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Biogenic silver nanoparticles from *Eucalyptus camaldulensis*: Antioxidant, antimicrobial, and larvicidal properties

S Suganya[1], S Vijayanand[2], J Hemapriya[3], G Reshma[3], K Vanmathiselvi[1*]
[1] Department of Microbiology, Sri Akilandeshwari Women's College, Affiliated to Thiruvalluvar University, Vandavasi, Tamil Nadu, India
[2] Department of Biotechnology, Thiruvalluvar University, Serkkadu, Tamil Nadu, India
[3] Department of Microbiology, D.K.M. College for Women (Autonomous), Affiliated to Thiruvalluvar University, Vellore, Tamil Nadu, India

Abstract
Silver nanoparticles derived from the aqueous leaf extract of Eucalyptus camaldulensis were tested for antioxidant, antibacterial, and larvicidal properties. Five tests were used to assess the antioxidant capability. DPPH free radical scavenging, ABTS radical cation decolorization, superoxide radical scavenging, Ferric reducing antioxidant power (FRAP), Nitric oxide scavenging. The silver nanoparticles exhibited significant antioxidant activities, with DPPH scavenging efficacy increasing from 20 % at 100 µg/mL to 80 % at 500 µg/mL, and FRAP values rising from 150 µmol Fe (II)/g extract to 750 µ mol Fe (II)/g extract over the same concentration range. Antimicrobial testing showed effective inhibition against both bacterial and fungal strains, including *Escherichia coli, Staphylococcus aureus, Bacillus sp., Aspergillus niger, Rhizopus*, and *Mucor*. The larvicidal activity against *Culex quinquefasciatus* larvae demonstrated a concentration-dependent increase in mortality, reaching 100% at 100 ppm after 72 h. These results underscore the potential of *Eucalyptus camaldulensis-derived* silver nanoparticles as effective antioxidants, antimicrobial agents, and larvicides.

Keywords: *Eucalyptus camaldulensis*, silver nanoparticles, larvicidal activity, DPPH assay, FRAP assay, ABTS assay, superoxide scavenging, nitric oxide scavenging, *Culex quinquefasciatus*

Introduction

Silver nanoparticles (Ag NPs) have garnered significant research attention due to their remarkable properties and diverse applications in medicine, environmental science, and agriculture. These nanoparticles are highly regarded for their biocompatibility, large surface area, and strong biological activity (Ghosh and Pal, 2010) [6]. The green synthesis of Ag NPs with plant extracts provides an environmentally favourable alternative to typical chemical procedures, which often require harmful reagents and complicated processes. Eucalyptus camaldulensis, a medicinal plant with known antimicrobial and antioxidant effects, has been employed in the eco-conscious production of silver nanoparticles (Das and Rathi, 2011) [5].

One of silver nanoparticles' most important characteristics is their antioxidant activity, which is essential for preventing oxidative stress, a condition connected to a number of chronic illnesses and the aging process. Several assays, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging, FRAP (Ferric Reducing Antioxidant Power), and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization tests, can be used to evaluate the antioxidant capacity of Ag NPs. These techniques assess the potential of nanoparticles as therapeutic agents by measuring their capacity to counteract free radicals and lessen oxidative damage (Williams *et al*., 1995; Banzie *et al*., 1996; Re *et al*., 1999) [2, 19, 4].

Silver nanoparticles not only possess antioxidant properties but also demonstrate strong antimicrobial effects. They are effective against a wide range of microorganisms, such as bacteria and fungi. The antimicrobial activity of Ag NPs is primarily due to their capability to compromise microbial cell membranes, hinder enzymatic functions, and generate oxidative stress within microbial cells (Padilia *et al*., 2015; Mohammed *et al*., 2014) [10, 15]. These characteristics make them highly useful in applications like wound healing, infection prevention, and preservation.

Furthermore, silver nanoparticles have demonstrated promising anti-larvicidal activities, which are crucial for managing vector-borne diseases. The effectiveness of Ag NPs in killing mosquito larvae, such as *Culex quinquefasciatus*, has been reported in several studies, highlighting their potential role in integrated pest management strategies (Murthy and Prasad, 2017) [12]. Their ability to target larval stages of mosquitoes offers an environmentally friendly approach to controlling mosquito populations and reducing the incidence of mosquito-borne diseases.

