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Research Article

SAGE AND THYME-MEDIATED ZINC OXIDE NANOPARTICLE SYNTHESIS AND ITS BIOMEDICAL APPLICATIONS

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ABSTRACT

This study focuses on the synthesis of zinc oxide nanoparticles using sage and thyme extracts as green reducing agents. The synthesized nanoparticles were characterized using UV-Vis spectroscopy. Furthermore, the biomedical applications of the synthesized nanoparticles were investigated, including their antibacterial activity against various pathogenic bacteria and their potential as a drug delivery system. The results showed that the synthesized nanoparticles had significant antibacterial activity against Gram-positive and Gram-negative bacteria. The nanoparticles also exhibited strong antioxidant and anti-inflammatory properties, indicating their potential for use in various biomedical applications. Overall, the study suggests that sage and thyme-mediated zinc oxide nanoparticles have promising antimicrobial, cytotoxicity activity, anti-inflammatory, and antioxidant activities, and could serve as potential candidates for the development of novel therapeutic agents.

Keywords: Zinc nanoparticle, *Salvia officinalis*, *Thymus vulgaris*, Brine shrimp lethality assay, Antimicrobial activity.

INTRODUCTION

In recent years, nanotechnology has played an integral part in our day-to-day lives. The principle of this revolutionary technology has been applied in various fields and products with nanomaterials, or claims of nano-based particles have become readily available these days (Soares *et al*., 2018). Pharmaceutical research is no exception; the usage of nanoparticles for medical applications is termed nanomedicine. Nanomedicine is described as the employment of nanoparticles to prevent, control, diagnose, and cure certain diseases and illnesses (Tinkle *et al*., 2014). *Salvia officinalis L.,* commonly known as sage, is an herb native to the Mediterranean region but is cultivated around various parts of the world. Recent research conducted on extracts found sage as a rich source of bioactive compounds. Further studies also revealed that sage possesses antioxidant and antimicrobial effects, such as antiviral and fungicidal properties. So, due to its strong antimicrobial potential and palatable taste, it is also used as a preservative in the food industry to inhibit the growth of certain foodborne pathogens (El-Feky *et al*., 2016; Abdelkader *et al*., 2014). Similarly, *Thymus vulgaris*, also known as thyme, is a perennial shrub commonly found in the Mediterranean region. It is also rich in bioactive compounds, the main phenolic compounds being thymol and carvacrol, constituting 68.1% and 10% respectively (Nieto, 2020). The essential oil of thyme acquired by the distillation of leaves is a major commercial product and it is used in products such as food flavorings, cosmetics, soaps, mouthwashes, toothpaste, pesticides, and pharmaceutical products (Singletary, 2016).

Thyme and its constituent compounds have been studied intensively and were proven to possess antimicrobial, antioxidant, analgesic, anticonvulsant, and anxiolytic properties (Zaïri *et al*., 2020). Since ancient times, Zinc oxide (ZnO) has been noted for its antibacterial efficiency; several historical records denote that zinc oxide has been used in ointments to treat boils and infections since 2000

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BC. Nowadays, it is used in solar cells, photoconductive materials, transparent transistors, LED, sunscreens, cosmetics, lotions, and memory devices, among many other things. The amount of ZnO being used in the field of medicine is relatively low compared to other fields. The FDA has classified (21 CFR 182.8991) zinc oxide to be safe (Siddiqi *et al*., 2018). Green synthesis of nanoparticles has emerged as a cost-effective, biocompatible, eco-friendly, and safe method to synthesize nanoparticles. Furthermore, it allows the production of ZnO NPs in large numbers and is free of impurities (Agarwal *et al*., 2017). The main objective of the study is to synthesize and characterize zinc oxide nanoparticles from the leaf extracts of sage and thyme through the green synthesis method and the evaluation of the antimicrobial, cytotoxic, anti-inflammatory, and antioxidant activity using specific assays to determine the biomedical potential of sage and thyme mediated ZnO NPs.

