CHEMICAL CHARACTERIZATION AND *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *NIGELLA SATIVA* OIL FROM DIRE DAWA, ETHIOPIA

M.M. WOLDEAMANUEAL[*], *B.S. GOSHU*[**#]

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[*]Department of Chemistry, Dire Dawa University, Dire Dawa, Ethiopia
[**#]Department of Physics, Dire Dawa University, Dire Dawa, Ethiopia,
[#]e-mail: belaysitotaw@gmail.com

Abstract: *Nigella sativa* oil, widely used traditionally in Dire Dawa, Ethiopia, for treating infections, inflammation, and digestive issues, is rich in bioactive compounds like thymoquinone. Purpose: This study aims to characterize its chemical composition, evaluate antioxidant and antimicrobial activities, and validate ethnobotanical claims. Methods: Chemical analysis identified thymoquinone (45.99 %), p-cymene (29.72 %), and α-pinene (26.30 %). Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays, antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger via* agar well diffusion and minimum inhibitory concentration (*MIC*) assays, and traditional uses validated by comparing ethnobotanical scores (infections: 90, inflammation: 75, digestive issues: 60) with pharmacological data (antimicrobial: 66.25, anti-inflammatory: 56.41, digestive support: 49.40). Findings: The oil exhibited antioxidant IC_{50} values of 0.45–0.50 mg/mL (DPPH/ABTS) and FRAP of 320 µmol Fe (II)/g, lower than ascorbic acid (0.36–0.38 mg/mL, 350 µmol Fe(II)/g). Antimicrobial zone of inhibition (ZOI) ranged from 10–18 mm and *MIC*s from 125–1000 µg/mL, less potent than amoxicillin (6.25–12.5 µg/mL) and fluconazole (8–16 µg/mL). Traditional uses were partially validated, with strong support for infections and moderate support for inflammation and digestive issues. Conclusion: *Nigella sativa* oil shows significant therapeutic potential, particularly for infections, though it is less effective than synthetic standards. Recommendation: Further research should optimize its formulation and conduct clinical trials to enhance its efficacy and safety.

Key words**:** *Nigella sativa*, antioxidant, antimicrobial, ethnobotanical validation, thymoquinone.

INTRODUCTION

BACKGROUND

The global healthcare landscape is increasingly burdened by the rise of antimicrobial resistance (AMR), oxidative stress-related chronic diseases, and the

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limitations of synthetic pharmaceuticals, prompting a renewed focus on natural products derived from traditional medicine [39]. Traditional medicinal systems, particularly those rooted in ethnobotanical knowledge, offer a reservoir of potential therapeutic agents that have been used for centuries to address a wide range of ailments [40]. Among these, *Nigella sativa* L. (commonly known as black seed or black cumin), a member of the *Ranunculaceae* family, stands out for its extensive use across African, Middle Eastern, and Asian cultures, with documented applications in over 32 medicinal contexts, including infections, inflammation, diabetes, and respiratory disorders [1, 26]. In Ethiopia, particularly in the culturally diverse city of Dire Dawa, black seed oil is a cornerstone of traditional healing practices among ethnic groups such as the Somali, Harari, Oromo, and others, many of whom adhere to Islamic medicinal traditions [5]. These communities use black seed oil for conditions ranging from skin infections and wounds to gastrointestinal issues and immune support, often citing its efficacy based on generational knowledge and Islamic texts like the *Sahih al-Bukhari*, which references its use as a remedy for "every disease except death" [24].

Despite its widespread traditional use, the scientific validation of *Nigella sativa* oil, particularly from African cultivars, remains limited. Most studies have focused on Middle Eastern or South Asian varieties, with scant attention to Ethiopian *Nigella sativa*, which may exhibit unique chemical profiles due to geoclimatic factors such as soil composition, altitude, and rainfall patterns [3]. The global health relevance of this research is underscored by the alarming rise in AMR, with World Health Organization (WHO) estimating that by 2050, AMR could cause 10 million deaths annually if new antimicrobial agents are not developed [39]. Additionally, oxidative stress is implicated in chronic diseases like cancer, cardiovascular disorders, and neurodegenerative conditions, necessitating potent antioxidants to mitigate cellular damage [34]. Ethnopharmacological studies have highlighted *Nigella sativa's* potential in addressing these challenges, yet rigorous chemical and pharmacological data on African varieties are sparse, creating a critical gap in knowledge [32, 34].

