

FREE RADICAL SCAVENGING ACTIVITY OF SILVER NANOPARTICLES SYNTHESISED USING LAVENDER ANGUSTIFOLIA AND CLITORIA TERNATEA

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ABSTRACT

INTRODUCTION

Nanotechnology is a vigorous research area in the current science field. Silver nanoparticles are used to treat infections in open wounds, chronic ulcers and in textiles, home water purification systems, medical devices, cosmetics, electronics and in medicine. Antioxidants act as radical scavengers, hinder lipid peroxidation and free radical unpaired procedure, and they protect the human body from various diseases accredited to the reaction of radicals. Lavender angustifolia, classified in the mint family, is an evergreen shrub native to southern Europe, primarily the Mediterranean region. Lavender essential oil, when diluted with a carrier oil, is commonly used as a relaxant with massage therapy. The flower Clitoria ternatea belongs to family Fabaceae and subfamily Papilionaceae is an herbaceous perennial, is a connective enhancer used in Ayurveda that is also known as Shanka Pushi.

AIM

The aim of the study is to evaluate the free radical scavenging activity of silver nanoparticles synthesised using Lavender angustifolia and Clitoria ternatea.

MATERIALS AND METHODS

DPPH assay was used to test the free radical scavenging activity of biogenic synthesised silver nanoparticles. The percentage of inhibition was determined from the following equation, % inhibition = (Absorbance of control - Absorbance of test sample) / Absorbance of control × 100. Hydroxyl radical scavenging assay and FRAP assay was also done.

RESULTS AND DISCUSSION

The study has resulted that activity of DPPH assay is significantly inhibited by the silver nanoparticle synthesis using Lavender angustifolia and Clitoria ternatea. Further, the synthesised nanoparticles also significantly inhibited H₂O₂ assay.

CONCLUSION

In conclusion, our results demonstrate that the synthesised silver nanoparticles using Lavender Angustifolia and Clitoria ternatea effectively inhibited the DPPH assay and H₂O₂ assay thereby exhibiting as a free radical scavenging agent.

KEYWORDS: Nanotechnology, free radical scavenging, Lavender angustifolia, Clitoria ternatea, Antioxidants, peroxidation.

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INTRODUCTION:

Nanotechnology is a vigorous research area in the current science field. Nanoparticles have

dimensions up to 100 nm and revelations of dissimilar properties constructed on their size and morphology(Kannan et al. 2014) . The maximum considered nanoparticles are those made from metal

such as Copper, Gold, Silver and Platinum is the metal of excellence for biological systems and medicine. The metal is synthesised by various biological techniques using plants and fungi (Shukla and Iravani 2018). Nanoparticles can be chemical or biological reduction (Jain et al. 2019). Nanoparticles have considerable application in the field of medicine, mechanical, chemical, electronic, optical, food, agriculture, environment, etc. A number of nanoparticles based therapeutics have been approved clinically for infection, vaccine and renal disease. (Krithiga et al. 2015). Silver nanoparticles are used to treat infections in open wounds, chronic ulcers and in textiles, home water purification systems, medical devices, cosmetics, electronics, household appliances, catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine (Ingle et al. 2020).

Lavender (*Lavandula angustifolia*), classified in the mint family (Lamiaceae), is an evergreen shrub native to southern Europe, primarily the Mediterranean region. It is commercially cultivated in, among other places, France, Portugal, Poland, Spain, Hungary, the UK, Bulgaria, Australia, China, and the USA (Cavanagh and Wilkinson 2005). The species name *angustifolia* is Latin for "narrow leaf". Previously, it was known as *Lavandula officinalis*, referring to its medicinal properties. It is a strongly aromatic shrub growing as high as 1 to 2 metres (3 ft 3 in to 6 ft 7 in) tall. The leaves are evergreen, 2–6 cm (1–2+1/2 in) long, and 4–6 mm (3/16–1/4 in) broad. The flowers are pinkish-purple (lavender-coloured), produced on spikes 2–8 cm (1–3 in) long at the top of slender, leafless stems 10–30 cm (4–12 in) long (Yegorova et al. 2019). Lavender is commonly grown as an ornamental plant. It is popular for its colourful flowers, its fragrance, and its ability to survive with low water consumption (Madan et al. 2026). It does not grow well in continuously damp soil and may benefit from increased drainage provided by inorganic mulches such as gravel. It does best in Mediterranean climates similar to its native habitat, characterised by wet winters and dry summers. It is fairly tolerant of low temperatures and is generally considered hardy to USDA zone 5. It tolerates acid soils but favours neutral to alkaline soils, and in some conditions it may be short-lived. The flowers and leaves are used as a herbal medicine in the form of lavender oil or as a herbal tea (de Oliveira et al. 2020). Lavender essential oil, when diluted with a carrier oil, is commonly used as a source relaxant with massage therapy (Braillko et al. 2017).