MATERIALS AND METHODS

Plant extract preparation

Dried samples of sage and thyme extracts were purchased from local markets around Chennai. The extraction procedure employed for the dried extracts is as follows g of thyme and 1g of sage were measured and taken. 100 mL of distilled water was added to the measured extracts, and it was boiled for about 10 minutes at 65-75 °C. The mixture was cooled and the contents were filtered using a filter cloth, a funnel, and a measuring flask.

Zinc oxide nanoparticle synthesis

15 Mm (0.445g) of zinc oxide (ZnO) was dissolved in 50 ml of distilled water and 50 ml of thyme and sage extract mixture. Then the solution was kept in a magnetic stirrer for 48 hours for nanoparticle synthesis. Subsequently, using a UV-visible double beam spectrophotometer, the colour changes in the mixture were observed at different wavelengths ranging from 250 nm to 650 nm and the graph was obtained. The mixture of sage and thyme-mediated zinc oxide nanoparticles was centrifuged at 8000 rpm for 10 minutes. The pellet was separated from the supernatant, collected, and stored in airtight vials for future usage.

Characterization of zinc oxide nanoparticles

The zinc oxide nanoparticles were characterized using a Double-beam U-V vis spectrophotometer. The sage and thyme-mediated nanoparticle solution was taken in a cuvette and analyzed at 400-650 nm frequency. The results were recorded, and a graphical analysis was done.

Antimicrobial activity using the well diffusion method

The well diffusion method is the most commonly used method to evaluate the antimicrobial potential of plant extracts and nanoparticles (Balouiri *et al*., 2016). Mueller Hinton Agar and Rose Bengal agar were prepared for the evaluation of antibacterial and antifungal activity, respectively. Both were measured accordingly, dissolved in distilled water and sterilized using an autoclave for 15 minutes. The sterile media was poured into Petri plates and allowed to solidify. Following solidification, microorganisms such as *Escherichia Coli*, *Enterococcus faecalis*, *Pseudomonas*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Lactobacillus* were inoculated using cotton swabs. T-shaped wells were made and the sample was pipetted in the following concentrations (25, 50, 100 µL). The dishes were incubated at 31°C and monitored for microbial growth. After 24 hours, the zone of inhibition was measured and the readings are furnished below

Cytotoxicity analysis using brine shrimp lethality assay

Cytotoxicity in general is described as the characteristic of being toxic to cells (Mithra *et al*., 2021). 5g of sea salt was mixed with 500 ml of distilled water. Ten newly hatched shrimp nauplii were taken into six separate wells and the prepared salt solution was added to the wells. Then, the sage and thyme-mediated nanoparticle solution was added to each of the wells in concentrations of 5, 10, 20, 40, and 80 µL. One well without the sage and thyme ZnO-NP solution and with live nauplii served as the control. The wells were kept undisturbed for 48 hours and after that number of live nauplii was counted and the graph was obtained.

Anti-inflammatory activity using protein denaturation assay

The reagent for the assay was BSA (Bovine serum albumin). Because BSA accounts for approximately 60% of all proteins in animal serum, it is widely used in cell culture (Francis *et al*., 2020). A non-embryonated egg about 4-6 days old was taken. The top layer was broken and shells were carefully removed with forceps. The egg white was carefully separated from the yolk till the albumin was collected. Following this, in a beaker, 2800 µl of 10x Tris-PBS (Phosphate Buffer Saline) was added to 200 µl of egg albumin and distilled water and pipetted to five test tubes labelled with varying concentrations (10, 20, 30, 40, and 50 µl). The plant extract NP solution was pipetted into the test tube with respect to their labelled concentrations. The inhibitory percentage was estimated from this formula