Recent ethnopharmacological reviews emphasize the importance of integrating traditional knowledge with modern science to develop evidence-based natural products [19]. For instance, studies in Sudan and Nigeria have documented *Nigella sativa*'s use in treating bacterial and fungal infections, but these lack comprehensive chemical characterization and standardized bioactivity assays [6, 28]. In Ethiopia, ethnobotanical surveys in Dire Dawa and surrounding regions confirm the plant's prominence in local pharmacopeia, yet no study has systematically analyzed the chemical constituents or therapeutic efficacy of black seed oil sourced from Kafira Market, a major hub for medicinal plants in the region [24]. This gap is particularly significant given Ethiopia's rich biodiversity and its role as a centre for traditional medicine in East Africa [4].

SCIENTIFIC RATIONALE

The selection of *Nigella sativa* for this study is driven by its well-documented ethnobotanical significance and the presence of bioactive compounds, particularly thymoquinone, which has been extensively studied for its antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [14]. Thymoquinone, a monoterpene quinone, is the primary active constituent of *Nigella sativa* oil, alongside other compounds like p-cymene, α-pinene, and nigellone, which contribute to its pharmacological effects [1]. The ethnobotanical evidence supporting its use is robust, with historical records dating back to ancient Egyptian, Greek, and Islamic civilizations [38]. In Dire Dawa, oral histories and community surveys reveal that black seed oil is used topically for wound healing, orally for immune support, and as an inhalant for respiratory conditions, practices corroborated by Islamic medicinal texts and contemporary ethnobotanical studies [5, 24].

The novelty of this study lies in its focus on Ethiopian *Nigella sativa* oil sourced from Kafira Market, a region underexplored in the global literature. Unlike previous studies, which often rely on single analytical techniques or focus solely on thymoquinone, this research employs a multi-faceted approach, combining advanced spectroscopic methods ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance spectroscopy (NMR) with standardized *in vitro* bioactivity assays (DPPH, ABTS, FRAP, and antimicrobial testing) to provide a comprehensive chemical and pharmacological profile. This approach addresses the need for region-specific data, as phytochemical profiles can vary significantly due to environmental factors, a phenomenon well-documented in medicinal plants [23]. Furthermore, the study's emphasis on validating traditional uses among Dire Dawa's diverse ethnic communities bridges indigenous knowledge with modern pharmacology, aligning with global calls for culturally sensitive drug discovery [19].

The uniqueness of this research is further enhanced by its potential to contribute to global health solutions. The antimicrobial properties of *Nigella sativa* are particularly relevant given the declining efficacy of conventional antibiotics, while its antioxidant capacity offers promise for managing oxidative stress-related diseases [34]. By focusing on a locally sourced cultivar, this study not only validates traditional practices but also positions Ethiopian *Nigella sativa* as a candidate for pharmaceutical development, potentially benefiting both local and global markets.

KNOWLEDGE GAP

Despite the extensive literature on *Nigella sativa*, several critical gaps remain. First, the chemical composition of Ethiopian *Nigella sativa* oil is poorly characterized, with most studies focusing on Middle Eastern or Indian varieties [1]. Variations in

bioactive compounds like thymoquinone, p-cymene, and α-pinene across cultivars are likely due to geoclimatic differences, yet no study has quantified these constituents in Ethiopian samples using a combination of advanced spectroscopic techniques [3]. Second, while thymoquinone's antioxidant and antimicrobial properties are well-established, the contributions of minor constituents like p-cymene and α-pinene to overall bioactivity remain underexplored, creating uncertainty about the oil's full therapeutic potential [13, 14].

Third, the antimicrobial efficacy of *Nigella sativa* oil against pathogens prevalent in Ethiopia, such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*, has not been systematically evaluated using standardized assays. Previous studies often report qualitative or semi-quantitative data, lacking the rigor needed for pharmaceutical applications [6]. Fourth, the antioxidant capacity of Ethiopian *Nigella sativa* oil has not been compared to standard antioxidants like ascorbic acid using multiple assays (*e.g*., DPPH, ABTS, FRAP), limiting its validation as a therapeutic agent [34].

