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ASSESSMENT OF ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF SILVER NANOPARTICLES FROM *NEPETA LAEVIGATA* (D.DON) HAND. -MAZZ. AND *NEPETA KURRAMENSIS* RECH. F.

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Abstract

The present work focusses on antimicrobial and antioxidant activities of silver nanoparticles ($AgNO_3$ NPs) from two important medicinal plants *Nepeta leavigata* and *Nepeta kurramensis*. The $AgNO_3$ NPs antimicrobial results revealed that both the plants showed good potential against bacterial and fungal pathogens. The $AgNO_3$ NPs from *N. leavigata* showed 20.5 ± 0.047, 19.8 ± 0.408 and 18.7± 0.037 mm zone of inhibition against *Amanita muscaria, Fusarium oxysporum, Aspergillus terreus* respectively at a concentartion of 11 µg/ml while the lowest zone of inhibition 7.93 ± 0.391 mm was given against fungal pathogen *Puccinia graminis.* Antifungal results given by $AgNO_3$ NPs from *N. kurramensis* projected that they showed 20.16 ± 0.067, 19.6 ± 0.045 mm inhibition zone at a concentration of 11 µg/ml against *Amanita muscaria, Fusarium oxysporum* respectively while lowest inhibition zones 6.8 ± 0.048 and 6.3 ± 0.037 was given against *Puccinia graminis, Geotrichum candidum.* Three different concentartion of $AgNO_3$ NPs from our research plants were tested against seven bacterial pathogens. Results revealed that $AgNO_3$ NPs from *N. leavigata* exhibited 21.8 ± 0.065, 21.1 ± 0.05 and 19.6 ± 0.048 at a concentration of 7 µg/ml against *P. aeruginosa, Listeria monocytogens* and *Salmonella typae* respectively. Lowest zone of inhibition 16.7 ± 0.04 was given against *Klebsiella pneumonia. The* $AgNO_3$ NPs from *N. kurramensis* showed highest zone of inhibition 21.8 ± 0.045 against *P. aeruginosa* while the lowest inhibition zone 16.8 ± 0.043 was given against *E. coli* at a concentration of 7 µg/ml. The antioxidant activities results showed that $AgNO_3$ NPs from *N. kurramensis* extract had a higher scavenging effect than $AgNO_3$ NPs from *N. laevigata.* $AgNO_3$ NPs from *N. laevigata* demonstrated the maximum % of inhibition, measuring 47.17 ± 0.032 at a dose of 9 µg/ml. This was followed by 43.86 ± 0.023 % inhibition at a concentration of 7 µg/ml, whilst the lowest percentage activity was detected at a concentration level of 3 µg/ml, which was measured as 34.56 ± 0.028. The maximum scavenging inhibition given by $AgNO_3$ NPs form *N. kurramensis* was 50.03 ± 0.073 % at a dose of 9 µg/ml, and it was followed by 46.7 ± 0.043 % inhibition at a concentration of 7 µg/ml.

Key words: Medicinal plants screening, *Nepeta kurramensis, Nepeta laevigata*, antimicrobial activities, antioxidant activities.

Introduction

Silver (Ag) nanoparticles are among the most successful inorganic nanoparticles within the nanotechnology field. They have particular uses in agriculture and medicine as antimicrobials and antioxidants (Kaszuba *et al*., 2020). Silver nanoparticles have already been studied for their catalytic activities, surface enhanced Raman scattering, antibacterial activity, excellent conductance, and chemical resistance (Kasithevar *et al*., 2017). Several experiments have characterized not just the ability of natural extracts and microbes to form Ag NPs but also their better antioxidant properties, particularly in comparison to surfaces. This action is thought to be caused by the preferential sorption of extract elements on the surfaces of the nanoparticles (Demirbas *et al*., 2016). The most common chemical methods for synthesizing silver nanoparticles include reducing agents that use a variety of organic and inorganic reductants, electrochemical methods, and radiolysis. Most of these methodologies are still in the development stage, and the challenges they pose include NP stability and accumulation, crystal growth control, morphological characteristics, size, and shape. Furthermore, the extraction and purification of generated NPs for subsequent applications remain major problems (Ovais *et al*., 2016, Nasar *et al*., 2019). Silver has low resistance and high conductivity; it's also natively ductile and bendable. It occurs in a variety of chemical forms. Ag with 0 or +1 is common and abundant, whereas Ag with +2 or +3 is uncommon and found in inconvenience this may have caused (Kasthuri *et al*., 2009, Khan *et al*., 2017). Silver nanoparticles are used to treat a variety of disorders, including nicotine addiction, gastroenteritis, gonorrhea, epilepsy, infectious diseases, and epilepsy (Hudlikar *et al*., 2012). Silver demand is rising because of new applications for the metal, such as in the polymer, textile, and healthcare industries, as well as in pharmaceuticals (Amin *et al*., 2012; Kasithevar *et al*., 2017).

