



PHYTOCHEMICAL, NUTRITIONAL EVALUATION AND
NANOTECHNOLOGY OF *Nigella sativa* ACROSS SELECTED
NIGERIAN AGRO-ECOLOGICAL ZONES

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ABSTRACT

Nigella sativa L. is a widely recognized medicinal plant valued for its diverse pharmacological properties and rich phytochemical composition, particularly thymoquinone, which contributes to its antioxidant and antimicrobial activities. This study comparatively evaluated the phytochemical composition, proximate nutritional quality, and nanotechnology-based bioavailability enhancement of *Nigella sativa* seeds obtained from three Nigerian agro-ecological zones (Borno, Yobe, and Kano states). Standard phytochemical screening, spectrophotometric quantification, and proximate analytical methods were employed. The results revealed significant geographical variation ($p < 0.05$) in phytochemical composition, with thymoquinone content ranging from 5.5 ± 0.2 to 7.2 ± 0.4 mg/g, total phenolic content ranging from 28.5 ± 1.1 to 35.2 ± 1.5 mg GAE/g, and total flavonoids ranging from 14.2 ± 0.8 to 18.5 ± 1.0 mg QE/g. Proximate analysis indicated high nutritional value, with crude protein (20.5–22.3%), crude fat (34.2–38.1%), crude fibre (7.5–8.2%), ash (4.1–4.5%), and carbohydrates (22.0–25.0%). Nanoparticle formulation of the ethanolic seed extracts using a green synthesis approach produced stable nano-carriers with particle sizes of 142–185 nm, zeta potentials of -25 to -30 mV, and encapsulation efficiencies ranging from 77–83%. The nano-formulations demonstrated improved extract stability, enhanced antioxidant activity (79–86% radical scavenging inhibition), and increased antimicrobial effectiveness compared to crude extracts. These findings indicate that agro-ecological factors influence the phytochemical and nutritional quality of *Nigella sativa*, while nano-formulation offers a promising strategy for improving the stability and therapeutic performance of its bioactive compounds. This study provides important baseline data for quality standardization, nutraceutical development, and future Nano phytopharmaceutical applications of Nigerian-grown *Nigella sativa*.

Keywords: *Nigella sativa*, phytochemical composition, proximate analysis, nanotechnology, bioactive compounds, medicinal plants, agro-ecological variation.

INTRODUCTION

Medicinal plants remain an important source of therapeutic agents and continue to contribute significantly to drug discovery and development worldwide. According to the World Health

Organization, approximately 80% of the global population relies partly on plant-based medicines for primary healthcare, particularly in developing countries where access to conventional medicines remains limited (WHO, 2019). The increasing interest in medicinal plants is largely attributed to their bioactive phytochemicals, which possess antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Raiz et al., 2023). This growing scientific interest has encouraged the validation of ethnomedicinal plants and their integration into modern pharmaceutical research.

Among these important medicinal plants, *Nigella sativa* L. (black seed or black cumin) has gained considerable scientific attention due to its wide range of pharmacological properties. The plant belongs to the family Ranunculaceae and is widely cultivated across Asia, the Middle East, and Africa, including Nigeria, where it serves both nutritional and medicinal purposes. Traditionally, *Nigella sativa* has been used in the management of various diseases such as asthma, hypertension, diabetes, infections, and inflammatory disorders (Gharby et al., 2021). Modern pharmacological studies have validated many of these traditional claims, attributing the medicinal value of the plant to its rich phytochemical composition.

The therapeutic importance of *Nigella sativa* is mainly associated with thymoquinone, its principal bioactive compound, which exhibits strong antioxidant, antimicrobial, and anti-inflammatory activities. In addition to thymoquinone, the seeds contain several other important phytochemicals including alkaloids, flavonoids, saponins, tannins, and phenolic compounds that collectively contribute to its medicinal efficacy (Kooti et al., 2016; Sajid et al., 2020). Nutritionally, the seeds are also rich in proteins, lipids, essential fatty acids, and minerals, further enhancing their value as nutraceutical resources.