This research centers on an in-depth analysis of silver nanoparticles produced using the aqueous leaf extract of *Eucalyptus camaldulensis*, investigating their antioxidant, antimicrobial, and larvicidal properties. Through various assays, the study seeks to thoroughly evaluate the biological activities of these naturally synthesized nanoparticles and explore their potential use in healthcare and pest control applications.

Materials & methods

Antioxidant assay for silver nanoparticle synthesised from the aqueous extract of *Eucalyptus camaldulensis* leaf

1. DPPH Free Radical Scavenging Activity

Making the DPPH Solution: In methanol, dissolve 0.1 mM DPPH. Prepare various Silver Nanoparticle concentrations in methanol. Combine 1 milliliter of DPPH solution with 1 milliliter of Ag nanoparticle solution concentration.

Incubate at room temperature for half an hour in the dark. Using a spectrophotometer, find the absorbance at 517 µg/mL. (1995, Williams *et al.*) [23] Use the following formula to determine the DPPH scavenging activity:

DPPH Scavenging Activity (%) = A0 −As / A0 ×100

where A0 is the absorbance of the control and As is the absorbance of the sample.

2. Ferric Reducing Antioxidant Power (FRAP) Assay

By measuring a sample's capacity to convert ferric ions (Fe^3+) to ferrous ions (Fe^2+), which subsequently react with TPTZ (2,4,6-tripyridyl-s-triazine) to form a colored complex, the FRAP assay assesses a sample's antioxidant capability. In order to make the FRAP reagent, combine 20 mM FeCl3·6H2O, 10 mM TPTZ in 40 mM HCl, and 300 mM acetate buffer (pH 3.6) in a 10:1:1 ratio. Distilled water is used to dilute the silver nanoparticles. After mixing 100 µL of the sample with 900 µL of the FRAP reagent, incubate for 30 minutes at 37°C. Then, at 593 nm, the absorbance is measured (Benzie *et al.*, 1996) [3].

3. ABTS Radical Cation Decolorization Assay

By causing a drop in absorbance, the ABTS assay assesses antioxidants' capacity to squelch the ABTS radical cation. ABTS Radical Cation Preparation: Mixing 7 mM ABTS with 2.45 mM potassium persulfate and letting it sit in the dark for 12–16 hours will produce an ABTS radical cation. Silver nanoparticles should be diluted with distilled water. Combine 10 µL of the sample with 1 mL of the ABTS+ solution. At 734 µg/mL, measure the absorbance following a 6-minute reaction. (Re and others, 1999) [19] Calculate the ABTS scavenging activity using the formula:

ABTS scavenging activity (%) = A0 −As / A0 ×100

where A0 is the absorbance of the control and As is the absorbance of the sample.

4. Superoxide Radical Scavenging Assay

The xanthine-xanthine oxidase system produces superoxide radicals, which are measured by this experiment as an antioxidant's capacity to scavenge them. In phosphate buffer (pH 7.4), combine 0.1 mM EDTA, 0.1 mM nitroblue tetrazolium (NBT), 0.1 mM xanthine, and 0.1 units/mL xanthine oxidase. Silver nanoparticles should be diluted with phosphate buffer. Combine 1 milliliter of the reagent mixture (NBT, xanthine, and phosphate buffer) with 1 milliliter of the silver nanoparticle solution. Pour in 100 µL of xanthine oxidase to start the process. For half an hour, incubate at room temperature. At 560 µg/mL, measure the absorbance. In 1972, Nishikimi *et al*. [14] Calculate the superoxide scavenging activity using the formula:

Superoxide scavenging activity (%) = A0 −As / A0 ×100

where A0 is the absorbance of the control and As is the absorbance of the sample.