The genus *Nepeta* is a member of family Lamiaceae. This family is represented by 200–250 species. Most of the plants belonging to this family are annual or perennial herbs with square stems, although a few species are woody shrubs or subshrubs. The leaves are usually simple and made arrangements in reverse directions. Almost all plants of this family contain volatile oils and have pleasant fragrance (Dienaitė *et al*., 2018). Members of this family are recognized as catnips or catmints because *Nepeta* species pleasurably helps to stimulate cats' pheromonic receptor. The genus is native to Europe, Asia, and Africa, with the largest species diversification in the Mediterranean area east to China (Ahmad *et al*., 2011). It is currently considered a weed in United States (Ullah *et al*., 2017). In Pakistan *Nepeta* species are frequently present in Swat and Kurram Agency. Locally these plant species are used to treat wound especially Leishmania infection.

In the current study, *Nepeta laevigata* and *Nepeta kurramensis* were chosen for the first time for the synthesis, characterization of silver nanoparticles and their antimicrobial, antioxidant screenings activities.

Material and Methods

Different concentrations of $AgNO_3$ NPs were subjected to antibacterial, antifungal and antioxidant activities under established protocols for their ability to combat bacteria, fungus, and free radicals.

Antimicrobial activity procedure: The agar disc diffusion technique (Salie *et al.*, 1996) was used to determine the antimicrobial effects of $AgNO_3$ NPs. Petriplates of uniform size were washed abd sterilized. Media wa prepared and poured in periplates. Respective baxteriun/fungus was swabbed on media. Wells were made on each petriplate. Samples were addedin respective wells. Antibiotic disc was placesd in cebreal well as positive control. Prior to the experiment, the microbial cultures were transferred to nutrient broth and kept in a shaker incubator (37 °C; 200 RPM) for 24 hours. Albicans and ampicillin discs were used as a positive control, and the microbial plates were incubated for 24/48 hours at 37°C. A vernier caliper was used to assess the zone of inhibition. Antimicrobial activity was evaluated at different concentration levels. The rest of the approach was carried out in accordance with Djahaniani *et al.*, (2017).

Antioxidant activity procedure: Spectrophotometric procedure (Friedman *et al.*, 2010) was used to examine the radical quenching capacity of synthesized $AgNO_3$ NPs using DPPH (2,2-diphenyl 1-picrylhydrazyl) as a stable free radical. Concentration levels of Ag NPs ranging from 200 g/mL to 1 g/mL were analyzed to determine free radical scavenging. Gallic acid and Dimethylformamide were used as positive and negative controls, respectively. After 20 minutes in the dark, optical density values at 517 nm were assessed using a BIOTEK micro plates. The equation can be used to calculate the percentage of free radical scavenging.

$$\%\ \text{DDPH} = \frac{1\text{- (Sample absorbance)}}{\text{(Control absorbance)}} \times 100$$

