ISSN: 2306-6091

International Journal of Pharmaceuticals and Health Care Research (IJPHR)

IJPHR |Vol.14 | Issue 3 | Jul - Sep -2026

www.ijphr.com

DOI : https://doi.org/10.61096/ijphr.v14.iss2.2026.594.615

Formulation and Evaluation of Garlic Oil–Zinc Oxide Nanoparticle Gel for In-Vitro Anti-Inflammatory and Antioxidant Activitiy

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Published on: 01.09.2026
Published by: Futuristic Publications

Abstract:

Background: Garlic oil (GO) contains organosulfur compounds with reported antioxidant and anti-inflammatory properties, whereas zinc oxide nanoparticles (ZnO NPs) are of interest for topical delivery systems.

Aim: To prepare GO-mediated ZnO NPs, formulate them into a topical gel, and evaluate in vitro antioxidant and anti-inflammatory activities.

Methods: GO-ZnO NPs were synthesized from zinc acetate using garlic oil and incorporated into a gel base. Appearance, odour, pH, and centrifugation stability were assessed. Antioxidant activity was evaluated by DPPH and FRAP assays; albumin denaturation assessed anti-inflammatory activity. Ascorbic acid and diclofenac sodium served as standards.

Results: The gel was pale white, garlic scented, and had pH 5; phase separation occurred after centrifugation. GO-ZnO NPs produced 78% DPPH inhibition at 10 mg (IC50 14.50 mg), whereas gel produced 90% inhibition at 5 mg (IC50 9.76 mg); ascorbic acid IC50 was 3.373 mg. At 15 mg, FRAP absorbance was 1.53 for NPs and 1.43 for gel. Albumin-denaturation inhibition was 55% at 5 mg for NPs (IC50 15.83 mg) and 62% at 10 mg for gel (IC50 6.09 mg); diclofenac IC50 was 12.129 mg. Alkaloids were detected.

Conclusion: Both preparations demonstrated activity; however, instability and non-linear responses require formulation optimization and validation before topical application.

Keywords: Garlic oil; Zinc oxide nanoparticles; Antioxidant activity; Anti-inflammatory activity; DPPH assay.

INTRODUCTION

Nanoparticles

Nanoparticles are tiny entities that measure between 1 and 100 nanometers in at least one dimension, positioning themselves in a distinct physical realm that lies between bulk materials and atomic structures. At this measurement range, the ratio of surface area to volume increases significantly, leading materials to display notably changed physical, chemical, and optical characteristics when compared to their larger forms. For example, normally unreactive substances such as gold can transform into highly effective catalysts, while materials that are normally opaque may become transparent. These alterations are attributed to quantum phenomena, which play a significant role in how these particles interact with light, heat, and electrical energy [1]

Zinc Oxide Nanoparticles

Zinc nanoparticles, particularly Zinc oxide nanoparticles (ZnO NPs), are widely used due to their unique properties like high surface area, strong UV absorption, and visible light emission. They find applications in various fields, including sunscreens, electronics, medicine, and environmental remediation.

Fig 1: Zinc Oxide Nanoparticles

Classification of zinc oxide nanoparticles:

Zinc oxide nanoparticles (ZnO NPs) can be classified based on their structure, size, and surface properties. Structurally, they can be categorized into hexagonal wurtzite (most common and stable), cubic zincblende, and cubic rocksalt. They can also be classified by size, with typical sizes ranging from 1 to 100 nanometers. Furthermore, ZnO NPs can be modified by coating with organic molecules to control surface chemistry and charge.

1. Structural Forms:

- **Hexagonal Wurtzite:** This the most common ans stable crystal structure of Zno at ambient conditions.
- **Cubic Zincblende:** This structure is less common and can be stabilized under certain conditions.
- **Cubic Rocksalt:** Another structure from of Zno nanoparticles.

2. Size-Based Classification:

- **Quantum dots:** These are typically very small, with sizes ranging from 1-10 nm.
- **Nanoparticles:** This category encompasses a broader range of sizes, generally up to 100 nm.
- **Nanowires/ Nanotubes:** These are elongated ZnO structures with varying aspect ratios.
- **Nanofibers:** ZnO structures with a high length-to-diameter ratio.

3. Surface-Modified ZnO NPs:

- **Surface modified ZnoNPs:** TheseZnO NPs have organic molecules attached to their surface, altering their surface chemistry and charge.
- **Coated ZnoNPs:** This is a specific type of surface modification where the ZnO NP is coated with a layer of another material.

4. Other Classifications:

- **Doped ZnoNPs:** ZnO nanoparticles with other elements incorporated into their structure, which can alter their properties.
- **Composite ZnoNPs:** ZnO nanoparticles combined with other materials to form composite structures[2].

Application of Zinc oxide Nanoparticles [3][4]

Zinc oxide nanoparticles (ZnO NPs) have diverse applications due to their unique properties like strong UV absorption, antimicrobial activity, and biocompatibility. They are used in sunscreens, textiles, biomedicine (drug delivery, bioimaging), and even in some industrial products like paints and coatings.

1. Sunscreens: ZnO NPs are a key ingredient in sunscreens because they effectively absorb ultraviolet (UV) light while remaining transparent to visible light. This makes them a popular choice for sun protection.

2. Textiles: ZnO NPs are incorporated into fabrics to provide antimicrobial and deodorant properties. They can also enhance UV resistance in textiles.

3. Biomedicine:

- **Drug Delivery:**ZnO NPs can be used as drug carriers due to their biocompatibility and ability to solubilize in acidic environments, making them potentially useful for targeted drug delivery.
- **Bioimaging:**Their inherent photoluminescence properties make them suitable for biosensing and bioimaging applications.
- **Tissue Engineering:** ZnO NPs are being investigated for their potential in tissue engineering and regenerative medicine due to their wound-healing, antioxidant, and other beneficial properties.
- **Cancer Therapy:** ZnO NPs can induce cell death in cancer cells through the generation of reactive oxygen species (ROS), making them a potential candidate for cancer therapy.
- **Dentistry:** ZnO NPs have applications in various dental fields, including restorative dentistry, endodontics, and regenerative endodontics.

4. Other Industrial Applications:

- **Coatings and Paints:** ZnO NPs are used as a component in coatings and paints due to their UV-protective and antimicrobial properties.
- **Rubber and Plastics:** They can be incorporated into rubber and plastics to improve their properties.
- **Ceramics:** The strong and stiff structure of ZnO NPs makes them useful in the ceramic industry.
- **Food Storage:** They can be used to enhance the shelf life of food products.
- **Catalysis:** ZnO NPs are used as catalysts in various chemical reactions, including photocatalysis.

5. Other potential applications:

- **Cosmetics:** ZnO NPs are used in cosmetics for their UV-blocking and antimicrobial properties.
- **Environmental Science:** They are being explored for their potential in water treatment and pollution management. **Agriculture:** ZnO NPs are being investigated for their potential in agriculture, including their use in fertilizers and pesticides [5].