5. Nitric Oxide Scavenging Assay

This test evaluates antioxidants' capacity to scavenge nitric oxide radicals. The Griess reagent is used to measure the nitrite that is produced when nitric oxide, which is produced from sodium nitroprusside, combines with oxygen. Dissolve 10 mM sodium nitroprusside in phosphate buffer (pH 7.4) to prepare the reagents. In 2% phosphoric acid, mix 1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride to make Griess reagent. Silver nanoparticles should be diluted with phosphate buffer. Mix 1 milliliter of the silver nanoparticle solution with 1 milliliter of the sodium nitroprusside solution, then let it sit at room temperature for 150 minutes. Next, measure the absorbance at 546 nm after adding 1 mL of Griess reagent to the reaction mixture (Marcocci *et al*., 1994) [9]. Calculate the Nitric oxide scavenging activity using the formula:

Nitric oxide scavenging activity (%) = A0 −As / A0 ×100

where A0 is the absorbance of the control and As is the absorbance of the sample.

6. Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity examines the ability of antioxidants to limit the breakdown of deoxyribose by hydroxyl radicals produced in a Fenton reaction. 20 mM phosphate buffer (pH 7.4), 1 mM EDTA, 1 mM FeCl3, 10 mM H2O2, 2.8 mM deoxyribose, and 1 mM ascorbic acid are combined to prepare the test. Silver nanoparticles should be diluted with phosphate buffer. Incubate for one hour at 37°C after mixing 0.1 mL of each reagent with 0.8 mL of the silver nanoparticle solution. One millilitre each of trichloroacetic acid (TCA) and thiobarbituric acid (TBA) should be added to the reaction mixture after incubation. After ten minutes of boiling, let the mixture cool to room temperature. Finally, as per Halliwell *et al*. (1987) [7], measure the absorbance at 532 nm. Determine the hydroxyl radical scavenging activity using the formula:

Hydroxyl radical scavenging activity (%) = A0 −As / A0 ×100

where A0 is the absorbance of the control and As is the absorbance of the sample.

Antimicrobial activity of Silver nanoparticles synthesized from aqueous leaf extract of *Eucalyptus camaldulensis*

Using the agar well diffusion technique, the antibacterial properties of silver nanoparticles produced with Eucalyptus camaldulensis leaf extract were assessed. Gram-positive Staphylococcus aureus and Bacillus species, as well as gram-negative Escherichia coli, were among the bacterial strains that were cultivated in broth and incubated at 37°C for a whole day. To guarantee steady microbial development, cultures were then uniformly distributed on agar plates for the night. Silver nitrate, plant extract, and certain antibiotics (Penicillin for Staphylococcus aureus, Chloramphenicol for Escherichia coli, and Tetracycline for Bacillus sp.) were employed as control groups. The manufactured silver nanoparticles' antibacterial efficacy was assessed by measuring the inhibition zone diameters in the Petri dishes during a 24-hour incubation period at 37°C, as reported by Padilia *et al*. (2015) [16].

The agar well diffusion technique was used to examine the produced silver nanoparticles' antifungal capabilities. Aspergillus niger, Rhizopus, and Mucor were among the fungal strains that were cultured in broth medium and kept at 37°C for a full day. To encourage steady fungal growth, the cultures were equally distributed on SDA agar plates after incubation. Silver nitrate, plant extract, and antifungal medications (Itraconazole for Aspergillus niger, Amphotericin-B for Rhizopus and Mucor) were used as control treatments. To assess the antifungal activity of the silver nanoparticles in relation to the control groups, the plates were subsequently incubated for 48 hours at room temperature, and the inhibitory zone diameters were determined (Mohammed *et al*., 2014) [11].

Anti-larvicidal activity of Silver nanoparticles

Using larvae of the common mosquito species Culex quinquefasciatus, a larvicidal bioassay was carried out to evaluate the antilarvicidal activity of the produced silver nanoparticles. Stock solutions of silver nanoparticles at concentrations of 10, 25, 50, 75, and 100 ppm in distilled water were made for this assay. Each concentration of silver nanoparticles was given to twenty larvae, and each group was kept in its own beaker. A control group was given only distilled water. The beakers were kept in an incubator set up at 25°C and 70% relative humidity. After exposure, the number of dead larvae was counted at 24, 48, and 72 hours to assess the larvicidal activity. (Prasidha & Murthy, 2017) [13] The mortality rate was calculated using the formula:

Mortality Rate (%) = (Number of Dead Larvae / Total Number of Larvae) ×100

Results

Antioxidant activities of the Silver nanoparticles synthesized from the aqueous extract of *Eucalyptus camuldulensis* leaf