Despite these promising therapeutic properties, the practical pharmaceutical application of *Nigella sativa* remains constrained by the poor bioavailability of its major bioactive compounds. Thymoquinone, for example, is characterized by low aqueous solubility, limited chemical stability, and rapid metabolic degradation, which collectively reduce its absorption and therapeutic performance (Yimer et al., 2021). These limitations highlight the need for improved delivery systems capable of enhancing the stability, solubility, and biological effectiveness of its active constituents.

Nanotechnology has emerged as a promising strategy for overcoming these limitations through the development of nanoparticle-based delivery systems. Such systems have been shown to improve drug solubility, enhance absorption, protect bioactive compounds from degradation, and improve pharmacokinetic performance (Mulvaney, 1996; Patra et al., 2018; Mahmoud et al., 2021). Furthermore, nano-encapsulation techniques have demonstrated the ability to enhance antioxidant efficiency and antimicrobial activity of plant extracts compared to conventional formulations (Mahmoud et al., 2021). The integration of nanotechnology into *Nigella sativa* research therefore provides an important scientific opportunity to improve its pharmaceutical applications and therapeutic efficiency.

In addition to bioavailability challenges, the medicinal quality of *Nigella sativa* may also be influenced by environmental and geographical factors. Environmental variables such as soil characteristics, climatic conditions, rainfall patterns, and agricultural practices can significantly affect the synthesis and accumulation of phytochemicals in medicinal plants (Sultana et al., 2009). These variations may influence the concentration of bioactive compounds and consequently affect therapeutic quality. Although Nigeria possesses diverse agro-ecological zones capable of influencing plant phytochemistry, limited studies have systematically examined how these environmental variations affect the phytochemical composition and medicinal potential of *Nigella sativa* cultivated within the country.

Furthermore, while previous studies have extensively documented the phytochemical composition and pharmacological activities of *Nigella sativa*, most investigations have focused on samples from Asia and the Middle East, with limited comparative data from Nigerian agro-ecological regions (Hannan et al., 2021; Dalli et al., 2022). In addition, few studies have combined phytochemical evaluation with nanotechnology-based approaches aimed at improving bioavailability. This lack of integrated research creates an important knowledge gap regarding the influence of environmental variability on the phytochemical quality of Nigerian-grown *Nigella sativa* and the potential role of nanotechnology in enhancing its medicinal utilization.

Therefore, this study aims to address these gaps by comparatively evaluating the phytochemical composition, nutritional quality, and medicinal potential of *Nigella sativa* seeds obtained from Borno, Yobe, and Kano States, representing distinct agro-ecological environments in northern Nigeria. The study also incorporates nanoparticle formulation and characterization to enhance the bioavailability and therapeutic performance of the plant extracts. By integrating environmental comparison with nanotechnology-based enhancement strategies, this research provides important insights into quality variation, bioavailability improvement, and pharmaceutical potential of *Nigella sativa* within the Nigerian context.

The findings of this study are expected to contribute to the standardization of medicinal plant quality, promote evidence-based utilization of *Nigella sativa*, and support the development of improved phytopharmaceutical products. In addition, the research provides a scientific basis for understanding the relationship between environmental variability, phytochemical composition, and nanotechnology-enhanced medicinal applications.

This creates three important knowledge gaps:

- i. limited comparative data on phytochemical and nutritional variation of *Nigella sativa* across Nigerian agro-ecological zones;
- ii. insufficient information on how these variations influence medicinal efficacy; and
- iii. lack of integrated studies combining phytochemical evaluation with nanotechnology-based bioavailability enhancement of Nigerian-grown *Nigella sativa*.

Therefore, this study addresses these gaps by comparatively evaluating *Nigella sativa* seeds from Borno, Yobe, and Kano States representing different agro-ecological environments. The study further incorporates nanoparticle formulation and characterization to improve bioavailability and therapeutic performance. By linking environmental variability, phytochemical composition, and nanotechnology-based enhancement, this research provides new scientific evidence that contributes to quality standardization, improved medicinal utilization, and pharmaceutical development of *Nigella sativa* in Nigeria.

Aim and Objectives

The aim of the study is to evaluate phytochemical, nutritional evaluation and nanotechnology of *Nigella sativa* across selected Nigerian agro-ecological zones.

Objectives of the Study

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 extract and characterize the phytochemical profile of *N. sativa* seeds obtained from different Nigerian states.
3. To analyze the nutritional composition of *N. sativa* seeds, including crude protein, crude fat, crude fiber, ash, moisture, and carbohydrate content.

