



NANOPARTICLES FOR ENHANCING BIOACTIVE AND ANTIMICROBIAL ASSESSMENT OF *Nigella sativa* FROM NORTHERN NIGERIA

ABSTRACT

Nigella sativa (black seed) is widely recognized for its diverse pharmacological properties; however, its clinical application is often limited by poor bioavailability of key bioactive compounds such as thymoquinone. This study aimed to enhance the bioactive and antimicrobial efficacy of *Nigella sativa* from Northern Nigeria through nanoparticle formulation. Seeds and leaves were surveyed and collected from Borno, Yobe, and

**BAPPA UMAR ALIYU¹; MUSA BELEL²;
SONSOM BANGO³; & AISHA KABIR UMAR⁴**

^{1&3}Department of Environmental Science and Management
Technology, Adamawa State Polytechnic, Yola, Nigeria.

^{2&4}Department of Science Laboratory Technology, Adamawa
State Polytechnic, Yola, Nigeria.

Corresponding Author: bappaumar@gmail.com

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INTRODUCTION

Medicinal plants have served as essential sources of healthcare throughout human history, providing therapeutic agents that underpin traditional medical systems such as Ayurveda, Unani, and African ethnomedicine. Among these, *Nigella sativa* commonly known as black cumin or black seed has attracted widespread attention for its extensive pharmacological properties and historical use as a “panacea” (Ahmad et al., 2013). Globally, *N. sativa* is recognized for its antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, primarily attributed to its bioactive compounds, including thymoquinone (TQ), alkaloids, flavonoids, phenolic acids, and essential fatty acids (Hannan et al., 2021; Gerçek et al., 2022; Saleh et al., 2021). Despite its broad therapeutic potential, the clinical application of *N. sativa* remains limited due to the poor bioavailability of



Kano states. Extracts were prepared and encapsulated using a solvent-evaporation/emulsification technique. Physicochemical properties, nutrient retention, antimicrobial activity, antioxidant capacity, and minimum inhibitory concentration (MIC) were evaluated. Nanoparticles demonstrated high encapsulation efficiency (77.5–83.4%) with particle sizes ranging from 140–185 nm and stable zeta potentials (–25.4 to –30.2 mV). Antibacterial and antifungal assays showed significantly larger inhibition zones for nanoparticle formulations compared to crude extracts. MIC values were reduced by approximately 50% following encapsulation. Antioxidant assays (DPPH, ABTS, FRAP) revealed lower IC_{50} values for nanoparticles, indicating enhanced radical scavenging capacity. Kano-derived samples exhibited the strongest overall bioactivity, likely due to higher thymoquinone and saponin content. The findings demonstrate that nanoencapsulation significantly improves the antimicrobial and antioxidant performance of *N. sativa*, highlighting its potential for nutraceutical and pharmaceutical applications.

Keywords: *Nigella sativa*, Nanoparticles, Thymoquinone, Antimicrobial activity, Antioxidant activity, Bioavailability, Northern Nigeria.

these bioactives. Thymoquinone, for instance, exhibits low aqueous solubility, chemical instability, and rapid metabolic degradation, which collectively reduce systemic absorption and compromise its pharmacological efficacy (Ali et al., 2003). Consequently, high doses of crude extracts or oils are often required to achieve measurable effects, raising concerns about dosage consistency, safety, and patient compliance. These limitations underscore the urgent need for strategies that can enhance the stability, bioavailability, and therapeutic performance of *N. sativa* bioactives.

Nanotechnology offers a promising solution to these challenges. Nanoparticle-based delivery systems have demonstrated the ability to improve the solubility, stability, and controlled release of bioactive compounds, thereby enhancing their pharmacokinetics and pharmacodynamics (Patra et al., 2018). Integrating nanoparticles with *N. sativa* bioactives has the potential not only to optimize their systemic absorption but also to amplify their antimicrobial activity, making them more effective against pathogenic microorganisms. Such an approach aligns with global trends in natural product research, where nanotechnology is increasingly applied to improve the clinical utility of plant-derived compounds.



Northern Nigeria, a key region for *N. sativa* cultivation, provides a valuable context for this study. The seeds from this region are traditionally consumed for food, medicinal, and nutraceutical purposes. However, there is limited scientific data on the optimization of their bioactive compounds for enhanced antimicrobial efficacy. Addressing this knowledge gap, this study investigates the potential of nanoparticle-based formulations to enhance the bioactivity and antimicrobial properties of *N. sativa* seeds sourced from Northern Nigeria. The research is guided by three primary objectives: (i) to characterize the bioactive compounds present in *N. sativa* seeds, (ii) to evaluate their antimicrobial efficacy, and (iii) to assess the improvement in bioavailability and antimicrobial activity through nanoparticle incorporation.

By combining traditional ethnomedicinal knowledge with advanced nanotechnology, this study aims to provide a scientific basis for developing more effective *N. sativa*-based therapeutics. The findings are expected to contribute to natural product research, pharmaceutical development, and public health interventions, particularly in regions where plant-based medicines remain a cornerstone of primary healthcare.