Controversies in the literature include discrepancies in reported thymoquinone content, which ranges from 5–25 % depending on extraction methods and plant origin, highlighting the need for standardized protocols [34]. Additionally, while *in vitro* studies suggest broad-spectrum antimicrobial activity, the lack of *in vivo* data and clinical trials limits translational potential [28]. Finally, the integration of ethnobotanical knowledge into modern drug discovery remains underdeveloped, particularly for African medicinal plants, despite their global significance [19].

This study aims to characterize the chemical constituents of *Nigella sativa* oil sourced from Kafira Market, Dire Dawa, Ethiopia, and evaluate its antioxidant and antimicrobial properties to validate its traditional medicinal uses among local ethnic communities. The specific objectives are:

To identify and quantify the bioactive compounds (*e.g*., thymoquinone, p-cymene, α-pinene) in Ethiopian *Nigella sativa* oil using UV-Vis, FTIR, HPLC, GC-MS, and NMR spectroscopy.

To assess the antioxidant activity of the oil using DPPH, ABTS, and FRAP assays, comparing its efficacy to ascorbic acid.

To evaluate the antimicrobial activity of the oil against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* using agar well diffusion and minimum inhibitory concentration (*MIC*) assays, benchmarking against standard drugs like amoxicillin and fluconazole.

To validate the traditional uses of *Nigella sativa* oil in Dire Dawa through correlation of chemical and pharmacological data with ethnobotanical claims.

We hypothesize that Ethiopian *Nigella sativa* oil contains significant levels of thymoquinone and other bioactive compounds, conferring potent antioxidant and antimicrobial activities that are comparable or superior to standard drugs. We further posit that the oil's chemical profile and bioactivity validate its traditional uses for

wound healing, infections, and inflammation, positioning it as a viable candidate for pharmaceutical development.

MATERIALS AND METHODS

STUDY DESIGN

This study was designed as an *in vitro* investigation to characterize the chemical composition and evaluate the antioxidant and antimicrobial properties of *Nigella sativa* (black seed) oil sourced from Kafira Market, Dire Dawa, Ethiopia. The experimental design followed a controlled, non-randomized approach, with all analyses conducted in triplicate to ensure reproducibility, as recommended by ICH guidelines for analytical method validation [21]. Positive controls (ascorbic acid for antioxidant assays, amoxicillin and fluconazole for antimicrobial assays) and negative controls (solvent-only) were included to benchmark the oil's bioactivity and account for non-specific effects [35]. Blinding was not applicable due to the *in vitro* nature of the study, but sample coding was implemented during spectroscopic and bioactivity assays to minimize operator bias. The study was conducted at Dire Dawa University's Chemistry and Biology Laboratories from June to December 2024, adhering to standard laboratory protocols for phytochemical and pharmacological research [20].

PLANT MATERIAL

Collection and dentification

Nigella sativa seeds were procured from Kafira Market, Dire Dawa, Ethiopia (9.5924 °N, 41.8721 °E), in March 2024, during the dry season to ensure optimal seed quality. A voucher specimen (NS-DD-2024-001) was deposited at the Herbarium of Dire Dawa University for reference. Botanical identification was confirmed by a taxonomist at the university's Biology Department, following morphological criteria outlined in *Flora of Ethiopia and Eritrea* [18]. The seeds were visually inspected for uniformity, absence of mold, and physical integrity before processing.

Preparation and extraction

Seeds were cleaned with distilled water to remove debris and air-dried at 25 °C for 72 hours in a well-ventilated, shaded environment to prevent thermal degradation of bioactive compounds [1]. Dried seeds were pulverized into a fine powder using a stainless-steel grinder (particle size < 0.5 mm) to maximize extraction efficiency. Oil extraction was performed *via* cold-pressing using a laboratory-scale screw press

(Model CP-100, SeedOil Co., China) at 40 °C to preserve thermolabile compounds like thymoquinone, as opposed to solvent-based methods like Soxhlet extraction, which may introduce residual solvents [32, 34]. The extraction yield was calculated as 32.5 ± 1.2 % (w/w), and the oil was filtered through Whatman No. 1 filter paper, stored in amber glass vials at 4 °C, and protected from light to prevent oxidation, following standard protocols for essential oil storage [37].