Garlic Oil

Herbs and spices can serve dual purposes, acting both as flavor enhancers and as ingredients with medicinal and healing qualities in snack items [6]. Garlic (Allium sativum L.; family Liliaceae) stands out as a key bulb cultivation known for its use as a seasoning and as a widely recognized traditional medicinal herb in India [7]. The health advantages associated with garlic are primarily linked to its sulfur-containing compounds, including allicin and S-allyl cysteine, along with its essential bioactive constituents, which encompass organosulfur compounds, thiosulfates, and allicin[8][9].

Garlic paste mixed with lime is applied to alleviate mouth ulcers, throat discomfort and can also be incorporated into toothpaste to help prevent tooth decay. Evidence has shown that garlic is a distinctive therapeutic food that may aid in managing infections related to the coronavirus disease (COVID-19) [10].Fresh garlic and its extracts in oil or powdered form can be employed as both functional and therapeutic foods. There is substantial evidence supporting garlic's preventive and healing roles in enhancing immune function, anti-cancer effects, as well as its antioxidant properties that defend the body against oxidative stress[11].

A diet that incorporates functional foods abundant in garlic has been shown to benefit human health. Garlic can influence blood anticoagulation levels and enhance the functionality of various body

systems, particularly those involved in respiration and digestion[12].Research from preclinical studies and clinical trials suggests that garlic intake has a pronounced effect on antihypertensive, antidiabetic, immune-modulatory, and lipid-lowering properties, making it highly advantageous for both medical and surgical applications. Moreover, it has been reported that garlic can reduce the prevalence of cryptosporidiosis, a gastrointestinal disease, in immunocompromised mice, along with mitigating inflammation[13].

Fig 2: Garlic Oil

Pharmacological studies:

Garlic oil (GO), commonly found in various garlic-based health products, is produced through steam distillation of crushed garlic, resulting in organo-sulfur compounds that account for roughly 0.09 to 0.35% of its fresh weight [14].GO exhibits biological actions such as the inhibition of angiotensin-converting enzymes, α-amylase, and α-glucosidase, demonstrating potential antihypertensive and antidiabetic effects [15].

In research trials, GO has shown notable effectiveness in cardiovascular diseases, addressing issues like calcium overload within cells, oxidative stress, inflammation, injury and dysfunction of vascular endothelial cells, as well as dyslipidemia[16].A molecule derived from allicin, known as allyl methyl sulfide, demonstrates a reasonable stability in the bloodstream, indicating that GO could be beneficial for treating various infectious diseases [17].In the food sector, fresh GO is employed as a natural preservative, flavor enhancer, and antibacterial agent, specifically effective against gram-negative bacteria such as Escherichia coli and Pseudomonas aeruginosa in processed meat and chicken products[18].

Natural substances, including rosemary essential oils and GO, have been effective in restricting the proliferation of aerobic microorganisms, including S. aureus, Salmonella species, B. thermosphacta, molds, yeasts, and coliforms, serving as natural meat preservatives [19].Research on the antioxidant properties of garlic, whether wet or dry heated at temperatures of 70, 100, and 121°C, indicated that heating reduces its antioxidant capability due to the degradation of phenolic and sulfur-containing compounds[20][21].It was discovered that even at a dosage of 200 mg of GO, it exhibited potent antioxidant effects comparable to those of vitamin C. In one clinical study, participants received gelatin capsules containing 8.2 mg of GO daily for 11 weeks, revealing that prolonged intake of GO led to a substantial reduction in blood sugar levels among the female participants, whereas male participants experienced an increase in their blood sugar levels [22].

A study indicated that there are gender differences in the advantages of garlic, suggesting that healthy males need to consume higher amounts of GO supplements to achieve the anticipated impacts on blood sugar and cardiovascular health [23].It has been documented that the compounds within GO, specifically diallyl sulfide and diallyl disulfide, inhibit mutagenesis by obstructing cytochrome P-450 2E1, an essential enzyme for converting carcinogenic cells [22].

Antioxidant and Anti-Inflammatory Potential of Garlic Oil:

1. Garlic Oil and Its Bioactive Constituents

Garlic (Allium sativum L.) is recognized as an important species of the genus Allium and has traditionally been utilized as both a food ingredient and a medicinal plant. Several of its biological effects have been associated with sulfur-containing phytochemicals that are produced or released during garlic bulb processing. Garlic oil is particularly characterized by the presence of volatile organosulfur compounds

such as diallyl sulphide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS). The proportions of these compounds may be influenced by factors such as garlic variety, cultivation conditions, extraction technique, and processing method. Consequently, variations in the chemical profile of garlic oil may affect its biological properties[24][25].Considerable scientific attention has been directed toward the organosulfur compounds found in garlic oil because antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and cytoprotective activities have been associated with them. Among the different sulfurcontaining constituents, DADS and DATS have been particularly investigated for their possible contribution to antioxidant and anti-inflammatory effects. Changes in cellular redox balance and inflammatory signaling have been observed following exposure to these compounds, indicating that the biological effects of garlic oil may be mediated through several interconnected mechanisms rather than a single molecular target[24][25][26][27].

2. Oxidative Stress and Antioxidant Activity

Oxidative stress is considered to occur when the generation of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defense systems. Under excessive oxidative conditions, membrane lipids, proteins, and nucleic acids may undergo oxidative damage, which can subsequently result in cellular dysfunction and tissue injury. A close association exists between oxidative stress and inflammation because ROS can stimulate intracellular signaling pathways responsible for the production of inflammatory mediators. Thus, maintenance of an appropriate redox balance is regarded as an important factor in preserving cellular homeostasis and reducing oxidative tissue damage associated with inflammation.

The antioxidant defense system has been shown to be modulated by several organosulfur compounds derived from garlic. Their activity is not necessarily restricted to direct scavenging of free radicals; instead, endogenous antioxidant mechanisms, including glutathione-associated defenses and redox-sensitive signaling pathways, may also be influenced. By strengthening cellular antioxidant capacity, prolonged protection against oxidative damage may be provided. Accordingly, modulation of oxidative stress andendogenous antioxidant defenses has been recognized as an important mechanism associated with the biological effects of garlic and its derivatives[26][28].

The influence of garlic oil on physiological antioxidant status has also been demonstrated experimentally. In a study conducted in rats, garlic oil and the major allyl sulfides DAS, DADS, and DATS were evaluated, and significant increases in erythrocyte glutathione (GSH) levels were observed following treatment with garlic oil, DADS, and DATS. Through these observations, modification of the endogenous antioxidant defense system by garlic oil was demonstrated, although differences were observed according to the compound and tissue examined[29]. Therefore, the antioxidant activity of garlic oil may involve physiological antioxidant mechanisms in addition to activity detected through chemical radicalscavenging assays. Antioxidant properties have also been demonstrated following direct examination of garlic essential oil. During chemical characterization, DATS and DADS were identified among the major constituents, while antioxidant activity was demonstrated using DPPH radicalscavenging and β-carotene bleaching assays[30].However, results obtained from chemical antioxidant assays cannot be directly equated with therapeutic effects in humans because biological activity may be affected by absorption, metabolism, tissue distribution, dose, stability, and the preparation method used for garlic oil[25][28].