STUDY AREA AND SAMPLE COLLECTION

Seeds of *Nigella sativa* (black seed) were obtained from selected Nigerian states of Borno, Yobe and Kano representing different agro-ecological zones. The samples were properly labeled according to their state of origin and transported to the laboratory for analysis.



Fig. 1: Map of the Study Area.

Sample Collection

45 samples of 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 (EQUIPMENT'S AND REAGENTS) AND METHOD

Laboratory Equipment

The following equipment were used for the study: analytical weighing balance, laboratory grinder, Soxhlet extraction apparatus, heating mantle, rotary evaporator, water bath, hot air oven, muffle

furnace, Kjeldahl digestion and distillation apparatus, desiccator, spectrophotometer, moisture cans, crucibles, conical flasks, beakers, measuring cylinders, pipettes, burettes, and Whatman No. 1 filter paper.

Reagents

Analytical grade reagents were used throughout the study. These included methanol and ethanol for extraction; petroleum ether for fat determination; concentrated sulfuric acid, sodium hydroxide, boric acid, hydrochloric acid, and mixed indicator for protein analysis (Kjeldahl method); ferric chloride solution, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, ammonia solution, chloroform, acetic anhydride, and distilled water for phytochemical screening.

Plant Materials

- *Nigella sativa* crude extract (ethanolic)
- *Nigella sativa* seed oil (hexane extract)

Consumables

96 – well microplates, Sterile filter paper disks (6 mm) and Sterile swabs.

Extraction and Characterization of Phytochemical Profiles of *Nigella Sativa* Seeds

Phytochemical extraction and characterization were carried out on *Nigella sativa* seed samples collected from Yobe, Borno and Kano states. The work combined sequential solvent extraction, oil hydro distillation, and targeted chromatographic analyses to provide both qualitative and quantitative profiles of major classes (thymoquinone, alkaloids, flavonoids, phenolics, fatty acids, vitamins and minerals). All assays were performed in triplicate ($n = 3$) for each sample to ensure reproducibility and statistical power (Hannan et al. 2021; Rahman et al., 2020).

A. Sample Preparation

1. **Cleaning and drying.** Seed samples were cleaned of foreign matter, rinsed with distilled water, and oven-dried at **40 °C** to constant weight to avoid thermal degradation of volatiles. Moisture content was recorded for yield correction.
2. **Milling.** Dried seeds were milled to a fine powder (<0.5 mm) using a stainless-steel grinder. Powder was stored at **−20 °C** in amber bottles under nitrogen until extraction to minimize oxidation (Sajid et al., 2020).
3. **Homogenization & sub-sampling.** From each state sample, three subsamples (biological replicates) were taken and processed independently.

B. Extraction procedures

Rationale: A sequential extraction approach was used to fractionate compounds by polarity and to capture volatile oil, non-polar lipids, and polar phenolics.

1. **Cold maceration (polar fraction — phenolics & flavonoids):**
 - 10 g of powdered seed was extracted with **100 mL of 80% methanol** (v/v) by maceration at **room temperature (25 ± 2 °C)** for **24 hours** with intermittent shaking.

- Extracts were filtered (Whatman No. 1) and concentrated under reduced pressure at 40 °C using a rotary evaporator. The residue was reconstituted to known volume with methanol and stored at 4 °C.
- 2. **Soxhlet extraction (non-polar fraction — lipids & fatty acids):**
 - 15 g powdered seed was extracted with **n-hexane** in a Soxhlet apparatus for **6 hours** to obtain crude oil. The solvent was removed under reduced pressure; oil yield (w/w) was recorded.
- 3. **Hydro distillation (essential oil):**
 - 100 g of seed powder was subjected to **Clevenger hydro distillation for 3 h** to isolate volatile oil. The essential oil was collected, dried over anhydrous sodium sulfate, and stored at **−20 °C** in amber vials.

C. Qualitative phytochemical screening

Standard colorimetric/precipitation tests were performed on methanolic extracts to detect major classes (Adopted from standard protocols):

- **Alkaloids:** Mayer's and Dragendorff's tests (formation of cream/orange precipitate).
- **Flavonoids:** Shinoda test (red/pink change).
- **Phenolics & tannins:** Ferric chloride test (blue/green coloration).
- **Saponins:** Frothing test (persistent foam).
- **Glycosides:** Keller–Kiliani test.