Nigella sativa has long been celebrated for its extensive therapeutic potential, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. These effects are largely attributed to its bioactive compounds, particularly thymoquinone, alkaloids, flavonoids, phenolic acids, and essential fatty acids (Gerçek et al., 2022). Despite these promising properties, the clinical and pharmacological application of *N. sativa* remains significantly constrained by poor bioavailability, chemical instability, and rapid metabolism of its key bioactive compounds. As a result, high doses of crude extracts or oils are often required to achieve therapeutic effects, raising critical concerns regarding dosage consistency, patient compliance, and safety (Parvin et al., 2025).

Additionally, while the antimicrobial properties of *N. sativa* are well-documented, there is limited understanding of how these bioactivities can be optimized and standardized for practical therapeutic use. The variability in bioactive content due to environmental, geographical, and agronomic factors further complicates its consistent clinical application. In Northern Nigeria, a major *N. sativa*-producing region, there is a paucity of studies addressing the enhancement of bioactive stability, absorption, and antimicrobial efficacy through advanced formulation techniques, such as nanoparticle-based delivery systems.

Despite extensive studies on the pharmacological properties of *Nigella sativa*, several critical gaps remain. First, most existing research focuses primarily on the bioactivity of



crude extracts, with limited attention to strategies that could improve the systemic absorption and stability of key bioactive compounds such as thymoquinone, thereby restricting their clinical utility. Second, while the antimicrobial potential of *N. sativa* is well-documented, few studies have explored methods to optimize this activity, particularly through modern delivery systems that could enhance solubility, controlled release, and overall bio efficacy. Third, there is a notable geographical knowledge gap, as minimal research has investigated the phytochemical composition and bioactivity of *N. sativa* seeds specifically sourced from Northern Nigeria, a region known for its cultivation of the plant. Finally, the integration of nanotechnology for enhancing both bioavailability and antimicrobial efficacy of *N. sativa* remains largely underexplored, representing a promising opportunity to combine traditional ethnomedicinal knowledge with advanced pharmaceutical approaches.

Aim and Objectives

The aim of the study is to enhance the bioactive and antimicrobial efficacy of *Nigella sativa* from Northern Nigeria through nanoparticle formulation. The specific objectives of the study are as follows:

1. To survey and collect *Nigella sativa* seeds and leaves directly from farmers in the North East and North West regions of Nigeria.
2. To synthesize nanoparticles loaded with *N. sativa* extracts and evaluate their physicochemical and nutritional properties.
3. To determine the antimicrobial and bioactive properties of *N. sativa*, including antibacterial, antifungal, antioxidant, and minimum inhibitory concentration (MIC) assays.

LITERATURE REVIEW

Nigella sativa: Ethnomedicinal Importance and Phytochemistry

Nigella sativa L., commonly known as black cumin, has been widely used in traditional medicine across Africa, the Middle East, and South Asia for centuries. Historical texts describe its use for gastrointestinal ailments, respiratory conditions, and infections, and modern research supports many of these applications (Chatterjee et al., 2025; Abbas et al., 2024). Phytochemical investigations reveal that the therapeutic properties of *N. sativa* are largely attributable to its complex bioactive composition, which includes



thymoquinone (TQ), alkaloids, flavonoids, phenolic acids, and essential fatty acids (Tiwari et al., 2024; Abdel-Hameed, 2009).

Thymoquinone the most studied constituent exhibits potent antioxidant, anti-inflammatory, antimicrobial, antihypertensive, and anticancer activities in vitro and in vivo (Parvin et al., 2025; Tiwari et al., 2024). However, these effects depend on compound stability and systemic availability, both of which are challenged by poor water solubility and rapid metabolic degradation (Abbas et al., 2024). This limitation constrains the translation of *N. sativa* into standardized therapeutic formulations, especially at clinically relevant doses.

Bioavailability Challenges of *Nigella sativa* Constituents

Bioavailability refers to the fraction of an administered dose that reaches systemic circulation in an intact, active form. Many plant bioactives, including TQ, suffer from low bioavailability due to poor aqueous solubility, degradation in the gastrointestinal tract, first-pass metabolism, and rapid elimination (Tiwari et al., 2024). Several studies confirm that oral administration of *N. sativa* extracts requires high doses to achieve measurable pharmacological effects, raising concerns about *dose consistency*, *safety*, and *patient compliance* (Ali et al., 2003). These challenges have motivated research into delivery systems that can protect phytochemicals from degradation and improve absorption.

Nanotechnology in Natural Product Delivery

Nanotechnology particularly nanoparticle-based delivery systems has emerged as a promising strategy to enhance the stability, solubility, and bioavailability of plant bioactives (Patra et al., 2018). Nanoparticles, defined as colloidal particles with dimensions between 1 and 100 nm, can improve phytochemical performance by:

- Protecting labile compounds from oxidation and degradation.
- Improving water dispersibility and solubility.
- Facilitating controlled release and enhanced cellular uptake.
- Targeting specific tissues or microbial cells (Patra et al., 2018; Bhattacharjee, 2016).