3. Anti-Inflammatory Activity of Garlic Oil

Inflammation is regarded as a coordinated biological response in which immune cells, cytokines, lipid mediators, nitric oxide (NO), and intracellular signaling pathways are involved. When inflammatory pathways are activated excessively or persistently, tissue damage and chronic inflammatory conditions may be produced. Several important inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), NO, and prostaglandin E_2 (PGE_2), are involved in this process. The nuclear factor-κB (NF-κB) pathway is considered a major regulatory mechanism controlling the expression of numerous inflammatory genes[27][31].

Anti-inflammatory effects have been reported for garlic oil and its organosulfur constituents in both cellular and animal experimental models. In LPS-stimulated RAW 264.7 macrophages, garlic oil-derived sulfur compounds were evaluated, and changes in inflammatory cytokine and mediator production were observed. The secretion of inflammatory cytokines was affected by DAS, DADS, and allyl methyl sulfide, while reductions in NO and PGE_2 production were also reported with selected compounds. These findings suggest that inflammatory responses mediated by macrophages can be modulated by organosulfur derivatives of garlic[32].

The interaction between oxidative stress and inflammation has also been investigated in relation to DADS. In an experimental model of allergic airway inflammation, attenuation of inflammatory responses was observed following DADS treatment. Reductions in inflammatory mediators and inflammatory cell accumulation were accompanied by decreased expression of NF-κB, iNOS, and matrix metalloproteinase-9. At the same time, increased activity of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase1 (HO-1) was observed. These findings indicate that antioxidant and anti-inflammatory effects may be mechanistically interconnected, with activation of the Nrf2/HO-1 pathway being associated with suppression of NF-κB-mediated inflammatory signaling [33]

MATERIALS AND METHODS

Collection of samples:

Garlic Oil collected from Yazhini Biotech Laboratory, Srivilliputhur, Tamilnadu for this investigation.

METHODOLOGY

Nanoparticle:

Preparation of Zinc Nanoparticles:

In a spotless beaker, 5.6 g of zinc acetate is dissolved in 100ml of purified water. This is done with a magnetic stirrer maintaining a temperature between 65 and 70 degrees Celsius. Introduce 40 ml of sample into the zinc acetate solution while continuously stirring for 30 minutes, followed by constant stirring for 2 to 3 hours. The solution is then left undisturbed overnight. The liquid on top is poured off or discarded. The nanoparticles that have settled at the bottom are rinsed with purified water three times, centrifuged at 5000 rpm, resuspended in purified water, and then dried at a controlled temperature for 2 hours. The resulting dried nanoparticles are then ready for subsequent investigations[34].

Phytochemical Analysis Test

Phytochemical screening is the process used to identify various compounds found in plant extracts. Plants contain numerous chemical constituents that can elicit different physiological responses and offer therapeutic advantages. Consequently, it is standard practice to evaluate plants for the existence of biologically active and medicinally significant phytochemicals. Constituents responsible for a particular biological activity. Some of the examples of phytoconstituents include alkaloids, steroids, carbohydrates, saponins, tannins, flavonoids etc [35][36].

Gel

Evaluation of Gel:

The physical traits are devoid of any coloration or fragrance. PH Evaluation involves the use of a digital pH meter to ensure it measures within the range of 5.5 to 6.0. The simplicity of application includes assessing how easily the gel is spread on the skin. The consistency is to identify if it possesses a viscous, gel-like feel, usually achieved through thickening agents like xanthan gum or carbomer[37][38].

Pharmacological Evaluation

In vitro Antioxidant activity

Antioxidant Activity by DPPH Assay

DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl- Hydrate) Assay

A popular way to evaluate the effectiveness of antioxidants in neutralising free radicals is through the DPPH assay. This process involves combining a stable free radical called DPPH assay with a plant extract and then observing the change in colour. A more noticeable colour shift suggests a greater antioxidant activity[39].

Principle:

The capacity of the extracts to neutralize free radicals was evaluated through the DPPH radical scavenging assay, following the methodology outlined by Blois and Desmarchelier[40][41].The potential of plant extracts to donate hydrogen atoms was examined by observing the decolorization of a methanol solution containing 2,2-diphenyl-1-picrylhydrazyl (DPPH).When antioxidants are present, the DPPH solution, which initially appears violet or purple, transitions to yellow hues[42].

Procedure:

A arrangement of DPPH at a concentration of 6×10-5M in methanol was made by dissolving 7.89mg in 100ml, which implies for 250ml, you'd utilize (7.89/100) ×250. At that point, 500µl from each test arrangement was set into an Eppendorf tube. Each concentration was tried three times. Following, 500µl of the DPPH solution was included to the test arrangement and blended well. This blend was shaken and cleared out at room temperature for half an hour. The absorbance was at that point measured at 520nm. Ascorbic corrosive served as the positive control, whereas refined water acted as the negative control. The rate restraint compared to the standard was calculated utilizing the condition underneath, and the IC50 values were too decided. Graph Pad Prism 9 software[40][43].

Analysis:

To analyse data statistically, you need to determine the actual absorbance of the plant material. This involves subtracting the blank plant absorbance from the assay absorbance of the plant. Essentially, after preparing your dilution series, you'll measure the colour intensity of the plant material using a spectrophotometer, which will give you the absorbance reading for the blank plant. For instance, after diluting the plant, it will have a certain colour, and when you measure its colour intensity, you might find the absorbance to be A for the blank plant. If the DPPH assay shows an absorbance of B, then the actual absorbance for the assay would be calculated as B minus A. So, the formula for actual absorbance is: Assay absorbance – Blank absorbance.

Next, you'll need to calculate the percentage inhibition values using the following equation:

% Inhibition = (Blank DPPH solution absorbance – Actual absorbance) x 100% / Blank DPPH solution absorbance.

To discover the absorbance of the clear DPPH arrangement, you'll degree it after planning the DPPH arrangement. You'll degree its absorbance spectrophotometrically.

By rehashing this process for positive and negative controls, you have got to plot a log-concentration time chart and the IC50 should be detected employing a nonlinear relapse demonstrate utilizing Graph Pad Crystal computer program. At that point by comparing the test comes about with controls, you'll be able decipher the information discoveries.

FRAP (Ferric Reducing Antioxidant Power) Assay

The ability of antioxidants to convert ferric ions into ferrous ions is evaluated through the FRAP assay. This method measures the reduction potential, allowing for an estimation of a sample's antioxidant capacity. An enhancement in reduction potential indicates a rise in antioxidant effectiveness[44].