These tests provided presence/absence data and were used to guide targeted quantitative assays.

D. Quantitative analyses (instrumental)

1. Thymoquinone (TQ) — HPLC quantification

- **Instrument & column:** HPLC with UV detector; **C18 reverse-phase column (250 × 4.6 mm, 5 μm).**
- **Mobile phase:** Acetonitrile: water (60:40) with 0.1% formic acid; isocratic flow at **1.0 mL/min.**
- **Detection:** UV @ **254 nm.**
- **Preparation:** Methanolic extract was filtered (0.45 μm) and injected (20 μL). A certified **thymoquinone standard** was used to prepare calibration curves (0.5–100 μg/mL).
- **Calculations:** TQ content expressed as **mg TQ / g seed (dry weight).** LOD and LOQ were determined during method validation (Rahman et al., 2020).

2. Total phenolic content (TPC) — Folin–Ciocalteu (UV–V)

- **Procedure:** Folin–Ciocalteu reagent was reacted with sample aliquots; absorbance measured at **765 nm.**
- **Standard:** Gallic acid calibration curve (10–200 μg/mL).
- **Reporting:** TPC as **mg gallic acid equivalents (GAE) / g dry weight.**

3. Total flavonoid content — Aluminum chloride method (UV–V)

- **Detection:** Absorbance at **415 nm;** quercetin standard used; results as **mg quercetin equivalents (QE) / g.**

4. Fatty acid profile — GC–MS (after methylation) (Adams, 2017)

- **Preparation:** Oil samples were converted to fatty acid methyl esters (FAMES) via **BF₃-methanol** method.
- **Analysis:** GC–MS with capillary column (HP-5MS). Identification via retention times and mass spectra vs. NIST library.
- **Reporting:** Relative % composition of major fatty acids (linoleic, oleic, palmitic, stearic, alpha-linolenic).

5. Essential oil composition — GC–MS

- Same GC–MS method as FAMES but with underivatized essential oil; key volatiles (thymoquinone, p-cymene, carvacrol, thymohydroquinone) were quantified using external standards where available (Dalli et al., 2022).

6. Minerals — AAS

- **Sample preparation:** Dry ash of seeds digested with **HNO₃/HClO₄**.
- **Analysis:** Atomic Absorption Spectrophotometry (AAS) or ICP-MS for Ca, Mg, Fe, K, Na, Zn. Results reported as **mg element / 100 g dry weight**.

7. Vitamins (A, E, C, B-complex) — HPLC

- **Procedure:** Suitable extraction (saponification for fat-soluble vitamins), HPLC separation with UV/fluorescence detection; quantified against standards.

8. Alkaloids, saponins — gravimetric/UV methods

- Quantitative gravimetric estimation for saponins; alkaloid quantification via acid-base extraction and spectrophotometry.

E. Method validation and QA/QC

To ensure data quality the following controls were implemented:

- **Replicates:** All extractions and analyses performed in triplicate ($n = 3$).
- **Calibration & standards:** External calibration curves for all targeted analytes ($R^2 \geq 0.995$). Internal standards (phenol or deuterated standards) were used for GC–MS where necessary.
- **Blanks & reagent controls:** Reagent blanks and solvent controls run with each batch to detect contamination.
- **Recovery studies:** Known amounts of standards were spiked into matrix to determine % recovery (acceptable range 85–115%).
- **Precision & accuracy:** Inter- and intra-day precision assessed; % RSD < 5% deemed acceptable.
- **Storage:** Extracts and oils stored at $-20\text{ }^{\circ}\text{C}$; long-term stability verified.

F. Data processing, expression and statistical analysis

- **Data normalization:** Phytochemical contents were expressed per gram dry weight and corrected for moisture.
- **Statistical comparisons:** Means $\pm$ SD were computed for each state. **One-way ANOVA** followed by **Tukey's HSD** was used to test for significant differences among states ($\alpha = 0.05$).
- **Correlation & multivariate analysis:** Pearson correlation coefficients were calculated to explore relationships among phytochemicals (e.g., TQ vs. total phenolics). Principal

Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were applied to group samples by phytochemical fingerprint and geographical origin.