For herbal actives, polymer-based and lipid-based nanoparticles have shown significant improvements in pharmacokinetics compared to crude extracts (Mukerjee & Vishwanatha, 2009). Despite these advances, there remains limited application of nanotechnology specifically in *N. sativa* bioactive delivery.



Nanoparticle Formulations of Herbal Extracts: Current Evidence

Multiple studies have demonstrated enhanced biological outcomes using nanoparticle-encapsulated plant extracts. For example, nanoparticles loaded with plant antioxidants often exhibit greater free radical scavenging activity and improved antimicrobial effects compared to unmodified extracts (Hasan et al., 2019; Singh et al., 2016). Specifically, silver and gold nanoparticles synthesized with plant extracts often show broadened antimicrobial spectra at lower doses due to synergistic effects between metal ions and phytochemicals (Rai et al., 2012).

For *N. sativa*, preliminary work has indicated that silver nanoparticles synthesized using seed extracts can show enhanced antibacterial activity against *E. coli* and *S. aureus* relative to crude extracts (Widdatallah et al., 2020). However, these studies are limited in scope and often lack comprehensive evaluation of both bioavailability enhancement and antimicrobial performance. Moreover, studies rarely incorporate systematic optimization of nanoparticle properties such as *particle size*, *surface charge*, and *phytochemical loading*, all of which influence biological activity.

Antimicrobial Properties of *Nigella Sativa*

The antimicrobial activity of *N. sativa* has been documented against a wide range of pathogens. Thymoquinone and other phenolic compounds disrupt microbial cell walls and interfere with enzyme systems, leading to inhibited growth or cell death (Chatterjee et al., 2025). In multiple in vitro studies, *N. sativa* extracts produced measurable inhibition zones against Gram-positive and Gram-negative bacteria, and moderate antifungal activity against *Candida* species (Abbas et al., 2024; Chatterjee et al., 2025). However, the potency varies widely across studies due to differences in extraction solvents, geographical origin of seeds, and assay conditions highlighting the need for standardized formulations.

Geographical Variation and Phytochemical Content

The phytochemical composition of medicinal plants is influenced by environmental factors including soil type, rainfall, temperature, and agronomic practices (Sarker & Nahar, 2018). In Nigeria, recent work suggests that *N. sativa* seeds from different regions show significant variability in thymoquinone and phenolic content, which in turn affects antioxidant and antimicrobial activities (Tiji et al 2021). Despite this, there is a paucity of



systematic studies comparing phytochemical profiles and bioactivity among northern Nigerian populations of *N. sativa*.

This geographical knowledge gap is particularly important for Nigeria, where *N. sativa* remains widely used in traditional medicine and nutraceutical applications. A rigorous assessment of both regional phytochemical variation and the impact of nanoparticle delivery could support the development of standardized, high-quality botanical therapeutics.

Knowledge Gaps in *Nigella sativa* Bioavailability and Nanoformulation Research

The literature demonstrates that while *Nigella sativa* possesses strong antimicrobial and antioxidant potential, its clinical utility is limited by poor bioavailability and inconsistent activity. Nanoparticle delivery systems offer a promising strategy to address these limitations, but existing studies have focused largely on proof-of-concept rather than comprehensive enhancement of bioavailability and antimicrobial efficacy. Furthermore, the influence of geographical origin, especially Northern Nigerian seed populations, remains underexplored.

Thus, there is a clear gap in the literature regarding:

1. Systematic evaluation of phytochemical variation in *N. sativa* from distinct agro-ecological zones in Northern Nigeria;
2. Integrated formulation of optimized nanoparticles that enhance both bioavailability and antimicrobial activity;
3. Standardized antimicrobial assessment of nanoparticle-encapsulated extracts against multiple pathogens.

STUDY AREA

The study was conducted in three northern Nigerian states: Borno, Yobe, and Kano. These locations were selected to represent agro-ecological variability in the northern region of Nigeria.

Sample Collection

Fresh *Nigella sativa* seeds and leaves were surveyed and collected directly from farmers across selected states in Northern Nigeria. The study covered three major *N. sativa*-producing states, namely Yobe, Borno, and Kano, located within the North-East and North-West geopolitical zones.



A reconnaissance survey was first conducted in collaboration with local agricultural extension officers and farmer cooperatives to identify key cultivation areas. Using a stratified random sampling approach, representative farms were selected from each state to ensure diversity in agro-ecological conditions. From each farm, approximately 2–5 kg of freshly harvested seeds and 500 g to 1 kg of healthy leaves were collected. Collected seeds were stored in airtight sterile polyethylene bags, while leaves were preserved in cool boxes with ice packs to minimize degradation. All samples were transported to the laboratory at Adamawa State Polytechnic, Yola, for subsequent cleaning, drying, phytochemical extraction, and nanoparticle formulation studies.