Procedure:

For ideal and steady comes about, as it were crisply gathered tests ought to be utilized. The extraction from plant materials can be performed with a assortment of solvents such as water, methanol, ethanol, or acetone. The fitting extraction procedure is decided by the nature of the particular test being prepared. A weakening arrangement was made with the plant substances. To each of the tubes, 2.5 ml of phosphate buffer (0.2 M, pH 6.6) was presented. Each tube's substance was blended altogether. In this way, 2.5 ml of a 1% potassium ferricyanideK3F(CN)6 arrangement was included to each test. Taking after this, each response blend was unsettled energetically employing a vortex shaker. The tests were set to hatch at a temperature of 50°C for roughly 20 minutes. Once the hatching was completed, 2.5 ml of 10% trichloroacetic corrosive (TCA) was presented to each test. The test tubes experienced centrifugation at 3,000 rpm for a term of 10 minutes. From the coming about centrifuged tests, 2.5 ml of supernatant was gotten in unmistakable test tubes. In those recently procured isolated test tubes, 0.5 ml of ferric chloride (FeCl3) was joined. This brought about within the arrangement of a somewhat blue tint. Hence, absorbance was evaluated at a wavelength of 700 nm. A test with the next concentration displayed more prominent absorbance, while the speak connected to tests with lower concentrations. Ascorbic corrosive served as the positive control, whereas refined water acted as the negative control. The test was rehashed three times. At long last, the rate of antioxidant action and IC50 values were computed utilizing Graph Pad Crystal 9 computer program[45][46]

***In-vitro* Anti-Inflammatory Activity**

Principle of In vitro Egg Albumin Denaturation Method

The most objective of the egg white's denaturation measure is to discover out in case certain operators or compounds can stop or moderate down the method of egg whites getting to be denatured beneath conditions. Denaturation alludes to the alter in a protein's structure that leads to a misfortune of its natural work. In this try, egg whites serve as a demonstrate protein, and denaturation happens when it is uncovered to extraordinary warm, changing pH levels, or other denaturing substances. Amid denaturation, the initial shape of egg whites is changed, influencing its physical properties and coming about in a misfortune of its utilitarian movement. The test surveys how well a medicate or compound can avoid or diminish the denaturation of egg whites, which makes a difference assess its anti-inflammatory impacts. The fundamental rule of the egg whites denaturation measure is that substances with anti-inflammatory properties might balance out protein structures and anticipate denaturation, a prepare regularly related with irritation and tissue harm. Hence, operators or compounds that altogether diminish egg whites denaturation in this test might possibly display anti-inflammatory impacts. Protein denaturation is accepted to be one of the components contributing to irritation. Non-steroidal anti-inflammatory drugs (NSAIDs) not as it were avoid protein denaturation but to restrain the COX enzyme at the same time. Different concentrations of the test can be blended with egg whites arrangement beneath controlled exploratory conditions, permitting the responses to occur sometime recently measuring the absorbance to calculate the rate of restraint[47].

Inhibition of Albumin Denaturation:

The response blend was made by combining 0.5 ml of Boswellic acid corrosive and a 0.45 ml fluid arrangement of 5% bovine egg whites. The pH of the blend, which was 6.3, was balanced with a bit of 0.1N HCl whereas keeping it at 37 ˚C for 20 minutes. After that, it was warmed to 57 ˚C for 30 minutes. Once cooled, the arrangement was exchanged to 96-well plates, and the absorbance was measured at 660 nm. Standard was utilized as Diclofenac sodium (1000µg/ml) and the control contain 0.05ml refined water. The rate of restraint of egg whites denaturation was calculated by the taking after equation,

$$Percentage\ of\ Inhibition\ (\%) = \frac{Absorbance\ of\ Control - Absorbacne\ of\ Test\ Sample}{Absorbance\ of\ Control}\ x\ 100$$

Where, Control – Reaction mixture except drug, Test Sample – Reaction mixture containing the sample

RESULTS

Nanoparticle:

Antioxidant activity

DPPH assay

In the current study, GO-ZnONPsexhibited in vitro antioxidant activity as assessed by the DPPH assays. At a concentration of 10 mg, the DPPH assay revealed an inhibition percentage of 78%, with an IC_{50} value of 14.50 mg, in contrast to the standard ascorbic acid, which had an IC_{50} value of 3.373mg. This indicates that GO-ZnONPsantioxidant activity via the DPPH assay is good when compared to Vitamin C.

FRAP assay

GO-ZnONPshad constituent's dose dependent in vitro antioxidant by FRAP assay. When compared with standard ascorbic acid under similar condition. At 15 mg (1.53-OD) concentration in the extract had good activity compared with standard Vitamin C.

Anti-inflammatory activity

To assess anti-inflammatory activity in vitro, the albumin denaturation method was utilized. At 5 mg, demonstrated a 55% inhibition and an IC_{50} value of 15.83mg, compared to the standard diclofenac sodium, which had an IC_{50} value of 12.129mg. During our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti-inflammatory properties ofGO-ZnONPs. When compared to diclofenac sodium,GO-ZnONPsshowed moderate anti-inflammatory effects.

Phytochemical studies

Initial phytochemical analysis of the GO-ZnONPshas revealed the presence of alkaloids.

Gel:

Evaluation of Gel

The Garlic Oil-Zinc Oxide Nanoparticle gel (GO-ZnONPs + gel) presented as a pale white, insoluble liquid with a distinct garlic fragrance and a skin-compatible pH of 5. While the formulation proved immutable under standard conditions, centrifugal testing induced phase separation, highlighting a need for optimized stabilizing agents.

Antioxidant activity

DPPH assay

In the current study, GO-ZnONPs+ gel exhibited in vitro antioxidant activity as assessed by the DPPH assays. At a concentration of 5 mg, the DPPH assay revealed an inhibition percentage of 90%, with an IC_{50} value of 9.76 mg, in contrast to the standard ascorbic acid, which had an IC_{50} value of 3.373mg. This indicates that GO-ZnONPs+ gelantioxidant activity via the DPPH assay is good when compared to Vitamin C.

FRAP assay

GO-ZnONPs+ gelhad constituent's dose dependent in vitro antioxidant by FRAP assay. When compared with standard ascorbic acid under similar condition. At 15 mg (1.43-OD) concentration in the extract had good activity compared with standard Vitamin C.

Anti-inflammatory activity

To assess anti-inflammatory activity in vitro, the albumin denaturation method was utilized. At 10 mg, demonstrated a 62% inhibition and an IC_{50} value of 6.09mg, compared to the standard diclofenac sodium, which had an IC_{50} value of 12.129mg. During our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti-inflammatory properties ofGO-ZnONPs+ gel. When compared to diclofenac sodium,GO-ZnONPs+ gel showed moderate anti-inflammatory effects.