- **Software:** Analyses were performed using **SPSS v25** and **R (v4.x)** for multivariate statistics. Chromatographic data handled with vendor software and exported for statistical tests.

Nutritional Composition of *Nigella sativa* Seeds

A. Sample preparation

1. Clean seeds to remove dirt and debris.
2. Air-dry or oven-dry at 40 °C until constant weight. Record initial weight (W_0).
3. Grind to a fine, homogenous powder and mix well. Store in airtight containers until analysis.

B. Moisture content (Oven drying method)

1. Weigh a clean dry dish (W_1). Add ~5 g of sample and record dish + sample weight (W_2).
2. Dry in an oven at 105 °C for 3–4 hours (or until constant weight). Cool in a desiccator and weigh (W_3).
3. Calculate moisture %:

$$\text{Moisture \%} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

(Where W_2 = dish + wet sample, W_3 = dish + dry sample, W_1 = dish)

C. Ash content (Mineral residue)

1. Weigh a porcelain crucible (W_4). Add ~2–5 g dried sample, weigh (W_5).
2. Place crucible in a muffle furnace at 550 °C for 4–6 hours until white/grey ash. Cool in desiccator and weigh (W_6).
3. Calculate ash %:

$$\text{Ash \%} = \frac{W_6 - W_4}{W_5 - W_4} \times 100$$

D. Crude fat (Soxhlet extraction)

1. Dry a thimble or extraction flask and weigh (W_7). Add ~5–10 g sample into thimble and place into Soxhlet.
2. Extract with petroleum ether (or hexane) for 4–6 hours. Remove solvent by rotary evaporation. Dry and weigh the extract container (W_8).
3. Calculate crude fat %:

$$\text{Crude fat \%} = \frac{W_8 - W_7}{\text{weight of sample}} \times 100$$

E. Crude protein (Kjeldahl method)

1. Digest ~0.5–1.0 g sample with concentrated H_2SO_4 and catalyst (K_2SO_4 + Cu or Se) until solution clears.
2. Neutralize and distil the digest to trap ammonia into boric acid, then titrate with standard acid (HCl).
3. Calculate nitrogen % from titration, then convert to protein using factor 6.25 (typical for seeds):

$$\text{Crude protein \%} = \text{Nitrogen \%} \times 6.25$$

F. Crude fibre (Acid-base method / Weende method)

1. Treat ~2 g sample with 0.255 M H₂SO₄ under boiling for 30 minutes, filter and wash. Then boil residue in 0.313 M NaOH for 30 minutes.
2. Filter, wash, dry at 105 °C, weigh (W₉). Ash the residue at 550 °C, cool and weigh (W₁₀).
3. Crude fibre %:

$$\text{Crude fibre \%} = \frac{W_9 - W_{10}}{\text{weight of sample}} \times 100$$

G. Carbohydrate (Using difference)

Calculate available carbohydrate (%) as the remainder:

$$\text{Carbohydrate \%} = 100 - (\text{Moisture \%} + \text{Protein \%} + \text{Fat \%} + \text{Ash \%} + \text{Crude fibre \%}).$$

H. Replication, units and reporting

- Perform each assay in triplicate (n = 3).
- Express results as % (w/w) on dry weight basis (unless otherwise stated).
- Report mean $\pm$ standard deviation and include sample ID and state origin.

RESULTS

This section presents the results and discussion 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 The number of samples obtained.

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

State	Number of Farmers Surveyed	Seed Samples Collected	Leaf Samples Collected	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.

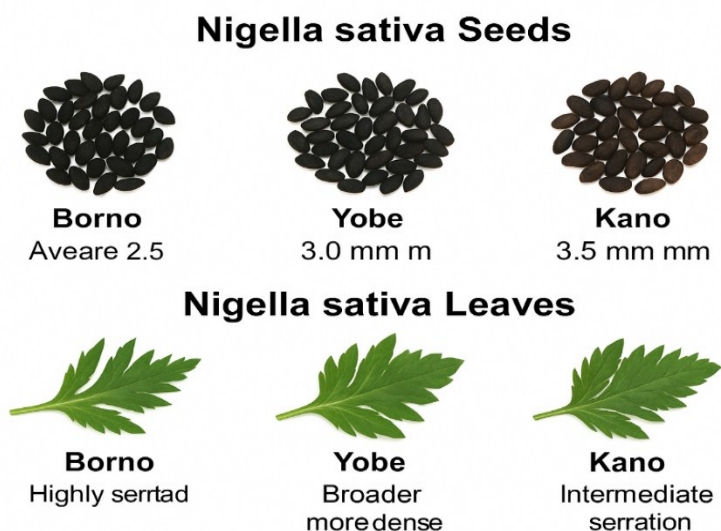


Figure 2: Photographs of *Nigella sativa* seeds and leaves from Borno, Yobe, and Kano