$$Inhibition\% = \frac{A0-A}{A0} \text{ x } 100$$

Antioxidant activity

DPPH 2,2-Diphenyl-1-picrylhydrazyl radical scavenging method

The antioxidant activity of sage and thyme-mediated ZnO NPS was determined in terms of free radical scavenging or metal ion chelating ability using the radical DPPH assay. Experiments were carried out using the procedure reported by Rajeshkumar *et al*. (2022). At absorbance 517 nm, the analysis of free radical scavenging was measured and

noted. The percentage of inhibition was calculated using the following equation:

$$Inhibition\% = \frac{A0-A}{A0} \text{ x } 100$$

Hydrogen peroxide activity

Five test tubes labelled with various concentrations were taken. 1000 µL of hydrogen peroxide H_2O_2 was added to each of the test tubes. Subsequently, the plant extract was also pipetted in the concentrations of 10, 20, 30, 40, and 50 µL in the five test tubes, respectively. Following that, the tubes were kept in the dark at 37° C to prevent oxidation. Finally, at absorbance 230 nm, OD values were recorded and noted. The percentage of inhibition was found and the graphical analysis was done.

RESULTS AND DISCUSSION

The formation of sage and thyme-mediated ZnO nanoparticles was observed with the help of a UV-Vis spectrophotometer. As the ZnO nanoparticle solution was added to the aqueous plant extract mixture, the colour was changed from dark brown to light brown (Figure 1A & B). The ZnO NPs were analysed within a wavelength range of 250-650 nm, and their peak (surface plasmon resonance) was noted at 352 nm (Figure 2). The colour change observed during the 48-hour incubation period confirms the formation of zinc oxide nanoparticles. From fig. 2, the absorption peak of the synthesized zinc oxide nanoparticles is around 352 nm. A study performed to analyze the characteristic properties of zinc oxide Nps states that the maximum absorption spectra of ZnO-NPs is around 352 nm and this is due to their huge excitation separation energy at those temperatures (Ngom *et al*., 2020; Talam *et al*., 2012). The absorption edge consistently moves to a higher energy or lower wavelength when the nanoparticle's size decreases (Manigandan *et al*., 2025). The antimicrobial activity of the mediated ZnO NPs was assessed using the agar well diffusion method with the sage and thyme plant extract acting as the control (Figure 3). A combination of Gram-negative, Gram-positive, along a fungus was the chosen microorganisms to determine the antimicrobial efficacy of the plant extract-mediated ZnO Nps. *Streptococcus mutans*, a gram-positive bacterium, showed the maximum sensitivity with a zone of inhibition of 25±1 nm at a concentration of 25 µl and 100 µl, which was similar to the control. Following that, *E. coli*, a gram-negative bacterium, was the second most sensitive bacteria with a zone diameter of 23±1 nm at 100 µL concentration, which was also higher compared to the control used. *Staphylococcus aureus* and *Enterococcus faecalis*, both belonging to gram-positive bacteria, exhibited their maximum zone of inhibition at 100 µL with a zone diameter of 22±1 nm and 13±1 nm, respectively. Lactobacillus and Pseudomonas, both gram-negative bacteria, also showed their maximum inhibition zone at 100 µl with a zone diameter measuring around 17±1 and 10±1 mm, respectively. Finally, the yeast used, *Candida albicans*, was the least sensitive among all the others, as its zone diameter at different concentrations was the same and remained unchanged, as depicted in figure 4 and Table 1. Gram-positive bacteria have a thick cell wall of about 20-80 nm consisting of multiple layers of peptidoglycan, whereas gram-negative bacteria have several layers of peptidoglycan underneath an outer membrane made up of a mixture of lipopolysaccharide, lipoproteins, and phospholipids with a thickness of about 8-10 nm (Fu *et al*., 2005). These cells are highly susceptible to damage since the nanoparticles are about the same size range and can easily pass through them (Sirelkhatim *et al*., 2015).