The flower *Clitoria ternatea* L. (Butterfly pea) belongs to family Fabaceae and subfamily Papilionaceae is an herbaceous perennial, is a connective enhancer used in Ayurveda that is also known as Shanka Pushi. It is native to South-east Asia and broadly dispersed throughout the world, mainly in tropical countries. Almost all the parts

have medicinal importance and are used traditionally for the treatment of various ailments (K et al. 2020). It is currently grown as a mixed ornamental, fodder and medicinal plant. The roots, leaves, and stems are all frequently used in Ayurveda, but for slightly different purposes. *Clitoria ternatea* L. is commonly used as a brain tonic in Ayurveda system of traditional Indian herbal medicine to ameliorate intelligence and intensity the memory function (Ponnusamy et al. 2010).

Antioxidants act as radical scavengers, hinder lipid peroxidation and free radical mediated procedure, and they protect the human body from various diseases accredited to the reaction of radicals (Duraisamy et al. 2024). In healthy individuals, the production of free radicals is balanced by the antioxidative defence system (Radwan et al. 2019). Major sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant source food antioxidants like vitamin C, vitamin E, carotenoids, phenolic acids, phytate and phytoestrogens have been documented as having the potential to reduce disease risk. Antioxidant based drug (Sharma et al. 2025) preparation are used for the anticipation and treatment of multifaceted diseases like diabetes, Alzheimer's disease, atherosclerosis, stroke and cancer (Jamil and Pa'ee 2018). The study is to estimate the free radical scavenging activity of silver nanoparticles synthesised using lavender *angustifolia* and *clitoria ternatea*.

MATERIALS AND METHODS:

1. DPPH METHOD

Antioxidant activity

DPPH assay was used to test the antioxidant activity of biogenic synthesised silver nanoparticles. Diverse concentrations (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) of *Lavandula angustifolia* and *clitoria ternatea* extract was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. Ascorbic acid was used as standard. The percentage of inhibition was determined from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

HYDROXYL RADICAL SCAVENGING ASSAY:

All solutions were prepared freshly. 1.0 mL of the reaction mixture contained 100 µL of 28 mM of 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µL solution of various concentrations of the *Lavandula angustifolia* and *clitoria ternatea* extract (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) 200 µL of 200 µM FeCl₃ and 1.04 mM EDTA (1:1 v/v), 100 µL H₂O₂ (1.0 mM) and 100 µL ascorbic

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acid(1.0mM).After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation at about 532nm against the blank solution . Vitamin E was used as a positive control.

FRAP ASSAY :

REAGENTS FOR FRAP ASSAY:

- Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water. b) TPTZ (2, 4, 6-tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). c) FeCl₃. 6 H₂O: (M.W. 270.30), 20 mM. The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. Standard was FeSO₄. 7 H₂O: 0.1 - 1.5 mM in methanol. All the reagents were prepared from Merck (Germany) company.
- FRAP solution (3.6 mL) is added to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution mixed with a certain concentration of the *Lavandula angustifolia* and *clitoria ternatea* extract (10µL,20µL,30µL,40µL,50µL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO₄, 7H₂O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions.

RESULTS:



Figure 1: Silver nanoparticles formation.

Effect of silver nanoparticles synthesised using *Lavender angustifolia* and *Clitoria ternatea* on DPPH assay:

In the present study, the activity of DPPH assay was significantly inhibited by silver nanoparticles synthesised using *Lavender angustifolia* and *Clitoria ternatea*.

Effect of silver nanoparticles synthesised using *Lavender angustifolia* and *Clitoria ternatea* on H₂O₂ assay:

In the present study, the activity of H₂O₂ assay was significantly inhibited by silver nanoparticles synthesised using *Lavender angustifolia* and *Clitoria ternatea*.