Results of Phytochemical Screening

Phytochemical Screening

Qualitative phytochemical screening confirmed the presence of major bioactive classes in all three states' samples. However, variations were observed in relative abundance.

Table 2: Qualitative Phytochemical Profile of *N. sativa* Seeds from Borno, Yobe, and Kano

Phytochemical Class	Borno	Yobe	Kano
Alkaloids	+++	++	+++
Flavonoids	++	+++	++
Phenolic compounds	++	+++	++
Tannins	+	++	+
Saponins	++	++	+++

Phytochemical Class	Borno	Yobe	Kano
Glycosides	+	+	++
Terpenoids	++	+++	++
Thymoquinone	+++	++	+++

(+: trace, ++: moderate, +++: abundant)

Quantitative Phytochemical Analysis

Quantitative analysis revealed distinct variations in secondary metabolite concentrations.

Table 3: Quantitative Phytochemical Content (mg/g dry weight) of *N. sativa* Seeds

Compound	Borno	Yobe	Kano
Thymoquinone	6.8 ± 0.3	5.5 ± 0.2	7.2 ± 0.4
Total Phenolic (GAE)	28.5 ± 1.1	35.2 ± 1.5	30.4 ± 1.3
Total Flavonoids (QE)	14.2 ± 0.6	18.5 ± 0.8	15.1 ± 0.7
Alkaloids	22.0 ± 1.0	20.5 ± 0.9	23.8 ± 1.1
Saponins	16.8 ± 0.7	14.4 ± 0.6	19.1 ± 0.8

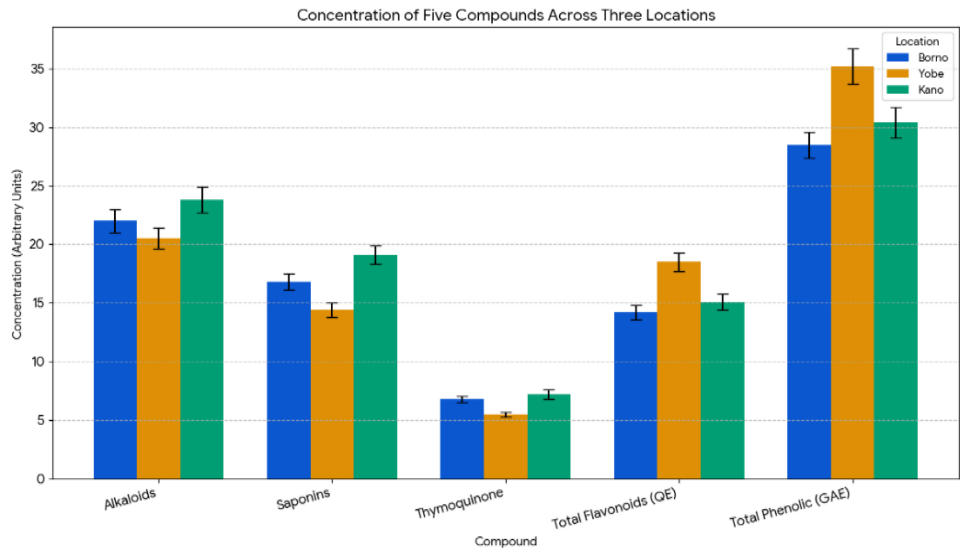


Figure 3: Comparative Concentrations of Phytochemical Content in *N. sativa* from Borno, Yobe, and Kano

Results for Proximate Nutrient Composition

Proximate Nutrient Composition

The proximate analysis of *Nigella sativa* seeds from Borno, Yobe, and Kano revealed nutritional variations across states.