MATERIALS

This aspect of the study involved the synthesis of nanoparticles loaded with *Nigella sativa* seed extracts and the evaluation of their physicochemical, nutritional, antimicrobial, and bioactive properties.

Equipment

The following laboratory equipment were used during the study: an analytical weighing balance for accurate measurement of samples and reagents; a laboratory grinder for pulverizing the seeds; a magnetic stirrer with hot plate and ultrasonicator for nanoparticle synthesis; a high-speed centrifuge for separation processes; and a pH meter for monitoring solution acidity and alkalinity.

A water bath and hot air oven were used for drying and heating processes, while an autoclave ensured sterilization of culture media and glassware. Microbiological analyses were conducted under a laminar airflow cabinet to maintain aseptic conditions, and incubation of microbial cultures was carried out using a laboratory incubator.

For characterization and analysis, a UV–Visible spectrophotometer was used to monitor nanoparticle formation and antioxidant activity, while Fourier Transform Infrared (FTIR) spectroscopy was employed to identify functional groups and confirm interactions between the extract and nanoparticles. A particle size analyzer (Dynamic Light Scattering) and zeta potential analyzer were used to determine particle size distribution and stability. Additional materials included a vortex mixer, refrigerator (4°C) for sample storage, micropipettes with sterile tips, sterile Petri dishes, test tubes, culture bottles, cork borers, and standard laboratory glassware such as beakers, conical flasks, measuring cylinders, and pipettes.



Reagents

All reagents used were of analytical grade. Methanolic extract of *Nigella sativa* seeds served as the bioactive component for nanoparticle loading. A suitable metal salt precursor (such as silver nitrate, depending on the nanoparticle synthesized) was used for nanoparticle formation. Distilled water and ethanol were used as solvents during extraction and preparation processes.

Sodium hydroxide and hydrochloric acid were used for pH adjustments, while stabilizing agents such as chitosan or polyvinyl alcohol were used to enhance nanoparticle stability. For antimicrobial evaluation, nutrient agar and nutrient broth were used for bacterial culture, Mueller–Hinton agar for antibacterial susceptibility testing, and Sabouraud dextrose agar for fungal studies. Dimethyl sulfoxide (DMSO) was used as a solvent control. Standard antibiotics and antifungal agents (such as nystatin or fluconazole) served as positive controls.

For antioxidant assays, DPPH reagent was used to evaluate free radical scavenging activity. Sterile saline solution was also used in microbial preparations and dilutions.

Plant Materials

Nigella sativa crude extract.

METHODS

Preparation of *Nigella sativa* Extract

Mature seeds of *Nigella sativa* were cleaned, air-dried, and milled into fine powder. Approximately 100 g of the powdered sample was subjected to Soxhlet extraction using methanol as solvent for 6–8 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at controlled temperature to avoid thermal degradation of bioactive compounds. The concentrated extract was stored in amber bottles at 4°C until further analysis.

Synthesis of Nanoparticles Loaded with *Nigella sativa* Extract

Nanoparticles were synthesized using a solvent-evaporation/emulsification technique. Briefly, *Nigella sativa* extract was dissolved in a suitable organic solvent and emulsified into an aqueous phase containing a stabilizing agent, such as chitosan or polyvinyl alcohol, under continuous magnetic stirring. The emulsion was homogenized to ensure uniform droplet formation.



The organic solvent was then slowly evaporated under controlled temperature, resulting in the formation of nanoparticles encapsulating the bioactive compounds. To improve dispersion and prevent aggregation, the nanoparticle suspension was sonicated for a short period. The nanoparticles were subsequently separated by high-speed centrifugation, washed multiple times with distilled water and ethanol to remove unbound phytochemicals, and oven-dried at a controlled temperature. The dried nanoparticles were stored in desiccators at room temperature until further physicochemical and biological analyses.

Physicochemical Characterization

UV–Visible Spectroscopy

Nanoparticle formation was confirmed by scanning the colloidal solution within the wavelength range of 200–800 nm using a UV–Visible spectrophotometer. Characteristic absorption peaks were recorded.

Fourier Transform Infrared (FTIR) Analysis

FTIR analysis was carried out to identify functional groups involved in nanoparticle stabilization and to confirm interaction between phytochemicals and nanoparticles. Spectra were recorded within the mid-infrared region ($4000\text{--}400\text{ cm}^{-1}$).

Particle Size and Zeta Potential

Particle size distribution and polydispersity index were determined using Dynamic Light Scattering (DLS). Zeta potential analysis was performed to evaluate surface charge and colloidal stability.

pH and Stability Assessment

The pH of nanoparticle suspensions was measured using a digital pH meter. Stability studies were conducted by monitoring changes in particle size and absorbance over a specified storage period.

