. Hatano, T.; Edamatsu, R.; Hiramatsu, M.; MORI, A.; Fujita, Y.; Yasuhara, T.; YOSHIDA, T.; OKUDA, T. Effects of the Interaction of Tannins with Co-Existing Substances. VI.: Effects of Tannins and Related Polyphenols on Superoxide Anion Radical, and on 1, 1-Diphenyl-2-Picrylhydrazyl Radical. Chem. Pharm. Bull. 1989, 37, 2016–2021.
24. Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant Activity of Dietary Polyphenols as Determined by a Modified Ferric Reducing/Antioxidant Power Assay. J. Agric. Food Chem. 2000, 48, 3396–3402.
25. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal. Biochem. 1999, 269, 337–341.
26. Mahendran, G.; Kumari, B.D.R. Biological Activities of Silver Nanoparticles from Nothapodytes Nimmoniana (Graham) Mabb. Fruit Extracts. Food Sci. Hum. Wellness 2016, 5, 207–218.
27. Qais, F.A.; Shafiq, A.; Khan, H.M.; Husain, F.M.; Khan, R.A.; Alenazi, B.; Alsalme, A.; Ahmad, I. Antibacterial Effect of Silver Nanoparticles Synthesized Using Murraya Koenigii (L.) against Multidrug-Resistant Pathogens. Bioinorg. Chem. Appl. 2019, 2019, doi:10.1155/2019/4649506.
28. Anwar, N.; Shah, M.; Saleem, S.; Rahman, H. Plant Mediated Synthesis of Silver Nanoparticles and Their Biological Applications. Bull. Chem. Soc. Ethiop. 2018, 32, 469–479.
29. Otunola, G.A.; Afolayan, A.J. In Vitro Antibacterial, Antioxidant and Toxicity Profile of Silver Nanoparticles Green-Synthesized and Characterized from Aqueous Extract of a Spice Blend Formulation. Biotechnol. Biotechnol. Equip. 2018, 32, 724–733, doi:10.1080/13102818.2018.1448301.

30. Mittal, A.K.; Bhaumik, J.; Kumar, S.; Banerjee, U.C. Biosynthesis of Silver Nanoparticles: Elucidation of Prospective Mechanism and Therapeutic Potential. J. Colloid Interface Sci. 2014, 415, 39–47.
31. Ramamurthy, C.H.; Padma, M.; mariya samadanam, I.D.; Mareeswaran, R.; Suyavaran, A.; Kumar, M.S.; Premkumar, K.; Thirunavukkarasu, C. The Extra Cellular Synthesis of Gold and Silver Nanoparticles and Their Free Radical Scavenging and Antibacterial Properties. Colloids Surfaces B Biointerfaces 2013, 102, 808–815, doi:10.1016/j.colsurfb.2012.09.025.
32. Kaviya, S.; Santhanalakshmi, J.; Viswanathan, B.; Muthumary, J.; Srinivasan, K. Biosynthesis of Silver Nanoparticles Using Citrus Sinensis Peel Extract and Its Antibacterial Activity. Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 2011, 79, 594–598, doi:10.1016/j.saa.2011.03.040.
33. Gopinath, V.; MubarakAli, D.; Priyadarshini, S.; Priyadharsshini, N.M.; Thajuddin, N.; Velusamy, P. Biosynthesis of Silver Nanoparticles from Tribulus Terrestris and Its Antimicrobial Activity: A Novel Biological Approach. Colloids Surfaces B Biointerfaces 2012, 96, 69–74, doi:10.1016/j.colsurfb.2012.03.023.
34. Prakash, P.; Gnanaprakasam, P.; Emmanuel, R.; Arokiyaraj, S.; Saravanan, M. Green Synthesis of Silver Nanoparticles from Leaf Extract of Mimusops Elengi, Linn. for Enhanced Antibacterial Activity against Multi Drug Resistant Clinical Isolates. Colloids Surfaces B Biointerfaces 2013, 108, 255–259, doi:10.1016/j.colsurfb.2013.03.017.
35. Malapermal, V.; Botha, I.; Krishna, S.B.N.; Mbatha, J.N. Enhancing Antidiabetic and Antimicrobial Performance of Ocimum Basilicum, and Ocimum Sanctum (L.) Using Silver Nanoparticles. Saudi J. Biol. Sci. 2017, 24, 1294–1305.
36. Xu, Z.P.; Zeng, Q.H.; Lu, G.Q.; Yu, A.B. Inorganic Nanoparticles as Carriers for Efficient Cellular Delivery. Chem. Eng. Sci. 2006, 61, 1027–1040.
37. Do\ugan, S. \cSahin; Kocaba\cs, A. Green Synthesis of ZnO Nanoparticles with Veronica Multifida and Their Antibiofilm Activity. Hum. \& Exp. Toxicol. 2020, 39, 319–327.
38. Swamy, M.K.; Akhtar, M.S.; Mohanty, S.K.; Sinniah, U.R. Synthesis and Characterization of Silver Nanoparticles Using Fruit Extract of Momordica Cymbalaria and Assessment of Their in Vitro Antimicrobial, Antioxidant and Cytotoxicity Activities. Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 2015, 151, 939–944, doi:10.1016/j.saa.2015.07.009.
39. Das, G.; Patra, J.K.; Debnath, T.; Ansari, A.; Shin, H.S. Investigation of Antioxidant, Antibacterial, Antidiabetic, and Cytotoxicity Potential of Silver Nanoparticles Synthesized Using the Outer Peel Extract of Ananas Comosus (L.). PLoS One 2019, 14, 1–19, doi:10.1371/journal.pone.0220950.
40. Nath, D.; Banerjee, P. Green Nanotechnology - A New Hope for Medical Biology. Environ. Toxicol. Pharmacol. 2013, 36, 997–1014, doi:10.1016/j.etap.2013.09.002.
41. Rajakumar, G.; Gomathi, T.; Thiruvengadam, M.; Rajeswari, V.D.; Kalpana, V.N.; Chung, I.-M. Evaluation of Anti-Cholinesterase, Antibacterial and Cytotoxic Activities of Green Synthesized Silver Nanoparticles Using from Millettia Pinnata Flower Extract. Microb. Pathog. 2017, 103, 123–128.
42. Abdel-Aziz, M.S.; Shaheen, M.S.; El-Nekeety, A.A.; Abdel-Wahhab, M.A. Antioxidant and Antibacterial Activity of Silver Nanoparticles Biosynthesized Using Chenopodium Murale Leaf Extract. J. Saudi Chem. Soc. 2014, 18, 356–363, doi:10.1016/j.jscs.2013.09.011.
43. Manam, D.; Kiran, V.; Murugesan, S. Biogenic Silver Nanoparticles by Halymenia Poryphyroides and Its in Vitro Anti-Diabetic Efficacy. J. Chem. Pharm. Res. 2013, 5, 1001–1008.