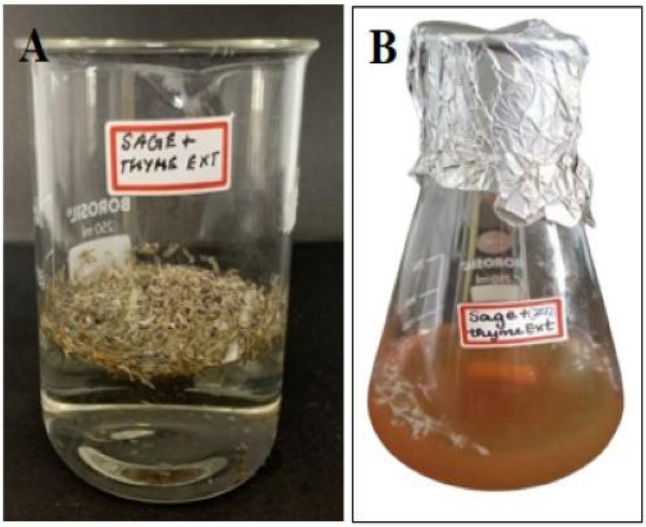

Figure 1. (A) Sage and thyme extract (B) Sage and thyme-mediated zinc oxide NPs

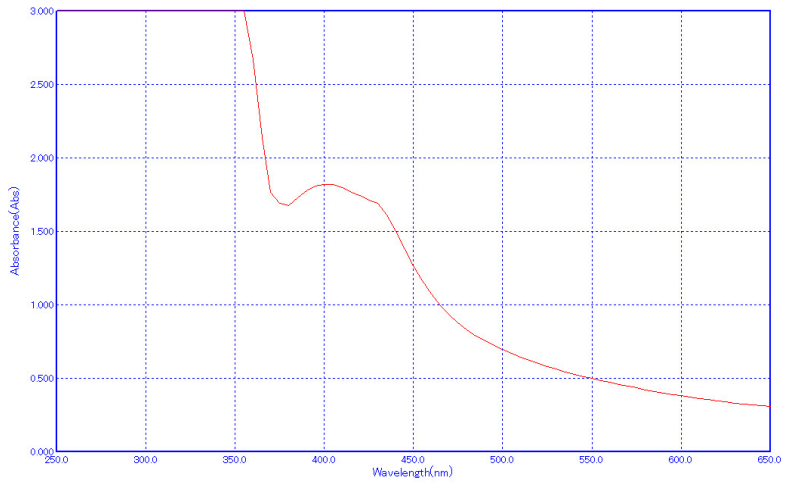

Figure 2. UV-vis spectroscopy of sage and thyme-mediated zinc oxide NPs

Foodborne diseases and food-related illnesses are becoming serious health concerns worldwide (Balaban *et al*., 2000). Staphylococcus aureus, an opportunistic pathogen known for a wide range of infections ranging from mild superficial skin infections to potentially fatal invasive diseases, is responsible for about 2.4 lakhs of foodborne disease cases in the United States alone. Thus, the necessity for novel approaches to prevent food spoilage and foodborne illnesses is rising more than ever due to increasing numbers of hospitalizations and deaths worldwide (Kadariya *et al*., 2014). Thyme is generally known to be a natural antioxidant and antimicrobial agent and it has been proven to be effective against the growth of *E. coli* and *S. aureus*. It is also considered to be generally safe for consumption (GRAS) by the FDA and is primarily used as a flavoring agent in foodstuffs (Magesh *et al*., 2022). Similarly, ZnO NPs are proven to be inhibitory towards the growth of pathogenic organisms such as *E. coli*, *E. faecalis*, *S. aureus*, and *P. aeruginosa*. Hence, owing to its strong antimicrobial potential, sage and thyme-mediated ZnO NPs can be employed as preservatives in food products to eliminate the growth of certain foodborne and spoilage-causing organisms (Sirelkhatim *et al*., 2015). The permeability and structure of the plasma membrane were impaired by ZnO nanoparticles, resulting in repressed cell growth. They produced reactive oxygen, leading to cell death. Light stimulation increased antimycotic action, and ROS quenching histidine decreased inhibition actions toward yeast cells. Active ROS participation in the process discloses the potential of ZnO nanoparticles for bacterial cell health (Magesh *et al*., 2022; Dulta *et al*., 2022).