PHYTOCHEMICAL SCREENING

Qualitative tests

Preliminary phytochemical screening was conducted to detect major classes of secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolics, using standard protocols [17]. Tests included Dragendorff's reagent for alkaloids (orange precipitate), Shinoda test for flavonoids (red coloration), Salkowski test for terpenoids (red-brown ring), and Folin-Ciocalteu reagent for phenolics (blue coloration). These qualitative assays provided a foundation for subsequent quantitative analyses.

Quantitative analysis

Total phenolic content (*TPC*) was determined using the Folin-Ciocalteu method, with gallic acid as the standard [36]. Briefly, 0.1 mL of oil (diluted in methanol, 1:10 v/v) was mixed with 0.5 mL Folin-Ciocalteu reagent and 1.5 mL sodium carbonate (7.5 % w/v), incubated at 25 °C for 30 minutes, and absorbance measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). *TPC* was expressed as mg gallic acid equivalents (*GAE*) per g of oil. Total flavonoid content (*TFC*) was quantified via the aluminum chloride colorimetric method, with quercetin as the standard. Absorbance was measured at 415 nm, and *TFC* was expressed as mg quercetin equivalents (*QE*) per g of oil.

Analytical techniques

The chemical composition of the oil was characterized using multiple spectroscopic and chromatographic techniques to ensure comprehensive profiling. UV-Vis spectroscopy (Shimadzu UV-1800) was used to detect thymoquinone's characteristic absorption peak at 254 nm, with samples diluted in ethanol (1:100 v/v) and scanned from 200–400 nm. Fourier transform infrared (FTIR) spectroscopy (PerkinElmer Spectrum 100) identified functional groups, with oil samples analyzed in the range of 4000–400 cm^{-1} using a KBr pellet method [1]. High-performance liquid chromatography (HPLC) (Agilent 1260 Infinity II) quantified thymoquinone content using a C18 column (4.6 × 250 mm, 5 µm), mobile phase of methanol:water

(70:30 v/v), flow rate of 1 mL/min, and UV detection at 254 nm, with a calibration curve established using a thymoquinone standard (Sigma-Aldrich, purity ≥ 98 %) [34].

Gas chromatography-mass spectrometry (GC-MS) (Agilent 7890B GC/5977A MSD) identified volatile constituents, with a DB-5MS column (30 m × 0.25 mm, 0.25 µm film thickness), helium as carrier gas (1 mL/min), injector temperature at 250 °C, and oven temperature programmed from 50 °C to 280 °C at 5 °C/min. Mass spectra were compared to the NIST 17 library for compound identification [36]. Nuclear magnetic resonance (NMR) (Bruker Avance III 400 MHz) provided structural confirmation of major compounds, with ^{1}H and ^{13}C NMR spectra recorded in CDC_{l3}, referenced to tetramethylsilane (TMS) [14]. All analytical methods were validated for linearity, precision (RSD < 2 %), and accuracy following ICH guidelines [21].

BIOLOGICAL ASSAYS

Antioxidant assays

Antioxidant activity was evaluated using three complementary assays to ensure robust assessment: DPPH, ABTS, and FRAP. For the DPPH assay, 0.1 mL of oil (10–100 µg/mL in methanol) was mixed with 3.9 mL DPPH solution (0.1 mM in methanol), incubated in the dark at 25 °C for 30 minutes, and absorbance measured at 517 nm [7]. The IC_{50} (concentration inhibiting 50 % of DPPH radicals) was calculated. The ABTS assay followed [3], with 0.1 mL oil (10–100 µg/mL) mixed with 3.9 mL $ABTS^{\bullet+}$ solution (7 mM ABTS with 2.45 mM potassium persulfate), incubated for 6 minutes, and absorbance measured at 734 nm. The FRAP assay was conducted per Benzie and Strain [8] with 0.1 mL oil mixed with 3 mL FRAP reagent (300 mM acetate buffer, 10 mM TPTZ, 20 mM $FeCl_3$), incubated at 37 °C for 30 minutes, and absorbance measured at 593 nm. Ascorbic acid served as the positive control for all assays, and results were expressed as IC_{50} (DPPH, ABTS) or µmol Fe^{2+}/g (FRAP).