Biological activity Results of silver nanoparticle

Antifungal activities results: Seven different fungal pathogens, *Alternaria nigra, Puccinia graminis, Fusarium oxysporum, Trichoderma harzianum, Geotrichum candidum, Aspergillus terreus* and *Amanita muscaria*, were tested at various concentrations of produced NPs from *Napata species.* The $AgNO_3$ NPs synthesized from *N. leavigata* showed 20.3 ± 0.47, 19.8 ± 0.37 and 18.7 ± 0.40 mm zone of inhibition against *Amanita muscaria, Fusarium oxysporum, Aspergillus terreus* respectively at a concentartion of 11 µg/ml while the lowest zone of inhibition 7.93 ± 0.39 mm was given against fungal pathogen *Puccinia graminis* at a dosage level of 2 µg/ml, while a moderate zone of inhibition of 15.7 ± 0.26 mm was recorded against *Geotrichum candidum* at a dosage level of 11 µg/ml. ((Fig. 1). In the case of *Nepeta kurramensis*, the maximum inhibition zone was recorded at 20.16 ± 0.67 mm at a dose of 11 µg/ml against *Amanita muscaria*, followed by a 19.6 ± 0.45 mm zone of inhibition against *Fusarium oxysporum*. At a concentration level of 5 µg/ml, *Trichoderma harzianum* had a moderate zone of inhibition of 13.6 ± 0.49 mm at a concentration level of 11 µg/ml, whereas *Geotrichum candidum* has the least efficacy zone of 6.3 ± 0.37 mm at a dosage level of 2 µg/ml. Antifungal drug is used as positive control. (Fig. 2).

Antibacterial activities results: The antibacterial activity of various concentration levels of $AgNO_3$ NPs from *Napata* species was tested against seven pathogenic bacteria, including *Staphylococcus aureus (ATCC 25923), Listeria monocytogens (ATCC 7644), Escherichia coli (ATCC 25922), Salmonella typae* (ATCC 1224)*, Klebsiella pneumonia* (ATCC 2214)*, Staphylococcus epidumis* (ATCC 7120) *and Pseudomonas aeruginosa* (ATCC 27853). Three different concentartion of $AgNO_3$ NPs from our research plants were tested against bacterial pathogens Results revealed that $AgNO_3$ NPs from *N. leavigata* exhibited 21.8 ± 0.065, 21.1 ± 0.05 and 19.6 ± 0.048 at a concentration of 7 µg/ml against *P. aeruginosa, Listeria monocytogens* and *Salmonella typae* respectively. Moderate zone of inhibition of 18.5 ± 0.41 mm was observed against *Staphylococus aureus* at a concentration of 7 µg/ml. Lowest zone of inhibition 16.7 ± 0.04 was given against *Klebsiella pneumonia.* (Fig. 3). Similarly, $AgNO_3$ NPs from *Nepeta kurramensis* showed 21.8 ± 0.45 mm zone of inhibition against *Pseudomonas aeruginosa* at 7 µg/ml followed by a 19.13 ± 0. 65 mm inhibition zone *Listeria monocytogens*. The minimum activity against *Escherichia coli* was detected at 13.4 ± 0.41 mm at a dosage level of 3 µg/ml, while a moderate zone of inhibition of 15.4 ± 0.41 mm was reported against *Salmonella typae* at a concentration level of 3 µg/ml. ampicillin was used as positive control drug. (Fig. 4).

Antioxidant activities results: Four different concentrations of $AgNO_3$ NPs synthesized from *N. leavigata and N. Kurramensis* were subjected to antioxidant activities. $AgNO_3$ NPs synthesized from *N. leavigata* demonstrated the maximum % of inhibition, measuring 47.17± 0.32 % at a dose of 9 µg/ml. This was followed by 43.86 ± 0.23 % inhibition at a concentration of 7 µg/ml. At a concentration level of 5 µg/ml, a moderate inhibition 38.6± 0.45 % was measured, whilst the lowest activity was detected at a concentration level of 3 µg/ml, which was measured as 34.56 ± 0.28%. The maximum inhibition activity for *Nepeta kurramensis* was 50.03 ± 0.73 % at a dose of 9 µg/ml, and it was followed by a 46.7 ± 0.43 % inhibition at a concentration of 7 µg/ml. At a concentration level of 5 µg/ml, a moderate inhibition 41.16 ± 0.65 % was seen, while the least activity was seen at a concentration level of 3 µg/ml, which was 38.16 ± %. As a positive control, gallic acid was used (Fig. 5).