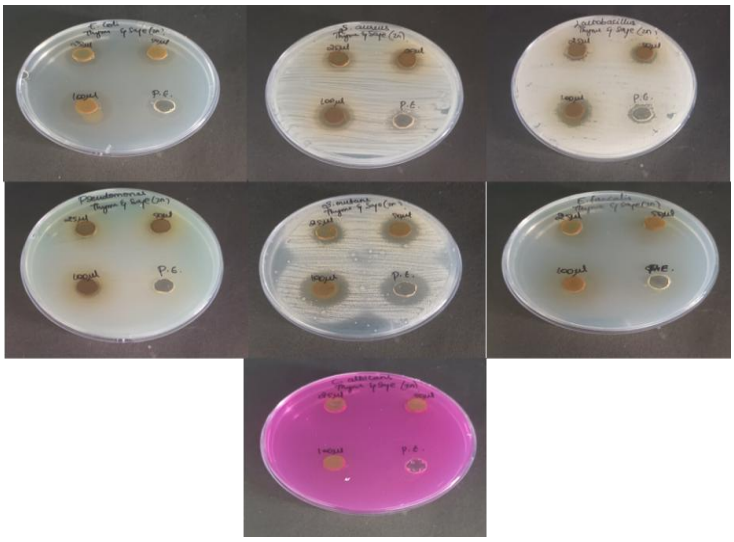

Figure 3. Zone of inhibition against selected pathogens.

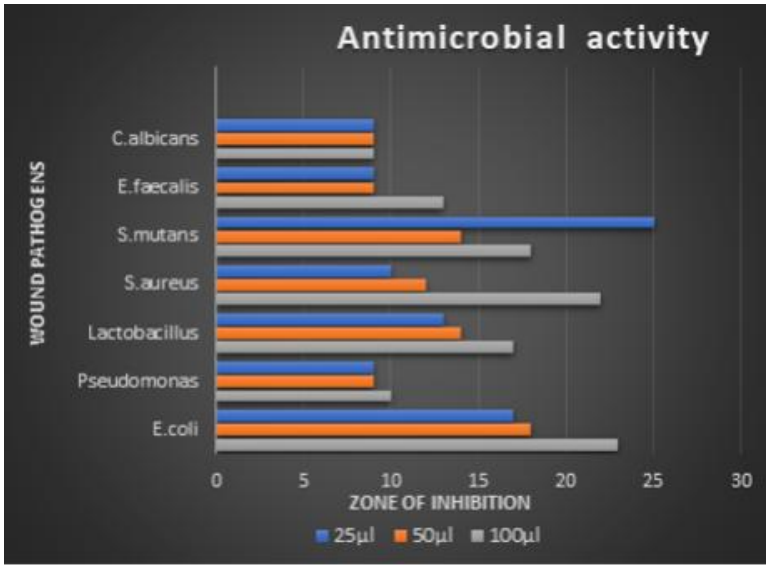

Figure 4. Antimicrobial activity against pathogens.

Table 1. Antimicrobial activity of the Zn nanoparticle against selected microbes.