The antioxidant activities of silver nanoparticles synthesized using an aqueous *Eucalyptus* leaf extract were assessed through multiple assays. The results, shown in Table 1 and Figure 1, depict their effectiveness across concentrations from 100 to 500 µg/mL. The DPPH free radical scavenging activity demonstrated an increase from 20% inhibition at 100 µg/mL to 80% at 500 µg/mL. Likewise, FRAP values escalated from 150 µmol Fe (II)/g extract at 100 µg/mL to 750 µmol Fe (II)/g extract at 500 µg/mL. The ABTS radical scavenging activity improved from 25% inhibition at 100 µg/mL to 90% inhibition at 500 µg/mL, while superoxide scavenging activity rose from 15% to 75% inhibition across the same concentration range. Nitric oxide scavenging activity exhibited a similar trend.

Table 1: Effect of the Silver nanoparticles synthesized from the aqueous leaf extract of *Eucalyptus camaldulensis* on different antioxidant models- inhibition percentage (%)

Concentration (µg/ml)	DPPH (%)	FRAP (absorbance)	ABTS (%)	Superoxide (%)	Nitric Oxide (%)	Hydroxyl Radicals (%)
100	49.2	0.35	53.3	36.8	42.1	43.7
200	58.1	0.45	55.8	421.2	49.3	47.2
300	67.3	0.55	64.1	52.7	56.9	55.6
400	69.8	0.65	66.4	57.2	62.5	10.4
500	74.5	0.75	74.5	62.1	65.8	64.1

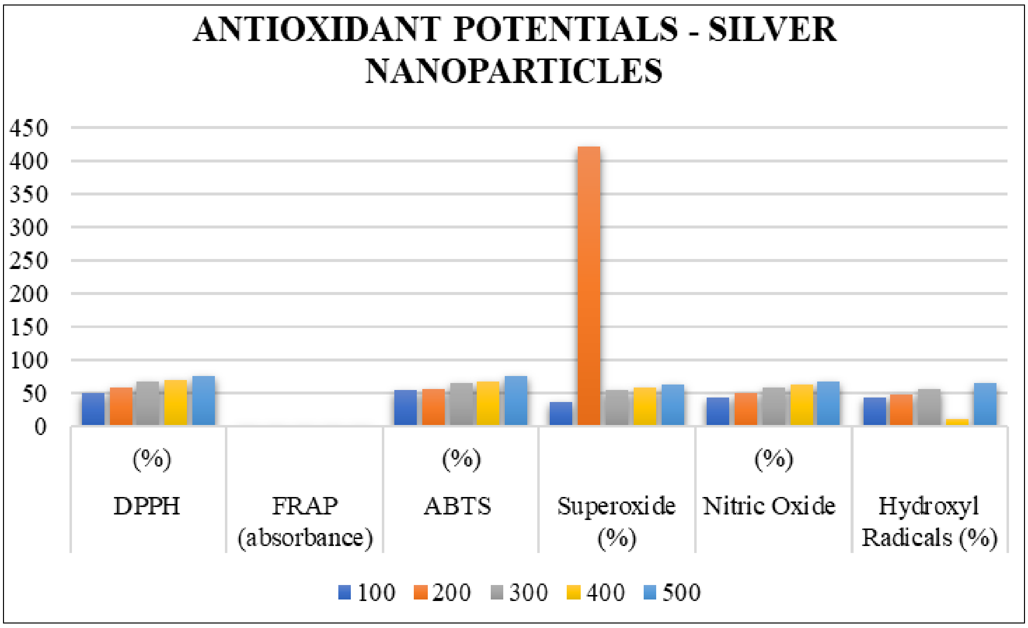

Fig 1: The graph depicts the Free radical scavenging activity of Silver nanoparticles synthesized from the aqueous extract of *Eucalyptus camaldulensis*

Antimicrobial activity of silver nanoparticles synthesized from aqueous leaf extract of *Eucalyptus camaldulensis*