TABLES

Nanoparticle:

Table 1: Kinetic studies GO- ZnONPs

	OD VALUE				
NANOMETER	**30 mins**	**1 hour**	**1.30 hours**	**2 hours**	**2.30 hours**
420 nm	0.08	0.15	0.16	0.05	0.04
480 nm	0.14	0.09	0.13	0.03	0.08
540 nm	0.07	0.14	0.08	0.02	0.05
620 nm	0.14	0.15	0.10	0.04	0.06
680 nm	0.11	0.11	0.18	0.04	0.03

Table 2: GO -ZnONPs *In vitro* antioxidants activity by DPPH assay

S. NO	Concentration (mg)	COD	SOD	%inhibition	Average	$IC_{50\ (mg)}$
1	5 mg	0.27	0.08	70%	70.3 ± 22.5	14.50 mg
2		0.27	0.14	48%		
3		0.27	0.02	93%		
1	10 mg	0.27	0.03	89%	77.6 ± 10.0	
2		0.27	0.08	70%		
3		0.27	0.07	74%		
1	15 mg	0.27	0.15	44%	49 ± 8.66	
2		0.27	0.15	44%		
3		0.27	0.11	59%		
***In vitro* Standard vitamin C**						
1	10 mg	0.27	0.03	88%	87 ± 1.73	

2		0.27	0.03	88%	
3		0.27	0.04	85%	

Table 3: GO -ZnONPs Antioxidant activity IC_{50} value (mg) compared to standard VIT C IC_{50} value (mg)

S. NO	Concentration (mg)	Average (%)	$IC_{50\ (mg)}$
1	5 mg	70%	14.50 mg
2	10 mg	78%	
3	15 mg	49%	
Standard Ascorbic acid vitamin C			
1	5 mg	91%	3.373 mg
2	10 mg	87%	
3	15 mg	86%	
4	20 mg	92%	
5	25 mg	84%	

Table 4: GO -ZnONPs In vitro antioxidants activity by FRAP assay

S.NO	Concentration (mg)	OD	Average
1	5 mg	1.35	1.34
2		1.34	
3		1.33	
1	10 mg	1.29	1.34
2		1.37	
3		1.38	
1	15 mg	1.54	1.53
2		1.55	
3		1.51	
Standard			
S.NO	**Concentration(mg)**	**OD**	**Average**
1	5 mg	1.55	1.55
2		1.57	
3		1.54	
1	10 mg	1.64	1.64
2		1.63	
3		1.65	
1	15 mg	1.67	1.66
2		1.65	
3		1.68	

Table 5: GO -ZnONPs *In vitro* anti-inflammatory activity

S. NO	Concentration (mg)	COD	SOD	%inhibition	Average	$IC_{50\ (mg)}$
1	5 mg	0.29	0.11	62%	55 ± 7	15.83mg
2		0.29	0.13	55%		
3		0.29	0.15	48%		
1	10 mg	0.29	0.14	52%	52 ± 7	
2		0.29	0.12	59%		
3		0.29	0.16	45%		
1	15 mg	0.29	0.13	55%	53 ± 4.04	
2		0.29	0.13	55%		
3		0.29	0.15	48%		
***In vitro* Standard Diclofenac sodium**						

1	10 mg	0.29	0.08	72%	73.3 ± 2.30
2		0.29	0.08	72%	
3		0.29	0.07	76%	

Table 6: GO -ZnONPs Anti-Inflammatory Activity IC_{50} Value (mg) Compared to Standard Diclofenac Sodium IC_{50} Value (mg)

S. NO	Concentration (mg)	Average (%)	$IC_{50\ (mg)}$
1	5 mg	55%	15.83mg
2	10 mg	52%	
3	15 mg	53%	
Standard Diclofenac sodium			
1	5 mg	91%	12.129 mg
2	10 mg	93%	
3	15 mg	85%	
4	20 mg	89%	
5	25 mg	88%	

Table 7: Phytochemical Studies
[- absence; + presence]

S. No	Phytochemical Test	GO -ZnONPs
1.	Alkaloids	+
2.	Flavonoids	-
3.	Phenols	-
4.	Proteins	-
5.	Saponin	-
6.	Carbohydrate	-

Gel:

Table 8: Evaluation of Gel

	GO -ZnONPs + gel
Colour	Pale White
Odour	Garlic Fragrance
Nature	Liquid
Solubility	Insoluble
p_H	5
Mutability	Immutable
Centrifuge	Separation

Table 9: GO -ZnONPs + gel *In vitro* antioxidants activity by DPPH assay

S.NO	Concentration (mg)	COD	SOD	%inhibition	Average	$IC_{50\ (mg)}$
1	5 mg	0.27	0.02	93%	90.3 ± 2.30	9.76 mg
2		0.27	0.03	89%		
3		0.27	0.03	89%		
1	10 mg	0.27	0.07	74%	74 ± 15	
2		0.27	0.11	59%		
3		0.27	0.03	89%		
1	15 mg	0.27	0.10	63%	61.6 ± 2.30	
2		0.27	0.10	63%		
3		0.27	0.11	59%		
***In vitro* Standard vitamin C**						

1	10 mg	0.27	0.03	88%	87 ± 1.73
2		0.27	0.03	88%	
3		0.27	0.04	85%	

Table 10: GO -ZnONPs + gel Antioxidant activity IC_{50} value (mg) compared to standard VIT C IC_{50} value (mg)

S. NO	Concentration (mg)	Average (%)	$IC_{50\ (mg)}$
1	5 mg	90%	9.76 mg
2	10 mg	74%	
3	15 mg	62%	
Standard Ascorbic acid vitamin C			
1	5 mg	91%	3.373 mg
2	10 mg	87%	
3	15 mg	86%	
4	20 mg	92%	
5	25 mg	84%	

Table 11: GO -ZnONPs + gel In vitro antioxidants activity by FRAP assay

S.NO	Concentration(mg)	OD	Average
1	5 mg	0.94	0.99
2		0.97	
3		1.08	
1	10 mg	1.26	1.26
2		1.28	
3		1.25	
1	15 mg	1.45	1.43
2		1.43	
3		1.42	
Standard			
S.NO	**Concentration(mg)**	**OD**	**Average**
1	5 mg	1.55	1.55
2		1.57	
3		1.54	
1	10 mg	1.64	1.64
2		1.63	
3		1.65	
1	15 mg	1.67	1.66
2		1.65	
3		1.68	

Table 12: GO -ZnONPs + gel *In vitr*o anti-inflammatory activity

S.NO	Concentration (mg)	COD	SOD	%inhibition	Average	$IC_{50\ (mg)}$
1	5 mg	0.29	0.15	48%	44.6 ± 3.51	6.09mg
2		0.29	0.17	41%		
3		0.29	0.16	45%		
1	10 mg	0.29	0.10	66%	62.3 ± 9.07	
2		0.29	0.14	52%		
3		0.29	0.09	69%		
1	15 mg	0.29	0.13	55%	54 ± 1.73	
2		0.29	0.13	55%		