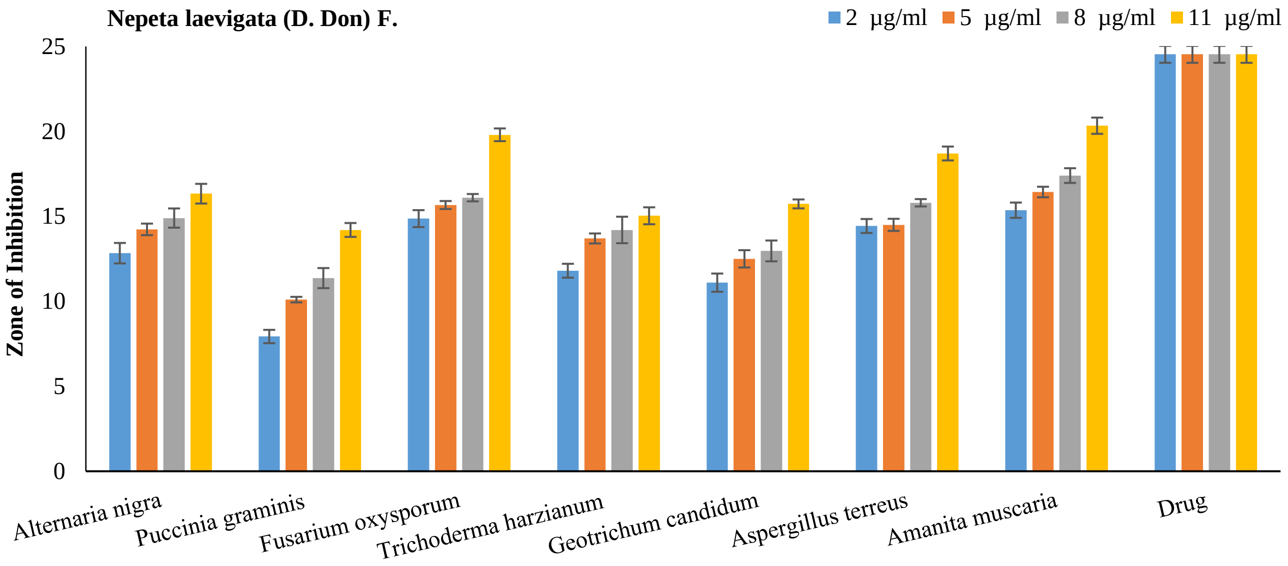

Fig. 1. Antifungal activity results of $AgNO_3$ NPs form *Napata aevigata*.

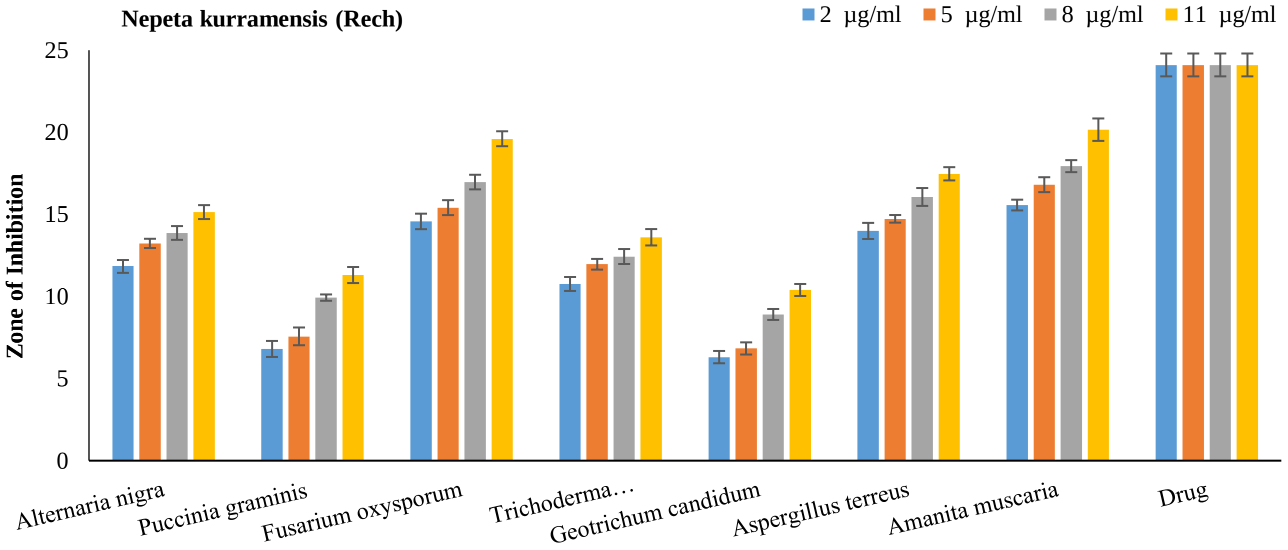

Fig. 2. Antifungal activity results of $AgNO_3$ NPs form *Napata kurramensis*.