Name of test organism	Zone of inhibition in mm ± SD			
	25 µL	50 µL	100 µL	Control
Escherichia coli	17 ± 1	18 ± 1	23 ± 1	19 ± 1
Pseudomonas aeruginosa	9 ± 1	9 ± 1	10 ± 1	9 ± 1
Lactobacillus acidophilus	13 ± 1	14 ± 1	17 ± 1	13 ± 1
Staphylococcus aureus	10 ± 1	12 ± 1	22 ± 1	20 ± 1
Streptococcus mutans	25 ± 1	14 ± 1	25 ± 1	25 ± 1
Enterococcus faecalis	9 ± 1	9 ± 1	13 ± 1	9 ± 1
Candida albicans	9 ± 1	9 ± 1	9 ± 1	9 ± 1

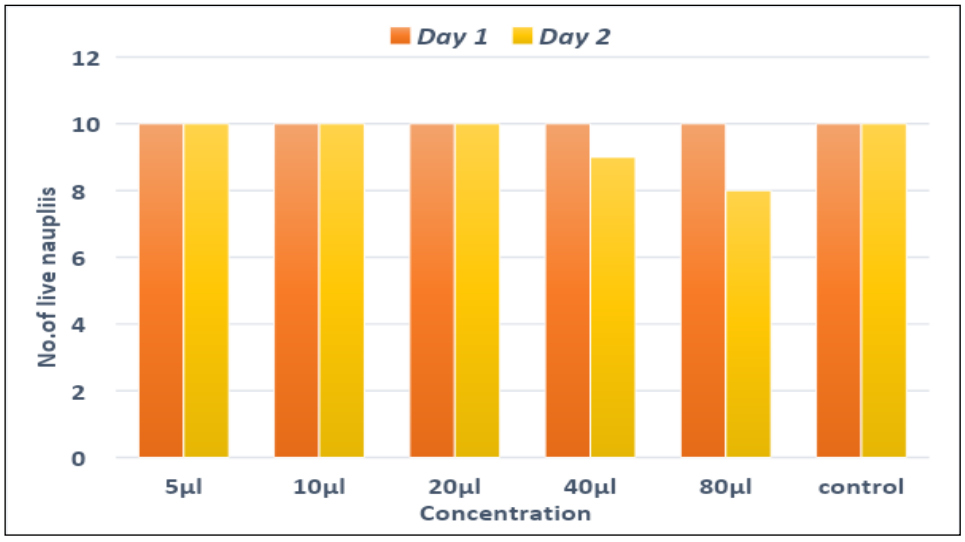

Figure 5. Cytotoxicity activity of sage and thyme-mediated ZnO NPs.

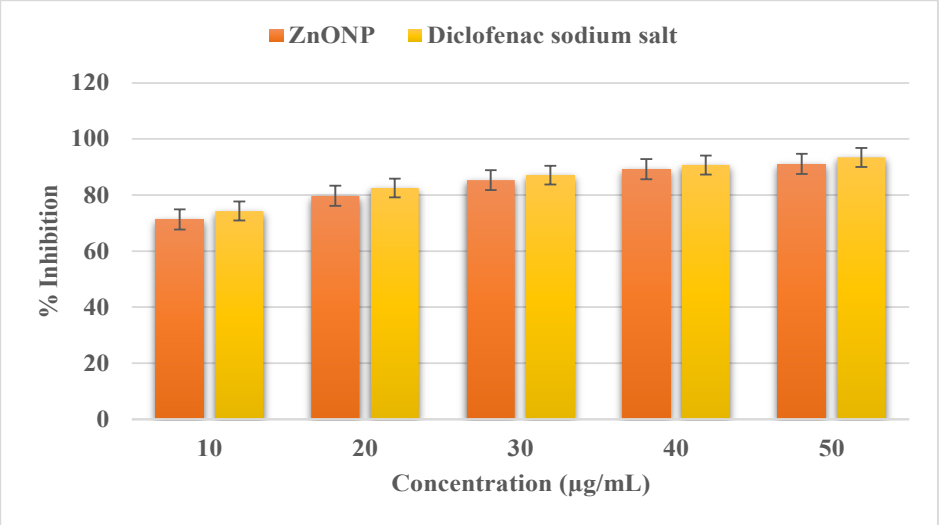

Figure 6. Anti-inflammatory activity of sage and thyme-mediated ZnO NPs.