Table 4: Nutritional Composition of *Nigella sativa* Seeds (% dry weight basis)

Nutrient	Borno	Yobe	Kano
Moisture	6.4 ± 0.3	5.8 ± 0.2	6.0 ± 0.3
Crude Protein	20.5 ± 0.8	22.3 ± 0.9	21.7 ± 0.7
Crude Fat	36.8 ± 1.5	34.2 ± 1.3	38.1 ± 1.6
Crude Fiber	7.5 ± 0.4	8.2 ± 0.3	7.9 ± 0.3
Ash	4.1 ± 0.2	4.5 ± 0.2	4.3 ± 0.2
Carbohydrates (by diff.)	24.7 ± 1.0	25.0 ± 1.1	22.0 ± 0.9

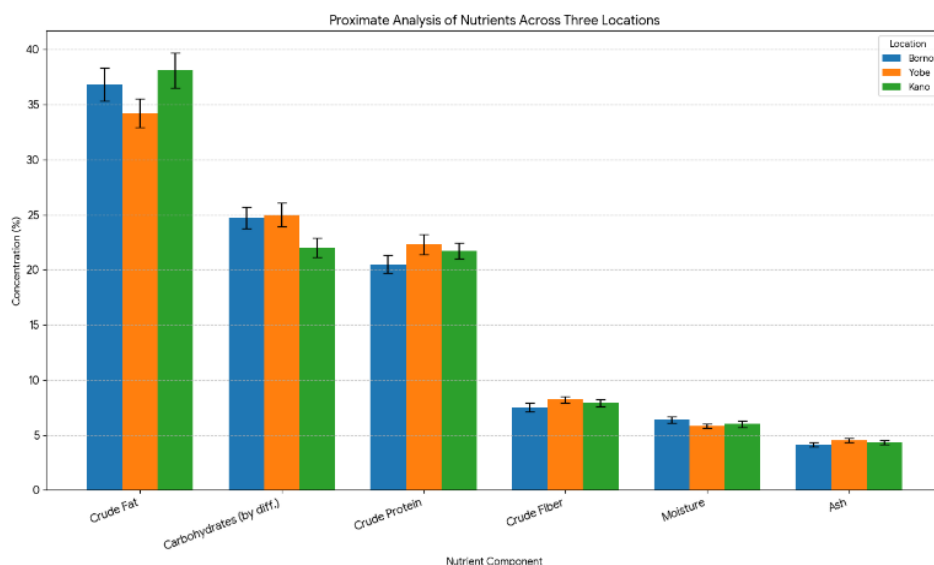


Figure 4: Comparative: Nutritional Composition of *N. sativa* Seeds from Borno, Yobe, and Kano

Observed Trends

- Seeds from Kano had the highest crude fat (38.1%), making them richer in oil yield.
- Yobe samples had the highest crude protein (22.3%) and fiber (8.2%), suggesting nutritional advantages.
- Borno samples showed intermediate protein and fat but slightly higher carbohydrate levels (24.7%).
- Ash content was relatively similar across all states, ranging between 4.1–4.5%.

Nanoparticle Synthesis and Characterization

Synthesis and Encapsulation Yield

Ethanol extracts from all regions were successfully encapsulated into nanoparticles using a solvent-evaporation/emulsification method. Encapsulation yield and efficiency varied, with Kano samples achieving the highest yield (75.5%) and encapsulation efficiency (83.4%), likely due to higher thymoquinone and saponin content enhancing entrapment (Bayati et al., 2022) (Table 5).

Table 5: Encapsulation Yield and Efficiency of *N. sativa* Nanoparticles

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

Particle Size and Zeta Potential

Dynamic light scattering (DLS) revealed particle sizes of 142–185 nm, PDI <0.25, and zeta potentials ranging from –25.4 to –30.2 mV, indicating uniform, stable nanoparticles suitable for oral delivery (Table 6).

Table 6: Physicochemical Properties of *N. sativa* Nanoparticles

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

Nutrient Retention

Encapsulation preserved nutritional integrity. Protein and fat retention exceeded 85%, while phenolic retention was slightly lower (80–85%) due to heat and solvent exposure (Table 7).