3		0.29	0.14	52%		
***In vitro* Standard Diclofenac sodium**						
1	10 mg	0.29	0.08	72%	73.3 ± 2.30	
2		0.29	0.08	72%		
3		0.29	0.07	76%		

Table 13: GO -ZnONPs + gel Anti-Inflammatory Activity IC_{50} Value (mg) Compared to Standard Diclofenac Sodium IC_{50} Value (mg)

S.NO	Concentration (mg)	Average (%)	$IC_{50\ (mg)}$
1	5 mg	45%	6.09mg
2	10 mg	62%	
3	15 mg	54%	
Standard Diclofenac sodium			
1	5 mg	91%	12.129 mg
2	10 mg	93%	
3	15 mg	85%	
4	20 mg	89%	
5	25 mg	88%	

Nanoparticle:

Fig 3: Garlic oil

Fig 4: GO – ZnONPs

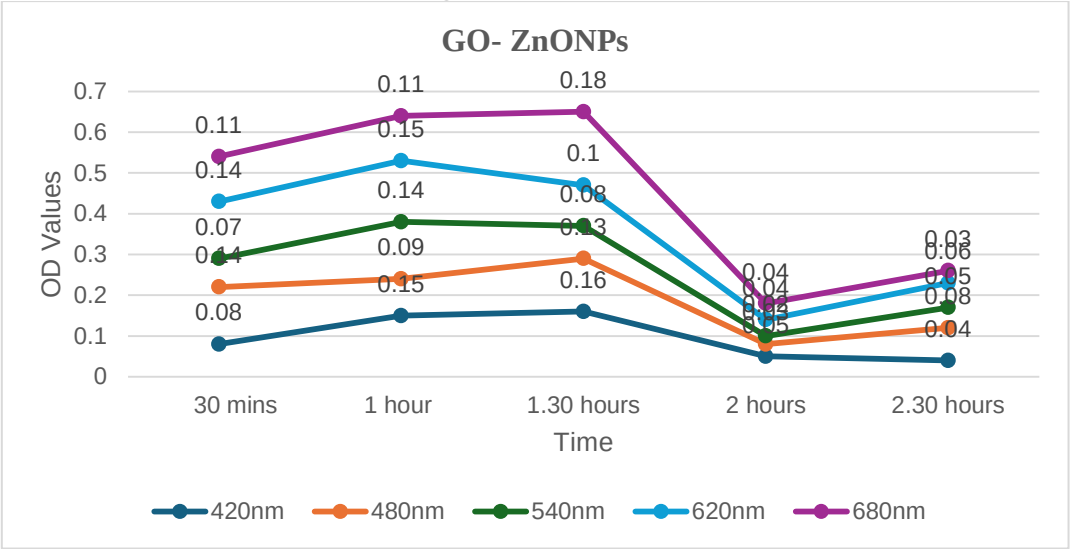

Fig 5: Kinetic studies GO- ZnONPs

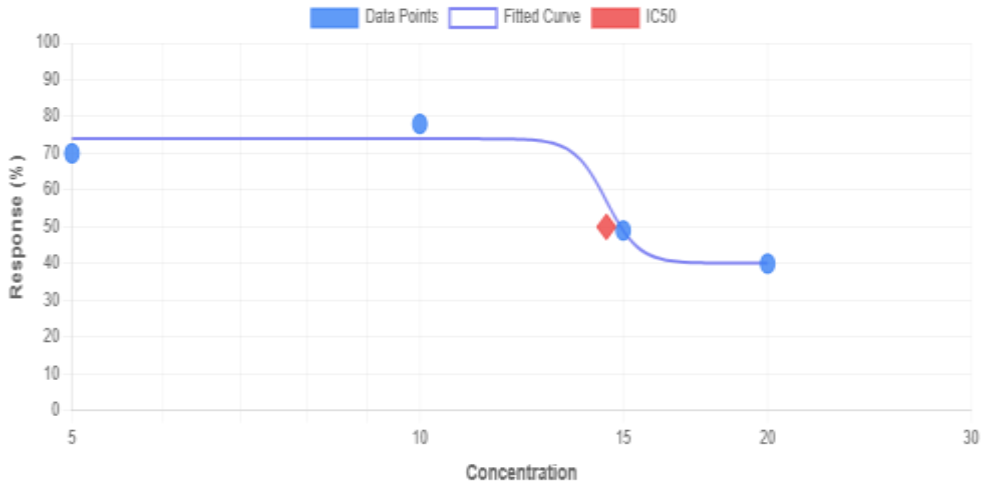

Fig 6: Anti-Oxidant Activity IC_{50} Value (mg)

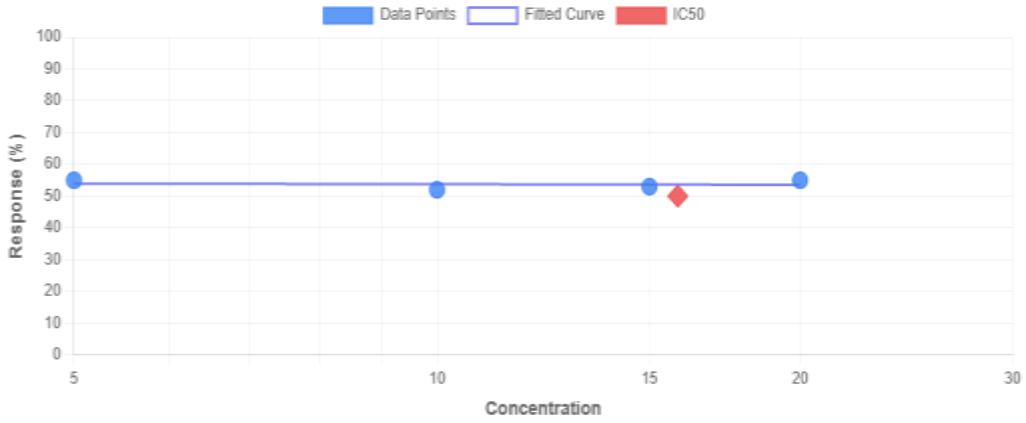

Fig 7: Anti-Inflammatory Activity IC_{50} Value (mg)