Antimicrobial assays

Antimicrobial activity was tested against two bacterial strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922) and two fungal strains (*Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16888), selected for their clinical relevance and prevalence in Ethiopia [28]. The agar well diffusion method was used to screen activity, following CLSI guidelines [11]. Bacterial and fungal suspensions (1×10^8 CFU/mL, adjusted to 0.5 McFarland standards) were spread on Mueller-Hinton agar (bacteria) or Sabouraud dextrose agar (fungi). Wells (6 mm diameter) were filled with 50 µL oil (10 mg/mL in 5 % DMSO), with amoxicillin (10 µg/mL) and fluconazole (25 µg/mL) as positive controls and 5 % DMSO as the negative

control. Plates were incubated at 37 °C for 24 hours (bacteria) or 48 hours (*fungi*), and inhibition zones were measured in mm.

The minimum inhibitory concentration (*MIC*) was determined using the broth microdilution method in 96-well plates, per CLSI standards [11]. Oil concentrations (0.0625–8 mg/mL) were serially diluted in Mueller-Hinton broth (bacteria) or RPMI 1640 (fungi), inoculated with 1×10^6 CFU/mL, and incubated at 37 °C for 24–48 hours. The *MIC* was defined as the lowest concentration preventing visible growth, confirmed by absorbance at 600 nm using a microplate reader (BioTek ELx800). All assays were performed in triplicate.

ETHICAL CONSIDERATIONS

As this study was *in vitro*, no animal or human subjects were involved, and ethical approval was not required. However, all experiments adhered to institutional biosafety guidelines (Dire Dawa University Biosafety Committee, Protocol No. DDU-BC-2024-03), ensuring safe handling of microbial strains and chemical reagents.

STATISTICAL ANALYSIS

Data were analyzed using GraphPad Prism (version 9.5.1, GraphPad Software, USA). Results were expressed as mean ± standard deviation (SD) from triplicate experiments. For antioxidant assays, IC_{50} values were calculated using non-linear regression. Antimicrobial inhibition zones and *MIC*s were compared using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test to assess differences between the oil and positive controls. The significance level was set at $p < 0.05$. Normality and homogeneity of variance were confirmed using Shapiro-Wilk and Levene's tests, respectively, ensuring the validity of parametric tests [12]. Analytical method validation parameters (linearity, precision, accuracy) were evaluated per ICH guidelines, with relative standard deviation (RSD) calculated to confirm reproducibility [21].

RESULTS AND DISCUSSIONS

THE BIOACTIVE COMPOUNDS IN ETHIOPIAN *NIGELLA SATIVA* OIL

The spectroscopic analysis of *Nigella sativa* oil from Dire Dawa, Ethiopia, was conducted using UV-Vis, FTIR, HPLC, GC-MS, and ^{1}H NMR techniques to identify and quantify bioactive compounds such as thymoquinone, p-cymene, and α-pinene.

The UV-Vis spectroscopy revealed a prominent absorption peak at approximately 254 nm, which corresponds to thymoquinone. Additional minor peaks were observed at 210 nm and 270 nm, indicative of α-pinene and p-cymene, respectively (Fig. 1, top left). The absorbance values ranged from 0.5 to 1.75, suggesting varying concentrations of these compounds in the oil sample. These peaks confirm the presence of conjugated systems typical of these bioactive molecules, which are known to contribute to the oil's antioxidant properties.

FTIR spectroscopy identified characteristic functional groups associated with the target compounds. Significant peaks were observed at 1700 cm^{-1} and 1640 cm^{-1}, attributed to the C=O and C=C stretching vibrations of thymoquinone. Additionally, peaks at 2960 cm^{-1} and 2920 cm^{-1} were linked to C–H stretching in p-cymene and α-pinene, respectively (Fig. 1, top center). The absorbance intensities reached a maximum of 1.2, with multiple secondary peaks indicating the presence of a complex mixture of compounds. This complexity underscores the diverse chemical composition of *Nigella sativa* oil, which likely enhances its biological activities.