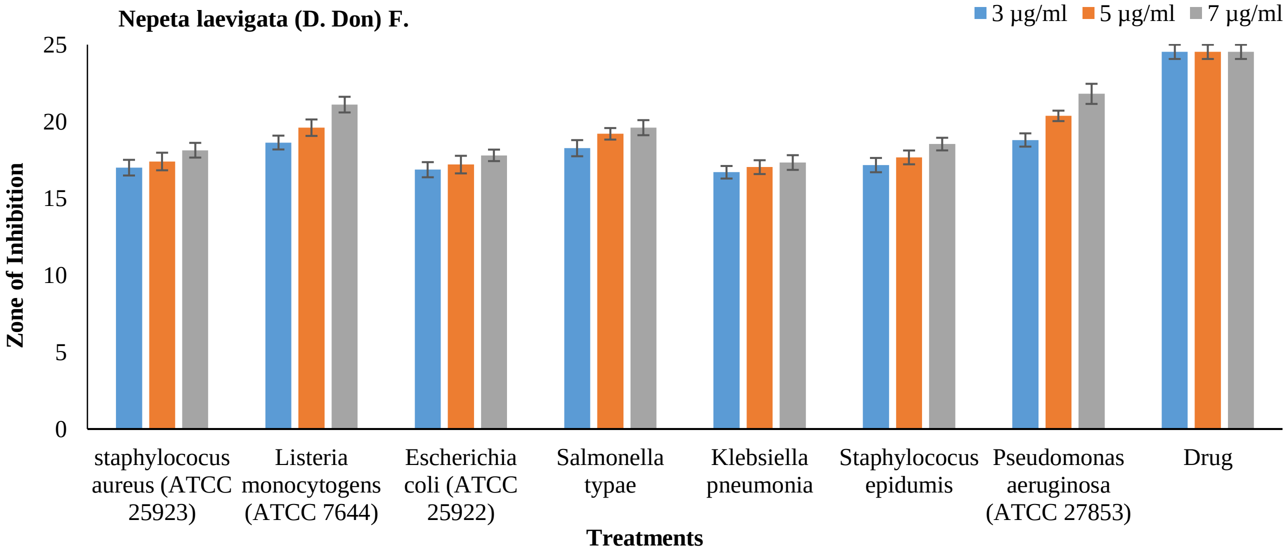

Fig. 3. Antibacterial activity results of $AgNO_3$ NPs form *Napata aevigata*

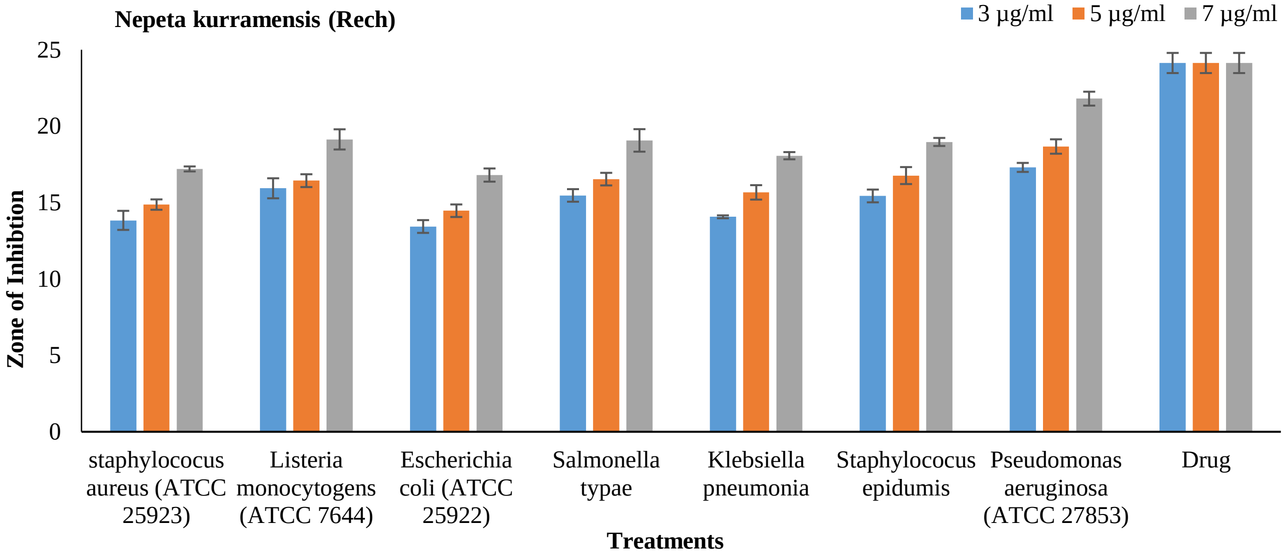

Fig. 4. Antibacterial activity results of $AgNO_3$ NPs form *Napata kurramensis*.

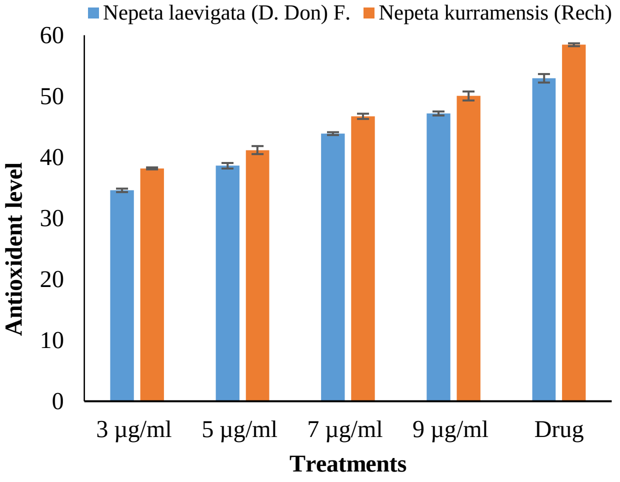

Fig. 5. Antioxidant activity results of $AgNO_3$ NPs form *Napata kurramensis* and *Napata laevigata.*

Discussion

The use of plant methanolic extracts in the synthesis of nanoparticles is a safer and simpler procedure. As a result, it is fashionable, and the plants are widely available as they are dispersed globally, are very safe to handle, and have specific therapeutic capabilities (Ahmed *et al*., 2014). Silver nanoparticles are well-known for their potency against a wide range of pathogens, including bacteria, fungus, and viruses. In the current study, two *Nepeta* species were employed to produce the nanomaterials. Both silver nitrate solutions changed colour from colourless to dark brown, suggesting the formation of silver nanoparticles. The existence of the surface plasmon, which results from the valence electron group oscillation in the magnetic field, accounts for the brown colour (Mulfinger *et al*., 2007).