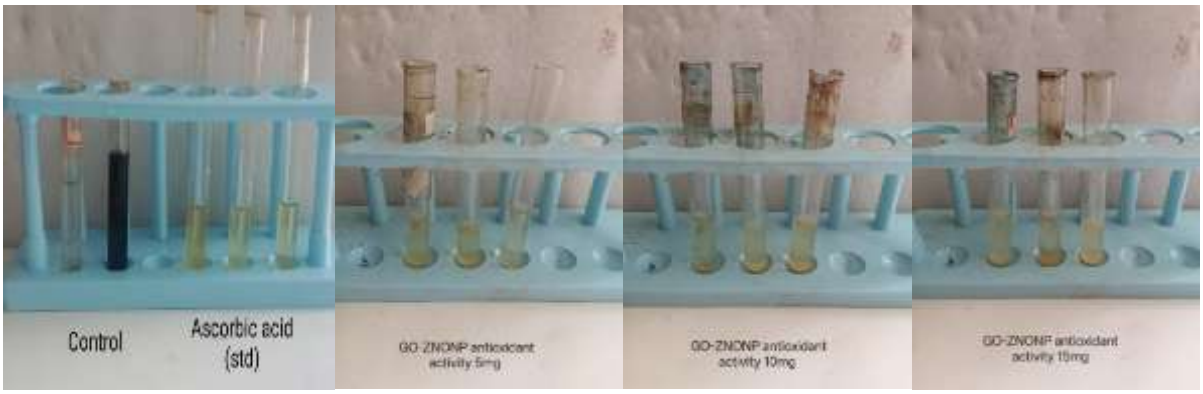

Fig 8a: In *vitro* antioxidants activity by DPPH assay

Fig 8b: *In vitr*o anti-inflammatory activity

Fig 9a: Antioxidant Activity by FRAP Assay

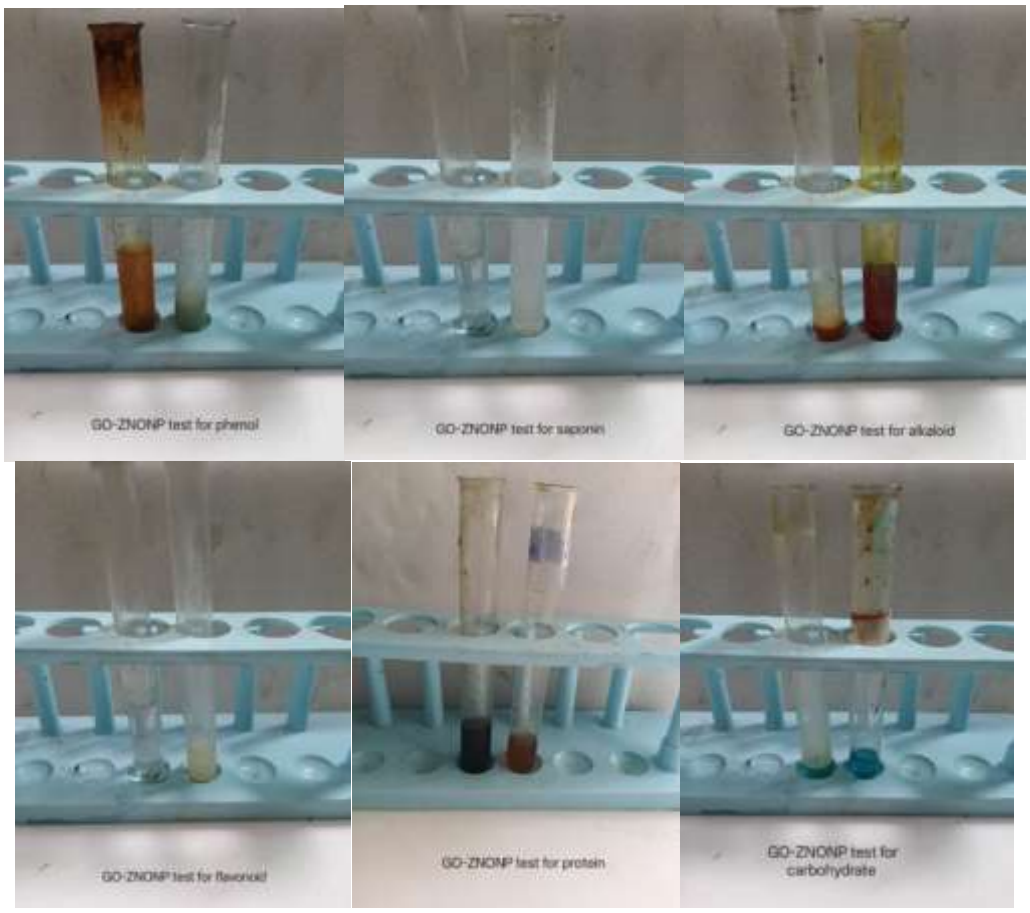

Fig 9b: Phytochemical Studies

Gel:

Fig 10: GO -ZnONPs +gel

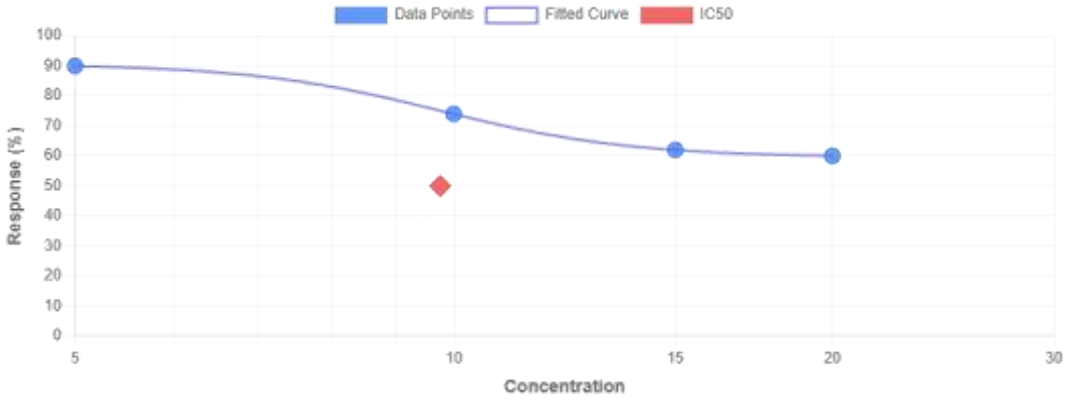

Fig 11: Anti-Oxidant Activity IC_{50} Value (mg)

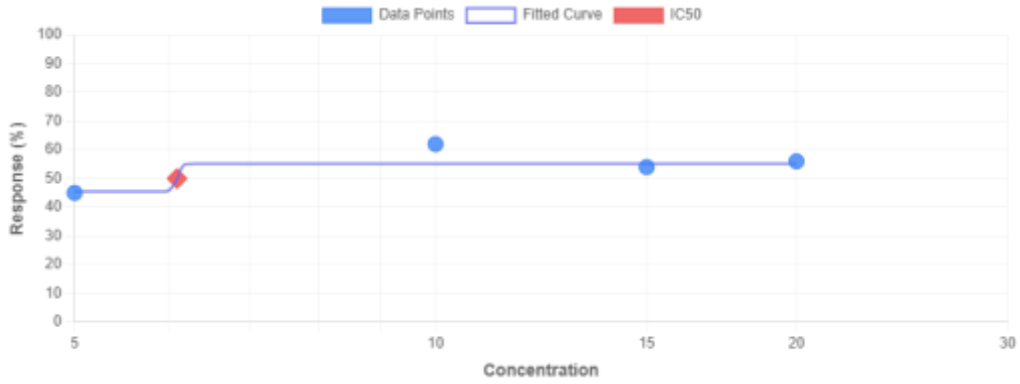

Fig 12: Anti-Inflammatory Activity IC_{50} Value (mg)