There was a total of 60 nauplii, with 10 nauplii evenly distributed among the six plates, including the control. The wells were kept undisturbed for 24 hrs to assess the activity of sage and thyme-mediated zinc oxide NPs. Eventually, the number of live nauplii was counted, and well 1, well 2, well 3 (concentrations of 10, 20, 30 µL respectively) and the control each had 10 alive nauplii and no dead nauplii were observed. Those three wells had the same amount of nauplii as they did the previous day. But well 4 and well 5 (concentrations of 40 and 50 µL) both showed a gradual decrease in the number of live nauplii, as they had 9 and 8 alive nauplii per well, respectively (Figure 5). The protein denaturation method is a significantly cheaper and effective method to assess the anti-inflammatory activity of any component (Dharmadeva *et al*., 2018). Sage and thyme-mediated zinc oxide nanoparticles were able to inhibit protein denaturation based on the amount of concentration (10, 20, 30, 40 and 50 µg/mL). Hence, the inhibitory response is entirely dependent on the concentration of the nanoparticle (Figure 6). The maximum inhibition of 91.1 % observed at a 50 µg/mL concentration. In comparison, the standard ascorbic acid displayed a 93.4% inhibition at the highest concentration. Even though there are anti-inflammatory benefits associated with nanoparticles, there are also the prospects of them having pro-inflammatory activities upon the intervention of reactive oxygen species (ROS). The contradictory activities outline the necessity for an integrative illustration of nanoparticle activities and applications of usefulness within anti-inflammatory applications (Varghese *et al*., 2024).

Oxidation is defined as the chemical reaction in which an atom, molecule, or ion loses its electron, ultimately increasing in oxidation state. The antioxidant potential of a compound is evaluated through its ability to act as a free radical scavenger that begins chain reactions, oxidative enzyme inhibitors, and metal ion chelators. Antioxidants are molecules that inhibit the oxidizing chain reactions and delay or stop the oxidation process of fats and lipids (Muthoni *et al*., 2020; Proestos *et al*., 2013). Test results for the above experiments were that the activity of iron oxide nanoparticles and normal ascorbic acid (Vit-C) at concentrations of 10–50 µg/mL greatly inhibited the action of these free radicals. ZnONPs in the present study exhibited a dose-dependent scavenging activity against DPPH radicals, with a maximum inhibition of 91.6 % observed at 50 µg/mL concentration (Figure 7). In comparison, the standard ascorbic acid displayed a 92.4% inhibition at the highest concentration. The H_2O_2 assay utilizes commonly adopted protocols to predict the antioxidant purity of the test samples. Though hydrogen peroxide is quite inert, it is dangerous to cells as it forms hydroxyl radicals, which are toxic. Antioxidants like phenols, polyphenols, and flavonoids have the capacity to scavenge H_2O_2 and thereby safeguard mammalian cells from harm induced by hydrogen peroxide. During one study that used a hydrogen peroxide assay, they noticed that ZnONPs showed an inhibition of free radicals by 87.1% at the 50 µg/mL concentration level, while the control antioxidant ascorbic acid showed a 90.1% inhibition of free radicals at the maximum concentration level (Figure 8). This evidence indicates that ZnONPs showed high antioxidant activity that was similar to that of ascorbic acid and thus showed great promise to serve as an antioxidant agent. Phenolic species found in plant extracts have generally been shown to have strong antioxidant qualities, which is a crucial feature for bioapplications (Anandan *et al*., 2013). Overproduction of free radicals in the body results in oxidative stress and damage to the biomolecules if the body's defense mechanism through antioxidants is insufficient. Antioxidant compounds suppress such excess free radicals in the system. The antioxidant behavior manifested by the newly synthesized zinc oxide nanoparticles makes them highly usable for applications through therapies for treating numerous ailments related to oxidative stress (Dulta *et al*., 2022).