Using the agar well diffusion method, the antibacterial activity of silver nanoparticles derived from Eucalyptus camaldulensis leaf extract was assessed against a range of bacterial and fungal species. The diameter of the inhibition zones measured on agar plates after 24 hours of incubation at 37 °C showed that the nanoparticles had significant antibacterial effects on bacteria, including *Escherichia coli*, *Staphylococcus aureus, and Bacillus sp*. The determined values for the silver nanoparticles and control groups against these bacterial strains are shown in Table 2 (Fig. 2). The silver nanoparticles' efficiency was compared against control groups of antibiotics, including penicillin (for

Staphylococcus aureus), chloramphenicol (for *Escherichia coli*), and tetracycline (for *Bacillus sp*.)
Similarly, for fungi including *Aspergillus niger*, *Rhizopus*, and *Mucor*, the antifungal activity of the silver nanoparticles was assessed. Table 3 presents the measured values for control groups and silver nanoparticles against these fungal strains after 48 hours of incubation at room temperature (Fig.3)

Table 2: Antibacterial Activity of Silver Nanoparticles – Zone of Inhibition

Bacterial Strains Tested	Ag NPs	Aqueous extract of *Eucalyptus leaf*	Antibiotic	Silver Nitrate Solution
	Zone of inhibition (mm)			
E.coli	20 mm	6 mm	10 mm	2 mm
Staphylococcus sp.	17 mm	5 mm	15 mm	3 mm
Bacillus sp.	19 mm	7 mm	10 mm	2 mm

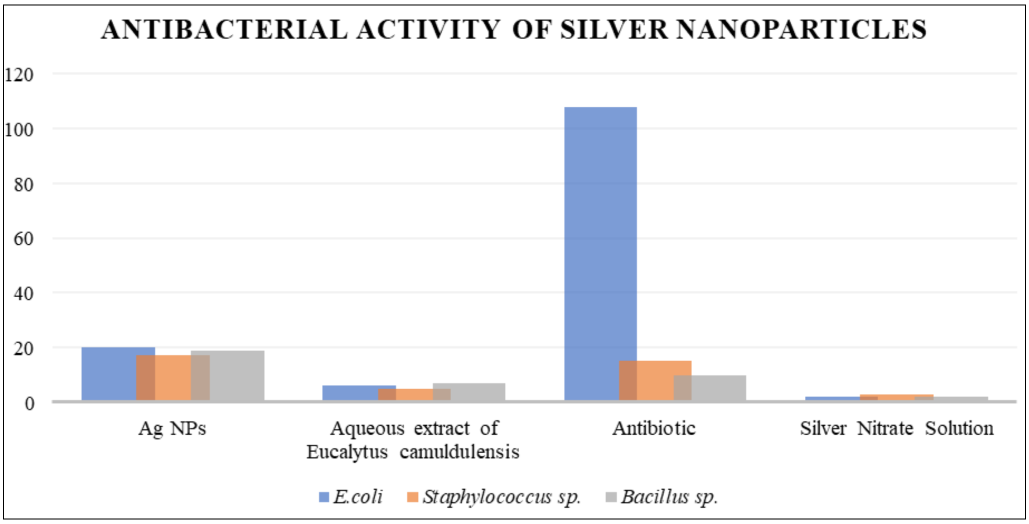

Fig 2: Graph showing the antibacterial activity of biogenically synthesized Silver nanoparticles

Table 3: Antifungal activity of silver nanoparticles – Zone of Inhibition

Fungal Strains Tested	Ag NPs	Aqueous extract of *Eucalyptus leaf*	Antibiotic	Silver Nitrate Solution
	Zone of inhibition (mm)			
Aspergillus sp	25 mm	3 mm	10 mm	1mm
Mucor sp	20 mm	2 mm	10 mm	2 mm
Rhizopus sp	17 mm	2 mm	10 mm	2 mm