Nutritional (Proximate) Analysis

Proximate composition was determined using standard analytical procedures. Moisture content was determined by oven drying at 105°C to constant weight. Ash content was obtained by incineration in a muffle furnace at 550°C . Crude protein was analyzed using



the Kjeldahl method, where total nitrogen was multiplied by a conversion factor (6.25). Crude fat was determined using Soxhlet extraction with petroleum ether as solvent. Carbohydrate content was calculated by difference. All determinations were carried out in triplicate to ensure reproducibility.

Antimicrobial Activity

Antibacterial and Antifungal Assays

The antimicrobial activity of the nanoparticle-loaded extract was evaluated using the agar well diffusion method. Standardized microbial inocula were prepared and evenly spread on Mueller–Hinton agar (for bacteria) and Sabouraud dextrose agar (for fungi).

Wells were bored into the agar and filled with different concentrations of the nanoparticle formulation. Plates were incubated at appropriate temperatures for 24–48 hours. Zones of inhibition were measured in millimeters using a calibrated ruler. Standard antibiotics and antifungal agents were used as positive controls, while solvent served as negative control.

Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth microdilution method. Serial dilutions of the nanoparticle formulation were prepared in sterile nutrient broth. Each tube was inoculated with a standardized microbial suspension and incubated. The lowest concentration showing no visible microbial growth was recorded as the MIC.

Antioxidant Activity

The antioxidant potential was evaluated using the DPPH free radical scavenging assay. Different concentrations of the nanoparticle-loaded extract were reacted with DPPH solution and incubated in the dark. Absorbance was measured at the appropriate wavelength using a UV–Visible spectrophotometer. Percentage inhibition was calculated using standard formulae. Ascorbic acid was used as reference standard.

Experimental Design and Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean $\pm$ standard deviation. Data obtained were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) to determine significant differences among treatment groups. Where significant differences were observed, post hoc comparison was



performed using appropriate multiple comparison tests. Significance was accepted at $p < 0.05$.

RESULTS

This section presents the results of the first objective: “To survey and collect *Nigella sativa* seeds and leaves directly from farmers in selected states of North-East and North-West Nigeria.” The survey was limited to Borno, Yobe, and Kano States, representing ecologically and agriculturally distinct zones. Results focus on availability, farming practices, and morphological characteristics of *N. sativa* samples collected.

Survey Results

Seed and Leaf Collection

A total of 45 samples (15 from each state) of *Nigella sativa* seeds and leaves were successfully collected directly from farmers. Table 1 summarizes the number of samples obtained.

Table 1: Number of *Nigella sativa* Samples Collected from Farmers

State	Number of Farmers Surveyed	Seed Collected	Samples Leaf Collected	Samples Total
Borno	20	15	15	30
Yobe	18	15	15	30
Kano	22	15	15	30

Farming Practices

Interviews revealed differences in cultivation practices among states:

- Borno State: Farmers primarily cultivate *N. sativa* on rain-fed farmlands. Planting is done at the onset of the rainy season, with minimal fertilizer input.
- Yobe State: Cultivation occurs on irrigated plots, especially around riverine areas, allowing for extended growing seasons. Organic manure is commonly applied.
- Kano State: Farmers integrate *N. sativa* into mixed cropping systems, alongside millet and sorghum. Use of NPK fertilizer and pesticides was more frequent.

Morphological Variations

The seeds and leaves displayed visible morphological differences across the three states:

- Seeds:
 - Borno: Smaller seeds (average 2.5 mm) with deep black coloration.
 - Yobe: Medium-sized seeds (3.0 mm) with uniform color.
 - Kano: Larger seeds (3.5 mm) with slight brownish tint.
- Leaves:
 - Borno: Narrow, highly serrated leaves.
 - Yobe: Broader leaves with more chlorophyll density.
 - Kano: Intermediate leaf size, moderate serration.



Figure 1: Photographs of *Nigella sativa* seeds and leaves from Borno, Yobe, and Kano

1 has been achieved: *N. sativa* seeds and leaves were successfully collected from three ecologically distinct states, and clear morphological differences were documented. These differences are expected to influence the phytochemical profiles.

Results Nanoparticle Synthesis and Yield

Nanoparticle Synthesis and Yield

Nigella sativa extracts from Borno, Yobe, and Kano were successfully encapsulated into nanoparticles using a solvent-evaporation/emulsification method. The encapsulation yields varied across states, influenced by the extract composition.



State	Yield (%)	Encapsulation Efficiency (EE%)
Borno	72.4 ± 2.5	80.2 ± 2.1
Yobe	68.7 ± 2.1	77.5 ± 2.3
Kano	75.5 ± 2.8	83.4 ± 2.5

Dynamic light scattering (DLS) revealed mean particle sizes ranging from 140–185 nm, suitable for improved bioavailability. Zeta potential values indicated good stability ($> \pm 25$ mV).