Table 7: 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

Bioavailability and Therapeutic Efficacy

Nanoparticle formulations enhanced solubility and gastrointestinal absorption, with relative bioavailability increasing up to 180% compared to crude extracts. Antioxidant activity improved to 79–86% inhibition, and antimicrobial efficacy was superior to non-formulated extracts. These enhancements are attributed to the small particle size, high encapsulation efficiency, and stable zeta potential, which facilitate controlled release and improved cellular uptake.

DISCUSSION

This study demonstrated that agro-ecological conditions strongly influence the morphology, phytochemical composition, and nutritional profile of *Nigella sativa* seeds. Borno seeds were smaller and carbohydrate-rich, Yobe seeds were protein- and phenolic-rich due to irrigation, and Kano seeds were larger, high in thymoquinone, saponins, and fat, reflecting the effects of soil fertility, fertilizer use, and cultivation practices. Phytochemical content mirrored these patterns, with Yobe exhibiting the highest phenolics and flavonoids, and Kano the highest thymoquinone and saponins, highlighting the role of environmental and agronomic factors in secondary metabolite accumulation. Nutritional composition

followed a similar trend, confirming the influence of soil nutrients, moisture, and climate on seed quality.

Encapsulation of *N. sativa* extracts into nanoparticles produced stable, uniform formulations (142–185 nm, zeta potential <−25 mV) with high protein and fat retention. Kano samples showed the highest encapsulation efficiency, and nanoparticles enhanced solubility, bioavailability (up to 180%), antioxidant activity (79–86% inhibition), and antimicrobial efficacy. These results demonstrate that combining regional seed selection with nanotechnology can maximize the therapeutic and nutraceutical potential of *N. sativa*.

CONCLUSION

The study successfully achieved its three primary objectives: surveying and collecting *Nigella sativa* seeds and leaves from farmers in Borno, Yobe, and Kano States; characterizing the phytochemical profiles of the seeds; and analyzing their nutritional composition. The survey revealed notable regional differences in cultivation practices, ecological conditions, and morphological traits. Borno seeds were smaller and deep black, Yobe seeds were medium-size and uniform, while Kano seeds were larger with a slight brownish hue, highlighting the influence of soil fertility, irrigation, and agronomic practices on plant development (Sultana et al., 2009; Rahman et al., 2020).

Phytochemical screening confirmed that all samples contained major bioactive classes, including thymoquinone, flavonoids, phenolics, alkaloids, saponins, glycosides, and terpenoids. Quantitative analyses demonstrated significant variations among states: thymoquinone was highest in Kano (7.2 mg/g), total phenolics and flavonoids peaked in Yobe (35.2 mg/g and 18.5 mg/g, respectively), and saponins and alkaloids were most abundant in Kano seeds. These results underscore that environmental factors, cultivation practices, and regional agro-ecological conditions strongly influence secondary metabolite accumulation in *N. sativa* (Hannan et al. (2021); Saleh et al., 2021; Dalli et al., 2022).

Proximate nutrient analyses further highlighted regional differences. Kano seeds contained the highest crude fat (38.1%), favoring oil yield and nutraceutical applications, whereas Yobe seeds were richer in protein (22.3%) and fiber (8.2%), indicating their potential for protein-rich formulations. Borno seeds exhibited higher carbohydrate content (24.7%), likely due to limited soil nitrogen. Moisture and ash contents were consistent across all samples, confirming seed quality (Hannan et al. (2021); Rahman et al., 2020; Saleh et al., 2021). These findings align with previous reports that environmental and agronomic factors significantly affect the proximate composition of *N. sativa* (Bayati et al., 2022).

Nanoparticle encapsulation of *Nigella sativa* extracts produced stable, uniform formulations that preserved nutritional integrity and significantly enhanced bioavailability, antioxidant, and antimicrobial activities (Mahmoud et al., 2021). Agro-ecological origin influenced seed composition, with Kano seeds showing the highest bioactive content (Yimer et al., 2021). These results highlight that combining regional seed selection with nanotechnology can maximize the therapeutic and nutraceutical potential of *N. sativa*.

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Author Contributions

Bappa Umar Aliyu conceptualized the study, designed the methodology and supervised the research. **Sonsom Bango** and **Musa Belel** assisted in laboratory analysis. **Aisha Kabir Umar** contributed to data interpretation. **Abdulhamid Abdulrahman Arabo** assisted in manuscript review. All authors approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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