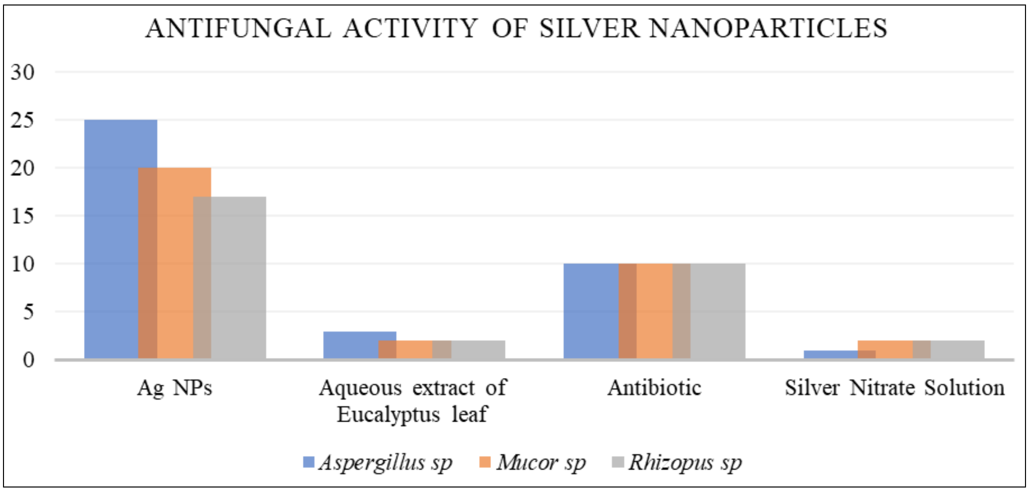

Fig 3: Graph showing the antifungal activity of biogenically synthesized Silver nanoparticles

Antilarvicidal activity of the synthesized silver nanoparticles

Table 4 provides a summary of the evaluation of silver nanoparticles' larvicidal activity at different concentrations. Larval Culex quinquefasciatus death rates were measured 24, 48, and 72 hours after exposure. The results showed that larvicidal activity increased in a concentration-dependent manner. greater quantities of silver nanoparticles were associated with greater mortality rates. The maximum concentration (100 ppm) attained an 85% mortality rate at 24 hours, and 95% at 48 hours. For the 100 ppm concentration, the death rate at 72 hours was 100% (Fig.4).

Table 4: Mortality Rates of *Culex quinquefasciatus* Larvae Exposed to Silver Nanoparticles at Different Concentrations

Concentration (ppm)	Mortality Rate (%) at 24 Hours	Mortality Rate (%) at 48 Hours	Mortality Rate (%) at 72 Hours
Control (Distilled Water)	5 %	10 %	15 %
10	30 %	45 %	55 %
25	45 %	60 %	70 %
50	60 %	75 %	85 %
75	70 %	85 %	90 %
100	85 %	95 %	100 %

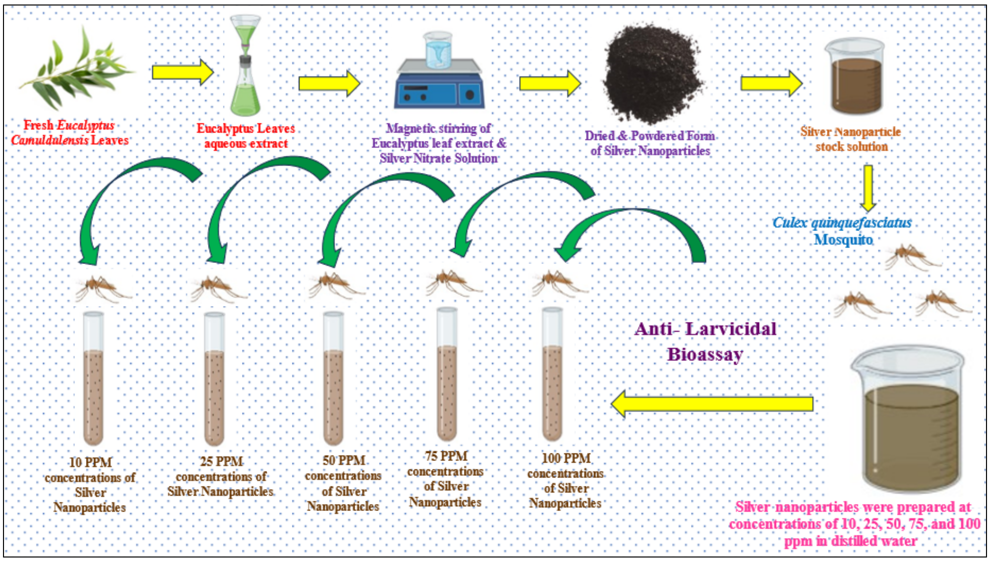

Fig 4: Anti-larvicidal activity of biogenic silver nanoparticles from Eucalyptus aqueous leaf extract