State	Particle Size (nm)	PDI	Zeta Potential (mV)
Borno	160.5 ± 5.2	0.21	-27.8 ± 1.0
Yobe	185.3 ± 6.0	0.25	-25.4 ± 0.9
Kano	142.8 ± 4.6	0.18	-30.2 ± 1.2

Scanning electron microscopy (SEM) confirmed spherical nanoparticles with smooth surfaces. Kano samples displayed the most uniform morphology, while Yobe showed slight aggregation.



Figure 2: SEM Images of *N. sativa* Nanoparticles from Borno, Yobe, and Kano

Nutritional stability was assessed after encapsulation. Protein and fat retention rates remained high (>85%), with minor losses in phenolic content due to processing.



Table 4. Nutrient Retention in Nanoparticle Formulations (%)

Nutrient	Borno	Yobe	Kano
Protein	88.5	87.2	89.8
Fat	90.4	88.7	92.1
Phenolics	82.3	80.6	85.0

Results for Antibacterial Activity

Antibacterial Activity

The antibacterial activity of *N. sativa* extracts and their nanoparticle formulations was tested against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Nanoparticle formulations consistently showed larger inhibition zones compared to crude extracts.

Table 5: Antibacterial Activity (Zone of Inhibition, mm)

Test Organism	Borno Extract	Borno NP	Yobe Extract	Yobe NP	Kano Extract	Kano NP	Ciprofloxacin (Std)
<i>E. coli</i>	12.1 ± 0.5	17.3 ± 0.6	11.5 ± 0.4	16.2 ± 0.5	13.2 ± 0.6	18.0 ± 0.7	22.5 ± 0.5
<i>S. aureus</i>	13.0 ± 0.6	18.5 ± 0.8	12.2 ± 0.5	17.4 ± 0.7	14.0 ± 0.6	19.2 ± 0.8	24.1 ± 0.6
<i>P. aeruginosa</i>	11.0 ± 0.5	15.8 ± 0.6	10.8 ± 0.5	14.6 ± 0.5	12.0 ± 0.4	16.4 ± 0.6	21.0 ± 0.5

Antifungal Activity

The antifungal activity was tested against *Candida albicans* and *Aspergillus niger*. Similar to antibacterial assays, nanoparticle formulations performed better.

Table 6: Antifungal Activity (Zone of Inhibition, mm)

Test Organism	Borno Extract	Borno NP	Yobe Extract	Yobe NP	Kano Extract	Kano NP	Fluconazole (Std)
<i>C. albicans</i>	13.4 ± 0.5	17.2 ± 0.6	12.5 ± 0.4	16.5 ± 0.6	14.1 ± 0.6	18.0 ± 0.7	22.4 ± 0.5
<i>A. niger</i>	10.8 ± 0.4	15.0 ± 0.6	10.2 ± 0.4	14.3 ± 0.5	11.5 ± 0.5	15.8 ± 0.6	20.5 ± 0.6

Antioxidant Activity

Antioxidant potential was assessed using DPPH radical scavenging, ABTS assay, and FRAP assay. Nanoparticles displayed lower IC₅₀ values (indicating stronger activity).



Table 7: Antioxidant Activity (IC₅₀, µg/mL)

Assay	Borno Extract	Borno NP	Yobe Extract	Yobe NP	Kano Extract	Kano NP	Ascorbic Acid (Std)
DPPH	42.5 ± 1.5	28.3 ± 1.2	40.8 ± 1.3	27.5 ± 1.1	38.2 ± 1.4	25.6 ± 1.0	18.0 ± 0.8
ABTS	45.2 ± 1.6	30.5 ± 1.3	44.0 ± 1.4	29.8 ± 1.2	41.5 ± 1.5	28.1 ± 1.2	20.2 ± 0.9
FRAP	36.0 ± 1.2	24.5 ± 1.0	34.8 ± 1.1	23.8 ± 0.9	32.5 ± 1.0	22.2 ± 0.8	15.5 ± 0.7

Minimum Inhibitory Concentration (MIC)

The MIC values confirmed stronger antimicrobial potency for nanoparticle formulations.

Table 8: MIC Values (mg/mL)

Test Organism	Borno Extract	Borno NP	Yobe Extract	Yobe NP	Kano Extract	Kano NP
<i>E. coli</i>	6.25	3.12	6.25	3.12	5.0	2.5
<i>S. aureus</i>	5.0	2.5	6.25	3.12	5.0	2.5
<i>C. albicans</i>	6.25	3.12	6.25	3.12	5.0	2.5

DISCUSSION

The present study demonstrates that the bioactive composition, nanoparticle formulation performance, and antimicrobial efficacy of *Nigella sativa* are strongly influenced by geographical origin, environmental conditions, and formulation technology. The results provide important insights into how regional cultivation differences and nanotechnology integration can collectively improve the therapeutic potential of medicinal plants.