The relevant fungi were isolated from sewage from clinics, households, and industrial plants: A. *niger*, *A. flavus*, *Fusarium oxysporium*, *A. terreus*, *Geotrichum candidum*, *F. solani*, *Trichophyton sp*, *Trichoderma harizianum*, *Rhizopus oryzae*, *Trichophyton*, *Mucor hiemalis*, and *P. chrysogenum. A. terreus* was the most common fungus, followed by Trichophyton sp. Some of the detected fungal species, such as *Geotrichum*, *Aspergillus*, *Trichophyton*, and *Trichophyton* sp, are also found in human tissue at various sites, including the intestine, which is vital for the body's regular activity. However, these advantageous bacterial types might occasionally result in major health problems if they proliferate (Alananbeh *et al*., 2017). Although some Aspergillus sp. species can infect people and produce aspergillosis, many of them are not poisonous and non-pathogenic. However, only a small number of them are more dangerous than other species, such as A. flavus, which is believed to be 100 times more toxic than other species in the same genus (Jha *et al*., 2009). Several of the species, including *A. niger*, are opportunists. This species is common in nature and colonises the human body. This can attack people with weakened immune systems or even other serious disorders (Abbaspour *et al*., 2015). Additional species, such as Trichoderma sp. (Kuhls *et al*., 1999) and Fusarium sp. (Boutati *et al*., 1997), *Geotrichum* sp. (Verghese & Ravichandran, 2003) and Penicillium sp. (Kajenthira, 2012) are fungi that can infect HIV patients. In addition, Mucor sp. and Rhizopus spare are accountable for mucormycosis and micromycetes, respectively, in immunosuppressed individuals (Kontoyiannis & Lewis, 2006). In the current study, we discovered that manufacturing wastewater has a higher concentration of fungal species than other kinds of wastewater. Metals, nitrogen, organic matter, phosphorus, grease, and oil are abundant in factory wastewater and are vital building blocks for fungus and other creatures (Sarwat *et al*., 2012). In addition, fungi identified from hospital waste have been found to have come from either diseased humans or animals. Because it may be combined with the source of drinking water, this water is regarded as the biggest hazard to population health (Abed & Alwakeel, 2007). It was discovered that there was no discernible difference between the two tests (which included seven different varieties of fungi) and their repetitions when targeted fungi were treated with the various quantities of silver nanoparticles of both manufactured particles. Nonetheless, a substantial difference was identified among the four factors. There is a distinction between the two variables. Furthermore, as compared to the other fungal strains, A. terreus showed a significant reduction in development. Furthermore, *Nepeta laevigata* had the maximum zone of inhibition against *Amanita muscaria*, measuring 20.3 mm at a dose of 11 µg/ml, followed by *Fusarium oxysporum*, with a zone of inhibition measuring 19.8 mm. The least activity was detected against *Puccinia graminis* at 7.93 mm at a dosage level of 2 µg/ml, while a moderate zone of inhibition of 12.5 mm was recorded against *Geotrichum candidum* at a dosage level of 2 µg/ml. In the case of *Nepeta kurramensis*, the maximum inhibition zone was recorded at 20.16 mm at a dose of 11 µg/ml against *Amanita muscaria*, followed by a 19.6 mm zone of inhibition against *Fusarium oxysporum*. At a concentration level of 5 µg/ml, *Trichoderma harzianum* had a moderate zone of inhibition of 11.9 mm at a concentration level of 5 µg/ml, whereas had *Geotrichum candidum* the least efficacy at 6.3 mm at a dosage level of 2 µg/ml. Previous research effectively used various nanoparticles and their dosage against a variety of other fungi, including *Alternaria alternata*, *Aspergillus*, and *Fusarium*, and they concluded that strong growth inhibition was achieved (Geoprincy *et al*., 2012). *Trichophyton mentagrophytes* (Keuk *et al*., 2009) as well as *Trichophyton rubrum* (Noorbakhsh *et al*., 2011) were also examined against the silver nanoparticle, and the silver nanoparticle effectively suppressed them. Additionally, Candida sp was tested against 1 mgL-1 of silver nanoparticle and found to have high antifungal activity against the identified fungi (Panacek *et al*., 2006). All properties, such as the present study findings, are like ionic silver, except for cytotoxicity (Panacek *et al*., 2009). The silver nanoparticle's mechanism of action can be described by a different method, including such metal deficiency, which would be linked to AgNPs and disrupt the pathogen's epithelial layer. This alteration in membrane fluidity leads to the release of the protein-membrane and endotoxin (Vijayakumar *et al*., 2013). Additionally, it was discovered that the nanoparticles created free radicals (Salem *et al*., 2014). Additionally, the biological activity of a NPS is dependent on the shape as well as the size of the produced nanoparticle, whereas the toxicity of the nanoparticle directly depends on the size and quantity of the nanoparticles (Panacek *et al*., 2006; Panacek *et al*., 2009; Morones *et al*., 2005).

NPs synthesized were also tested for their antibacterial profile. The inhibition zone for *Nepeta laevigata* against *Pseudomonas aeruginosa* was 21.8 mm at a dose of 7 µg/ml, followed by a 21.1 mm zone of inhibition for *Listeria monocytogens*. At a dosage level of 5 µg/ml, a moderate zone of inhibition of 17.4 mm was observed against *Staphylococus aureus*, whereas the least efficacy was shown against *Klebsiella pneumonia* at 16.7 mm at 2 µg/ml. Similarly, to *Nepeta kurramensis*, 21.8 mm zone of inhibition at 7 µg/ml followed by a 19.13 mm inhibition zone at a dose of 7 µg/ml against *Pseudomonas aeruginosa* and *Listeria monocytogens* respectively. The minimum activity against *Escherichia coli* was detected at 13.4 mm at a dosage level of 3 µg/ml, while a moderate zone of inhibition of 15.4 mm was reported against *Salmonella typae* at a concentration level of 2 µg/ml. This finding is consistent with earlier research (Uhrin *et al*., 2018; Kim *et al*., 2009).