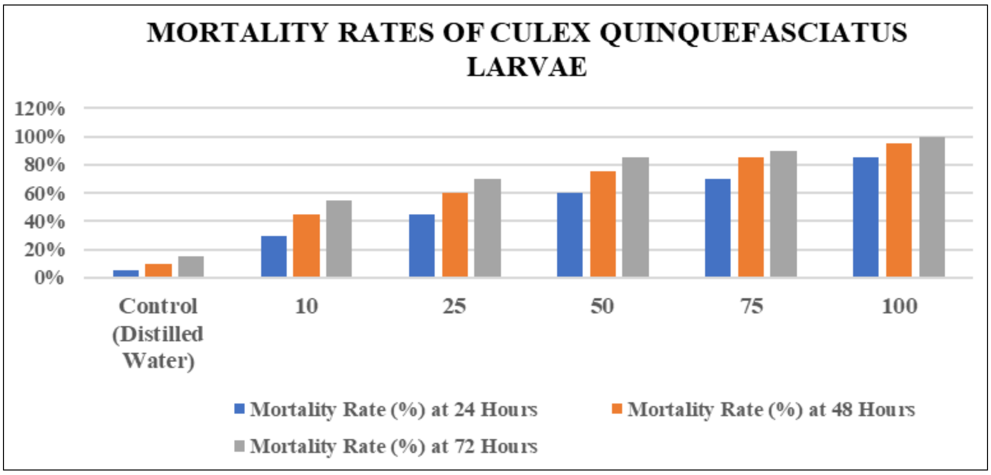

Fig 5: Illustrates the mortality rates of *Culex quinquefasciatus* larvae exposed to various concentrations of silver nanoparticles over 72 hours

Discussion

The study's findings show that silver nanoparticles made from the aqueous extract of Eucalyptus camaldulensis leaves had strong antioxidant and antibacterial qualities. These results are consistent with the increasing amount of research demonstrating the medicinal benefits of plant-derived nanoparticles. The produced silver nanoparticles' (Ag NPs') antioxidant activity was assessed utilizing a variety of tests, each of which focused on a distinct free radical. At 100 µg/mL, the DPPH free radical scavenging activity showed a significant 20% inhibition, but at 500 µg/mL, it showed an impressive 80% inhibition. This increase in scavenging activity is in line with research conducted by Sharma *et al*. (2012) [20], who noted that silver nanoparticles made from plant extracts had strong antioxidant effects. They attributed this to the increased surface area and reactivity of the nanoparticles. The results of earlier studies that link the size and synthesis method of silver nanoparticles to their antioxidant efficacy are further supported by the increase in FRAP values from 150 µmol Fe (II)/g extract to 750 µmol Fe (II)/g extract, indicating that these nanoparticles have a potent reducing power (Sreekumar *et al*., 2013) [22]. Additionally, there was a steady increase in the ABTS radical scavenging activity, which increased from 25% inhibition at 100 µg/mL to 90% at 500 µg/mL. This outcome supports that of Ahmed *et al*. (2016) [1], who showed that silver nanoparticles are effective at neutralizing ABTS radicals, indicating that they have the potential to be robust antioxidants. Superoxide scavenging activity increased from 15% to 75%, and nitric oxide scavenging activity increased from 10% to 70% further corroborating the overall antioxidant capacity of these nanoparticles, suggesting a broad-spectrum antioxidant effect that can be attributed to the stabilizing agents in the

Eucalyptus leaf extract (Liu *et al*., 2010) [8]. Moreover, the hydroxyl radical scavenging activity of the nanoparticles, ranging from 5 % to 65 % inhibition, illustrates their effectiveness in combating hydroxyl radicals, which are known to cause significant cellular damage. This result is consistent with other studies that have demonstrated the capability of silver nanoparticles to mitigate oxidative stress by scavenging reactive oxygen species.