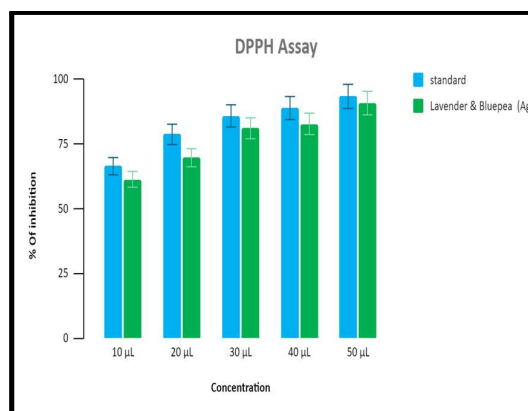


Figure 2: Effect of silver nanoparticles synthesised using *Lavender angustifolia* and *Clitoria ternatea* on DPPH assay.

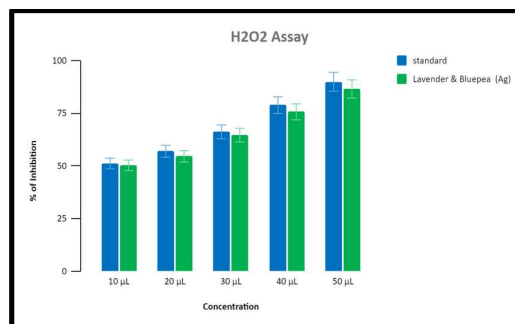


Figure 3: Effect of silver nanoparticles synthesised using *Lavender angustifolia* and *Clitoria ternatea* on H₂O₂ assay.

DISCUSSION:

Silver nanoparticles are the most often used nanoparticles among the others. It was used in personal care products, sensors, antimicrobial creams, and biomedical applications. Their potential toxicity is a concern given their diverse variety of uses. The two anti-inflammatory drugs that are used most frequently are ibuprofen and diclofenac sodium, both of which are NSAIDs(Swathy et al. 2020). Materials known as nanoparticles (NPs) have properties that set them apart from their bulk and molecular counterparts. Using a physical approach,

nanoparticles have been used to alter and enhance the pharmacokinetic and pharmacodynamic characteristics of a number of pharmaceutical drugs. To increase treatment efficiency while lowering side effects, several polymers have been used to create nanoparticles for drug delivery research (Banfield and Navrotsky 2018).

Although the prognosis for these drugs is good, they typically have gastrointestinal side effects that can include gastric ulcers and cardiovascular problems. Due to their safety characteristics and wide range of applications, notably in the biological sciences, silver nanoparticles produced through biosynthesis are preferred to those produced through other methods of synthesis. Biosynthesized AgNPs are used in pharmaceutical research (Kumar et al. 2021). The synthetic Lavender and Clotoria extract may have antioxidant capabilities without the usage of nanoparticles, according to research contradicting Chaudhary's direction (Chaudhary et al. 2013).

By lessening the cellular oxidative damage brought on by free radicals, antioxidants serve as a defence mechanism to stop the body from developing serious chronic diseases. To assess the scavenging abilities of the silver nanoparticles synthesised from Lavender angustifolia and Clitoria ternatea, the DPPH assay was carried out (Rajeshkumar et al. 2021). The potential of silver nanoparticles to significantly reduce cell viability in lung cancer cell lines (HepG2) and liver cancer cell lines (A59). According to Rajeshkumar's research, the free radical scavenging activity was concentration-dependent as well. Due to the presence of antioxidative compounds in its tissues, *Lavandula angustifolia* and *Clitoria ternatea* is well known for its health-promoting qualities (Zulfiqar et al. 2022). The i.a. cultivation method, growth environment, climatic circumstances, and plant genotype all have an impact on the chemical makeup of plant metabolites and their antioxidative characteristics (Imtiaz et al. 2021). The present study concluded that *Lavender angustifolia* and *Clitoria ternatea* showed significant free radical scavenging activity by DPPH assay and H₂O₂ assay.

CONCLUSION:

The *Lavender angustifolia* and *Clitoria ternatea* mediated Silver nanoparticles showed remarkable and considerable free radical scavenging activity when compared with the standard values. The antioxidant property of the extract mediated Silver nanoparticles, was applicable in nanomedicine through the help of advanced technologies to support the medical voided fields. The properties of the synthesised nanoparticles were further implied in nanotechnology which may be useful for other sourcing fields. More research is needed to fully assess the potential of plant-mediated nanoparticles. More scientific study must be done to develop our understanding on free radical scavenging activity of

silver nanoparticles synthesised using *Lavender angustifolia* and *Clitoria ternatea*.

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CONFLICT OF INTEREST:

The authors would like to declare no conflict of interest in the present study.

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