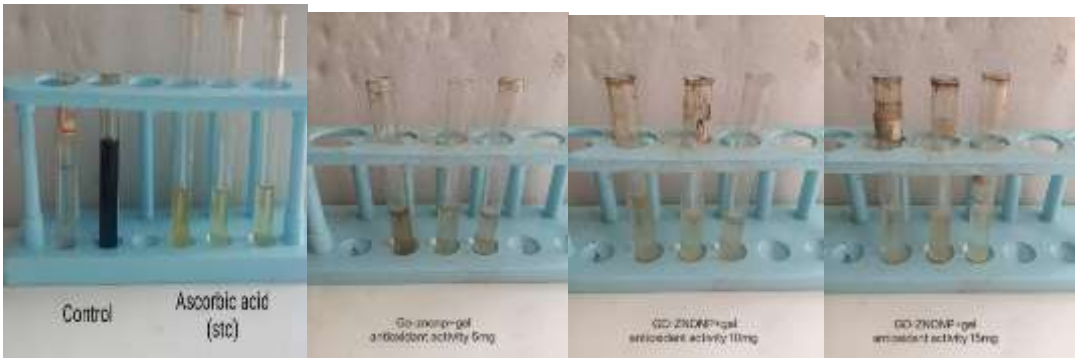

Fig 13: In *vitro* antioxidants activity by DPPH assay

Fig 14: Antioxidant Activity by FRAP Assay

Fig 15: *In vitr*o anti-inflammatory activity

DISCUSSION

This study demonstrates that garlic oil, rich in organosulfur compounds such as diallyl sulfide, diallyl disulfide, and diallyl trisulfide, can serve as a reducing/capping agent for green synthesis of ZnO nanoparticles, and that these nanoparticles can be incorporated into a topical gel base.

Antioxidant activity. DPPH inhibition for GO-ZnONPs from 70% (5 mg) to 78% (10 mg) but fell to 49% at 15 mg, while the gel showed a separate, monotonically declining pattern (90% → 74% → 62% from 5 to 15 mg). Neither formulation showed a simple concentration-dependent response, which likely reflects turbidity, nanoparticle aggregation, or matrix interference rather than true loss of radical-scavenging capacity at higher doses — this should be resolved with formulation blanks and independently prepared replicates in future work. Both preparations remained less potent than ascorbic acid (IC50 = 3.373 mg). FRAP absorbance generally increased with concentration in both preparations (NP: 1.34 → 1.34 → 1.53 OD; gel: 0.99 → 1.26 → 1.43 OD, at 5/10/15 mg respectively), though the NP values were flat between the lowest two doses. These are raw absorbance values rather than standard-equivalent concentrations, and cross-formulation comparisons should be treated cautiously given differences in turbidity and light scattering between the NP suspension and gel matrix.

Anti-inflammatory activity. The gel's IC50 (6.09 mg) was notably lower than diclofenac sodium's (12.129 mg), indicating it outperformed the standard on an IC50 basis, whereas the NP suspension alone

(15.83 mg) remained less potent than diclofenac. Inhibition at individual concentrations was not strictly dose-dependent for either preparation, and percentage inhibition at comparable doses remained below the standard's peak values. The gel's apparent superiority to diclofenac on IC50 should therefore be read as preliminary rather than conclusive without repeated trials and confidence intervals.

Gel improved over NP suspension — but why is unclear. Both antioxidant and anti-inflammatory IC50 values improved after gel incorporation, possibly due to better dispersion or sustained availability of active constituents within the matrix. Critically, no blank gel (vehicle-only) control was reported, so this improvement cannot yet be attributed to GO-ZnONPs activity specifically rather than to the gel base itself.

Formulation and characterization gaps. The gel was pale white, garlic-scented, and measured pH 5 — below the formulation's own stated target range of 5.5–6.0 described in the methods, a discrepancy that should be addressed or explained. This pH remains within the broader physiological range of skin (approximately 4.5–6.5), but the mismatch with the stated spec is worth flagging. The gel also showed phase separation on centrifugation, indicating inadequate physical stability that will need addressing through excipient/stabilizer optimization. Phytochemical screening detected only alkaloids, with flavonoids, phenols, proteins, saponins, and carbohydrates absent — a qualitative result that doesn't rule out the organosulfur constituents most relevant to garlic oil's bioactivity. Although XRD, SEM, UV-DRS, and PIXE were described in the methods, no corresponding characterization results were reported; without these, nanoparticle identity, size, morphology, and elemental composition remain unconfirmed.

Overall, the data provide preliminary *in vitro* evidence of antioxidant and anti-inflammatory activity for GO-ZnONPs and GO-ZnONPs + gel, with the gel outperforming the bare nanoparticle suspension. These findings should be presented as an initial screening study rather than confirmed therapeutic efficacy, pending blank-gel controls, structural characterization, stability testing, and *in vivo* validation.

CONCLUSION

This study demonstrated the green synthesis of garlic oil-mediated zinc oxide nanoparticles (GO–ZnO NPs) and their incorporation into a topical gel. Both GO–ZnO NPs and the gel showed *in vitro* antioxidant and anti-inflammatory activities. The gel showed better DPPH antioxidant activity, with 90% inhibition and an IC_{50} value of 9.76 mg, compared with GO–ZnO NPs, which showed 78% inhibition and an IC_{50} value of 14.50 mg. However, both formulations were less potent than the standard ascorbic acid.

In the albumin denaturation assay, the gel showed greater anti-inflammatory activity, with 62% inhibition and an IC_{50} value of 6.09 mg, than GO–ZnO NPs alone, which showed 55% inhibition and an IC_{50} value of 15.83 mg. The gel was pale white, had a characteristic garlic odour, and showed a pH of 5. However, phase separation after centrifugation indicated that formulation stability needs improvement.

Overall, GO–ZnO NP gel has preliminary potential as a topical antioxidant and anti-inflammatory formulation. Future studies should confirm nanoparticle size, morphology, crystallinity, and surface charge using appropriate characterization techniques. Further optimization of the gel with suitable stabilizers is required to prevent phase separation. Blank-gel controls, antimicrobial studies, drug-release studies, skin-irritation testing, stability studies, and *in vivo* pharmacological and toxicity studies should also be performed to establish the safety, stability, and therapeutic potential of the formulation.

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