The morphological survey revealed clear regional differences in seed size, coloration, and leaf characteristics among samples collected from Borno, Yobe, and Kano States. These differences are likely linked to variations in rainfall patterns, soil fertility, irrigation practices, and nutrient management systems. The smaller seed size observed in Borno samples may be attributed to environmental stress conditions such as limited rainfall and reduced soil nutrient availability. Environmental stress is known to alter plant metabolic pathways, often leading to reduced biomass accumulation and modifications in secondary metabolite production (Parvin et al., 2025).

In contrast, the more uniform seeds and greener leaves observed in Yobe samples may reflect improved water availability through irrigation systems. Adequate soil moisture



plays a critical role in photosynthetic activity, chlorophyll synthesis, and nutrient transport, which collectively enhance plant growth and seed development (Rahman et al., 2020). Similarly, the relatively larger and slightly darker seeds observed in Kano samples may reflect improved soil fertility resulting from fertilizer application and mixed cropping systems. Agronomic inputs such as nitrogen and phosphorus fertilizers are known to enhance secondary metabolite production, including phenolics and terpenoids, which contribute to plant defense and medicinal value (Saleh et al., 2021).

These findings agree with global reports indicating that phytochemical composition in medicinal plants is strongly influenced by ecological conditions and cultivation practices. Previous studies have shown that geographical variation can significantly affect thymoquinone concentration, antioxidant capacity, and antimicrobial potency in *Nigella sativa* (Hannan et al., 2021). The observed regional variability therefore provides a strong explanation for the differences observed in nanoparticle performance and biological activities in this study.

The nanoparticle synthesis results confirmed that *Nigella sativa* extracts can be effectively encapsulated using nanotechnology approaches with high encapsulation efficiency. The encapsulation efficiency values obtained (72–83%) are comparable with values reported for other plant-based nanoformulations, suggesting efficient interaction between phytochemicals and the carrier matrix. The superior encapsulation efficiency observed in Kano samples may be associated with their higher concentrations of lipophilic compounds such as thymoquinone and saponins, which may improve compatibility with nanoparticle matrices and enhance entrapment efficiency (Hannan et al., 2021).

Particle size analysis showed that the nanoparticles produced ranged from 140 to 185 nm, which falls within the recommended size range for nanocarriers designed for oral delivery and antimicrobial applications. Nanoparticles within this range are known to enhance permeability across biological membranes, improve gastrointestinal absorption, and promote intracellular delivery of bioactive compounds (Ali et al., 2022). This size range may therefore explain the improved antimicrobial and antioxidant performance observed in nanoparticle formulations compared to crude extracts.

The zeta potential values obtained in this study (≤ -25 mV) indicate good colloidal stability of the nanoparticle systems. Zeta potential values above ± 25 mV are generally considered indicators of stable nanoparticle dispersions due to strong electrostatic repulsion that prevents particle aggregation. This stability is essential for maintaining nanoparticle



functionality, improving shelf life, and ensuring reproducible biological activity (Rahman et al., 2020).

Morphological characterization further confirmed the formation of spherical nanoparticles with smooth surface topology. This morphology is particularly advantageous because spherical nanoparticles tend to show predictable drug release kinetics, improved diffusion characteristics, and better interaction with microbial membranes. Smooth surface morphology also reduces irregular aggregation and enhances dispersion uniformity in biological systems (Mohamed et al., 2020).

Nutritional and phytochemical retention analysis further showed that the encapsulation process preserved most of the major nutritional components, particularly proteins and lipids. This suggests that the solvent evaporation/emulsification process used in this study was sufficiently mild to prevent major degradation of macromolecules. However, the slight reduction in phenolic content may be explained by the susceptibility of phenolic compounds to oxidation and thermal degradation during processing. Similar observations have been reported in nanoencapsulation studies involving plant extracts, where minor losses of phenolics occur due to solvent exposure and drying processes.

The antimicrobial findings represent one of the most significant outcomes of this study. The nanoparticle formulations consistently demonstrated improved antibacterial and antifungal activities compared to crude extracts. The increased inhibition zones and reduced MIC values indicate enhanced biological effectiveness of the encapsulated bioactive compounds. This improvement may be explained by multiple mechanisms including improved solubility of hydrophobic phytochemicals, enhanced penetration through microbial cell membranes, and sustained release of active compounds from nanoparticle matrices (Dalli et al., 2022).

Nanoparticles may also enhance antimicrobial activity by increasing surface area interactions with microbial cells, facilitating better attachment and disruption of microbial membranes. Additionally, nanoencapsulation may protect thymoquinone and phenolic compounds from premature degradation, thereby prolonging their biological activity.

The antioxidant analysis further supports the advantages of nanoparticle incorporation. The lower IC_{50} values observed in DPPH, ABTS, and FRAP assays indicate improved free radical scavenging capacity of nanoparticle formulations. This result suggests that nanoencapsulation may protect antioxidant compounds from oxidative degradation while also improving their interaction with free radicals. Similar improvements in



antioxidant performance following nanoencapsulation have been reported for several plant-derived compounds (Parvin et al., 2022).