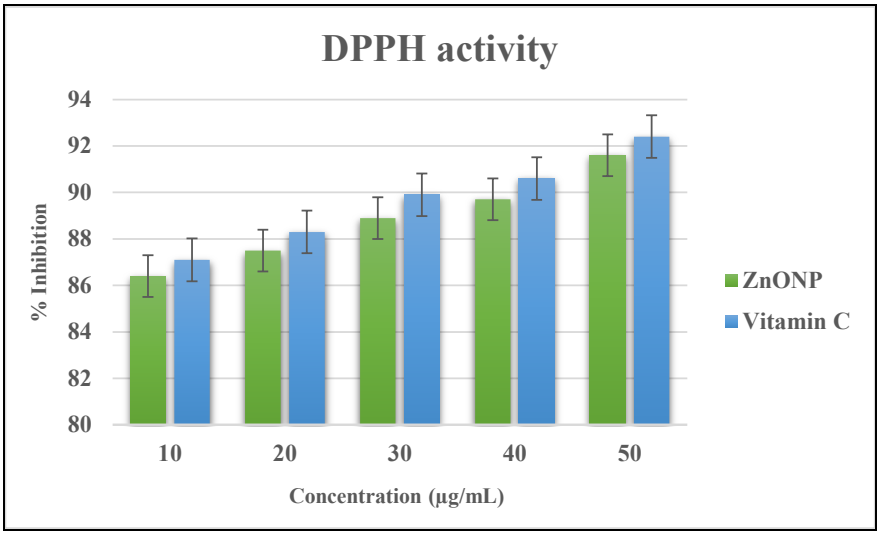

Figure 7. Antioxidant activity evaluated using the DPPH assay of sage and thyme mediated ZnO NPs.

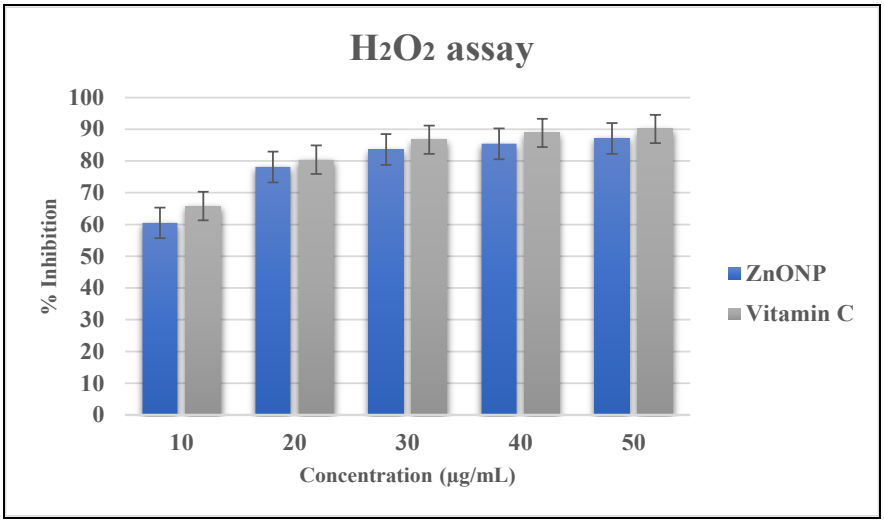

Figure 8. Antioxidant activity evaluated using the Hydrogen peroxide assay of sage and thyme mediated ZnO NPs.

CONCLUSION

This study shows that zinc oxide nanoparticles can be successfully synthesized using sage and thyme extracts in an environmentally friendly manner. The nanoparticles displayed strong antibacterial effects against both Gram-positive and Gram-negative bacteria, along with marked antioxidant and anti-inflammatory properties. These results suggest that plant-based ZnO nanoparticles hold real promise as future antimicrobial and anti-inflammatory agents. Thyme, rich in phenolic compounds like carvacrol and thymol, is effective against various cancer cell lines. Further research is needed to explore the anticarcinogenic activity of sage and thyme-mediated ZnO-NPs and their potential as anticancer or chemotherapeutic agents.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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