However, other research reports conflicting findings (Lee *et al*., 2016). Furthermore, as we increase, the quantity of silver nanoparticles in the zone of inhibition rises. Furthermore, silver nanoparticles from *Nepeta laevigata* exhibit a larger zone of inhibition compared to other plant species. However, there was no meaningful statistical change. The mechanism of action of silver nanoparticles is not fully understood. NPs, however, enter bacteria because of their small size, reducing the action of the strains' respiration enzyme and hence decreasing development (Chen *et al*., 2017; Zibaii *et al*., 2014). Phosphate uptake may be a factor in E. coli inhibition because it alters the structure of the bacteria's cells and triggers cell damage when it enters the cell (Morones *et al*., 2005). Along with this, the stronger peptidoglycan in the bacteria's cell wall was the cause of those types that the NPS could not block. Therefore, to stop their proliferation, a larger quantity of nanoparticles was needed (Dobias *et al*., 2013). The particle's continued reduction in size makes it easier for it to enter the bacterial cell, which can effectively stop the bacteria from growing (Alananbeh *et al*., 2017). Several other researchers employed various origins of NPs against other pathogenic organisms, who used silver nanoparticles from the Cassia tora and concluded that it has better anti-microbial activity against gram-negative bacteria than positive bacteria, because of the presence of a thinner wall in the gram-negative as opposed to positive, which has a higher density composed of denser peptidoglycan (Asmathunisha *et al*., 2010).

The DPPH free radical test is used to determine antioxidant capacity. This is one of the stable compounds that the hydrogen in silver nanoparticles may swiftly decrease. DPPH is among the most extensively performed procedures for determining antioxidant properties (Mittal *et al*., 2014). When the IC50 is high, it suggests that there is less scavenging effect than when it is low. When a purple solution turns yellow with the introduction of a silver nanoparticle or another prepared solution, this implies that it is scavenged (Flieger *et al*., 2021).

When two manufactured NPs were evaluated for scavenging effect, the results revealed that *Nepeta laevigata* outperformed *Nepeta kurramensis*. Furthermore, as the concentration was increased, so did the scavenging effect of the produced nanoparticle. *Nepeta laevigata* had a lower IC50 value, demonstrating its promise as an ideal scavenger. A little research was carried out to assess its ability as a scavenger. Research performed by Yarjanli *et al*., (2017) revealed that AgNPs have a stronger scavenging effect than walnut extract and it was hypothesised that the increased activity of the silver nanoparticle is linked to the presence of polyphenols, terpenoids, and flavonoids (Sepehr *et al*., 2017). After recording the very same outcome, the Costus (Ekrikaya *et al*., 20). Another explanation was put forth by Vijayan *et al*., (2020), who claim that the fact that nanoparticles have more antioxidant effects than leaf extract is due to the bioactive chemicals in leaf extract adhering to their spherical shape counterparts. Similar to this, AgNPs made with *Melia azedarach* extract showed increased antioxidant potential according to extract (Elemike *et al*., 2017).

Scientists conducted follow-up research in which they produced two types of nanoparticles and assessed three methodologies (NOx, DPPH, and ABTS) for antioxidant capacity, concluding that the DPPH had the highest radical scavenging and the ABTS had the lowest. In contrast, moderate activity was documented with the NOx, which could be related to various organic compounds that are important in stabilizing and limiting the NPS. Otunola published a similar study in which he synthesized silver nanoparticles from *Zingiber officinale*, *Capsicum frutescens*, and *Allium sativum* (Tian *et al*., 2020).

Conclusion

In the current study $AgNO_3$ NPs from *Nepeta leavig*ata and *Nepeta kurramensis* were subjected to biological activities. Remarkable antimicrobial and antioxidant activities were found in $AgNO_3$ NPs. Based on our finding we are confident enough about the safe traditional use of these plants extract for wound healing especially against leishmania infection.

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