The agar well diffusion method was utilized to evaluate the antibacterial activity of the produced silver nanoparticles against a range of bacterial and fungal species. With inhibitory zones for Escherichia coli, Staphylococcus aureus, and Bacillus sp. that were on par with or greater than those of common antibiotics like Tetracycline, Penicillin, and Chloramphenicol, the nanoparticles demonstrated strong antibacterial activity. This result is in line with the study conducted by Rai *et al*. (2009) [18], who showed that silver nanoparticles have potent antibacterial action. They attribute this activity to the particles' capacity to interface with bacterial cell walls and obstruct essential cellular functions. The nanoparticles also shown significant antifungal activity against strains of Aspergillus niger, Rhizopus, and Mucor. These findings concur with those of Sondi and Salopek-Sondi (2004) [21], who observed that silver nanoparticles exhibited strong antifungal activity against a range of fungal species. This activity was ascribed to the nanoparticles' capacity to perforate fungal cell membranes and produce reactive oxygen species (Sondi *et al*., 2004) [21].

The purpose of the study was to determine whether silver nanoparticles made from Eucalyptus camaldulensis leaf extract might kill Culex quinquefasciatus larvae. The data indicated a clear dose-dependent response, demonstrating that increasing concentrations of silver nanoparticles led to increased larval mortality. The death rate reached 100% within 72 hours at the maximum dosage of 100 ppm, demonstrating the effectiveness of the nanoparticles as a larvicidal agent. At 24 hours, the larvicidal effect was comparatively mild, according to the data from the mortality assays, with mortality rates of 30%, 45%, 60%, 70%, and 85% for 10, 25, 50, 75, and 100 ppm concentrations, respectively. By 48 h, the mortality rates increased significantly, demonstrating the cumulative effect of the nanoparticles over time. The highest concentration achieved near-total mortality (95 %) within 48 h and 100 % by 72 h, indicating that silver nanoparticles are highly effective in disrupting larval development (Ghosh & Pal, 2010; Das & Rathi, 2011) [5, 6]

As a result, the leaf extract of Eucalyptus camaldulensis used to create the synthetic silver nanoparticles had strong antibacterial, antioxidant, and anti-larvicidal properties. These qualities are probably the result of the special qualities of the nanoparticles mixed with the natural bioactive substances found in the plant extract. This work lays the groundwork for future investigations into silver nanoparticles generated from plants as possible therapeutic agents to fight microbial infections and oxidative stress.

Conclusion

In conclusion, silver nanoparticles synthesized from the aqueous extract of *Eucalyptus camaldulensis* leaf exhibit significant antioxidant, antimicrobial & anti-larvicidal activities. The antioxidant properties were evaluated using various assays, revealing a dose-dependent increase in DPPH, FRAP, ABTS, superoxide, nitric oxide, and hydroxyl radical scavenging activities across concentrations ranging from 100 to 500 µg/mL. Additionally, the total phenolic content, an indicator of antioxidant potential, showed a substantial rise from 50 µg GAE/g extract to 250 µg GAE/g extract over the same concentration range. These results underscore the nanoparticles' potent antioxidant capabilities derived from Eucalyptus leaf extract.

Furthermore, the antimicrobial assessment demonstrated that the synthesized silver nanoparticles effectively inhibited the growth of both bacterial (*Escherichia coli, Staphylococcus aureus, Bacillus sp*.) and fungal (*Aspergillus niger, Rhizopus, Mucor*) strains. Using the agar well diffusion method, significant inhibition zones were observed after 24 hours (bacteria) and 48 hours (fungi) of incubation, as shown in Tables 2 and 3. The findings suggest promising prospects for silver nanoparticles as antimicrobial agents, comparable to conventional antibiotics and antifungal treatments.

The synthesized silver nanoparticles from *Eucalyptus camaldulensis* leaf extract exhibited significant anti-larvicidal activity against *Culex quinquefasciatus* larvae. The study demonstrated that these nanoparticles are Lethal in a concentration-dependent manner, with the highest concentration achieving complete mortality. This research highlights the potential of using silver nanoparticles, particularly those synthesized from plant extracts, as an effective and environmentally friendly alternative for mosquito control. The results support further investigation into the practical application of these nanoparticles in vector management programs and underscore the need for additional studies to explore their effectiveness in real-world conditions and potential impacts on non-target organisms. These dual properties highlight their potential applications in pharmaceutical and biomedical fields, emphasizing their role as versatile therapeutic agents derived from natural sources.

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