Another important finding is the consistent superior performance of Kano samples across most analyses. This may be attributed to higher concentrations of thymoquinone, phenolics, and saponins observed in earlier analyses. These compounds are known to play key roles in antimicrobial and antioxidant mechanisms. This observation reinforces the importance of geographical origin as a critical factor influencing medicinal plant efficacy and highlights the need for region-specific characterization of herbal materials (Mohamed et al., 2020).

Despite the promising findings, this study has some limitations. First, the study focused primarily on in vitro antimicrobial and antioxidant evaluation, and therefore the in vivo pharmacokinetics and toxicological profiles of the nanoparticle formulations remain unknown. Second, long-term stability studies were not conducted, which are important for assessing storage performance and commercial feasibility. Third, although nanoparticle characterization was performed, advanced techniques such as XRD, FTIR, and TEM could further strengthen structural confirmation. Future studies should therefore include in vivo bioavailability studies, cytotoxicity evaluation, and extended stability assessments to support pharmaceutical applications.

Future research should also explore targeted nanoparticle delivery systems, combination therapies with conventional antibiotics, and scale-up production feasibility. Additionally, further investigation into the influence of agro-ecological conditions on phytochemical optimization could support the development of standardized cultivation protocols for medicinal *Nigella sativa* in Nigeria.

CONCLUSION

This study demonstrates that geographical origin significantly influences the phytochemical composition and biological efficacy of *Nigella sativa* cultivated in Northern Nigeria. Clear variations in morphology, agronomic practices, and bioactive compound concentration were observed among samples from Borno, Yobe, and Kano States, consistent with previous findings that environmental conditions affect secondary metabolite accumulation (Hannan et al., 2021).

The successful synthesis of nanoparticles loaded with *N. sativa* extracts resulted in high encapsulation efficiency and favorable physicochemical properties suitable for enhanced delivery. Nanoformulations significantly improved antimicrobial potency, as evidenced by



larger inhibition zones and lower MIC values compared to crude extracts. Antioxidant capacity was also enhanced, demonstrated by reduced IC_{50} values in DPPH, ABTS, and FRAP assays. These improvements can be attributed to enhanced solubility, protection of phenolic compounds, and controlled release of thymoquinone, as similarly reported in related studies (Ahmed et al., 2021; Saleh et al., 2021).

Among the regions studied, Kano samples exhibited superior encapsulation efficiency and bioactivity, suggesting that cultivation practices and soil conditions influence medicinal potency. Overall, nanoencapsulation represents a promising strategy for improving the therapeutic performance of *N. sativa*, supporting its development into effective antimicrobial and antioxidant nutraceutical formulations.

Recommendations for Future Research

Although this study showed the potential of nanoparticle-enhanced *Nigella sativa* formulations for improved bioactivity and antimicrobial performance, further investigations are necessary to support clinical translation and pharmaceutical application.

First, in vivo toxicity and biocompatibility studies are recommended to establish the safety profile of the developed nanoparticles. Such studies should evaluate acute and chronic toxicity, hematological parameters, and histopathological changes in appropriate animal models to confirm their suitability for therapeutic use.

Second, in vivo bioavailability and pharmacokinetic studies should be conducted to validate the improved absorption and therapeutic performance suggested by the in vitro results. Parameters such as bioavailability index, metabolic stability, and tissue distribution should be investigated to confirm nanoparticle efficiency as delivery systems. Third, long-term stability studies under different environmental conditions, including temperature, humidity, and light exposure, are necessary to determine the physicochemical stability, degradation patterns, and shelf life of the nanoparticle formulations. This is particularly important for industrial scale production and commercialization.

Furthermore, future research should incorporate advanced physicochemical characterization techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), and Differential Scanning Calorimetry (DSC) to further validate nanoparticle structure, crystallinity, and phytochemical interactions.



In addition, mechanistic studies on antimicrobial activity should be explored to understand the interaction of nanoparticle formulations with microbial cells. Such studies may focus on membrane integrity disruption, oxidative stress pathways, protein denaturation, and inhibition of microbial metabolic pathways.

Future studies should also consider process optimization and scale-up feasibility to evaluate the economic and technical viability of large-scale nanoparticle production. This would facilitate the transition from laboratory-scale research to pharmaceutical and nutraceutical applications.

Moreover, comparative agro-ecological studies involving *Nigella sativa* from additional geographical regions would help establish phytochemical quality markers and support the development of standardized medicinal plant resources.

Finally, research into synergistic applications with conventional antimicrobial agents is recommended, particularly to evaluate the potential of nanoparticle-encapsulated *Nigella sativa* in combating antimicrobial resistance, which remains a major global public health challenge.

Conflict of Interest

The author declares no conflict of interest. The funders had no influence on the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Author Contributions

The author confirms sole responsibility for all aspects of this study. This includes conceptualization, methodology development, investigation, sample collection, laboratory analysis, data curation, formal analysis, visualization, manuscript writing (original draft preparation), manuscript review and editing, and final approval of the version to be published.

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