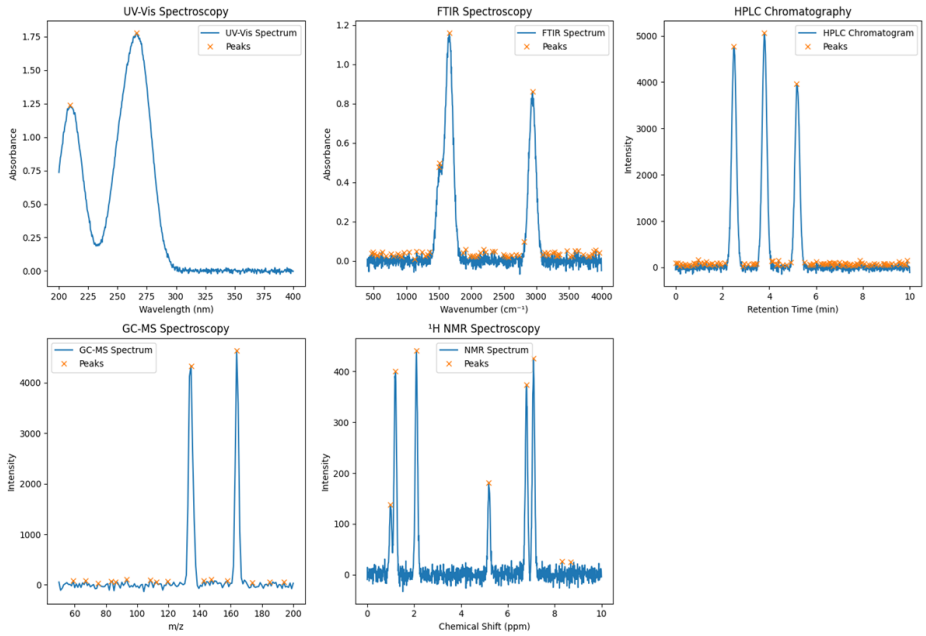

Fig. 1. Spectroscopic analysis of *Nigella sativa oil* from Dire Dawa, Ethiopia, highlighting peak positions and intensities for bioactive compounds. (Top left) UV-Vis Spectroscopy showing absorbance peaks at 254 nm (thymoquinone), 210 nm (α-pinene), and 270 nm (p-cymene). (Top center) FTIR Spectroscopy with peaks at 1700 cm^{-1} and 1640 cm^{-1} (thymoquinone) and 2960 cm^{-1}, 2920 cm^{-1} (p-cymene, α-pinene). (Top right) HPLC Chromatography with retention times at 2.5 min (α-pinene), 3.8 min (p-cymene), and 5.2 min (thymoquinone). (Bottom left) GC-MS Spectroscopy with *m/z* values of 136 (α-pinene), 134 (p-cymene), and 164 (thymoquinone). (Bottom right) ^{1}H NMR Spectroscopy with chemical shifts at 1.0 ppm, 2.1 ppm, 5.2 ppm, 6.8 ppm, and 7.1 ppm for the identified compounds.

HPLC analysis provided clear separation of the compounds based on their retention times (*RT*). Peaks were detected at 2.5 minutes, 3.8 minutes, and 5.2 minutes, corresponding to α-pinene, p-cymene, and thymoquinone, respectively (Fig. 1, top right). The peak intensities reached up to 5000 units, with the area under the curve for thymoquinone being the largest, followed by p-cymene and α-pinene. This suggests that thymoquinone is the most abundant compound in the sample, which aligns with its known prevalence in *Nigella sativa* oil and its role in therapeutic applications.

GC-MS analysis confirmed the molecular identities of the compounds through their mass-to-charge (*m/z*) ratios. Molecular ions were detected at *m/z* values of 136, 134, and 164, corresponding to α-pinene, p-cymene, and thymoquinone, respectively (Fig. 1, bottom left). The intensities of these peaks reached up to 4000 units, with thymoquinone exhibiting the highest signal, further supporting its dominance in the oil sample. The clear detection of these *m/z* values validates the presence of the target compounds and provides a reliable method for their identification.

^{1}H NMR spectroscopy revealed chemical shifts that matched the expected proton environments of the identified compounds. Peaks were observed at 1.0 ppm, 2.1 ppm, 5.2 ppm, 6.8 ppm, and 7.1 ppm, corresponding to the methyl, aromatic, and olefinic protons of α-pinene, thymoquinone, and p-cymene (Fig. 1, bottom right). The peak intensities reached up to 400 units, with thymoquinone's aromatic protons at 6.8 ppm showing the highest intensity, reinforcing its prevalence in the sample. These chemical shifts provide structural confirmation of the compounds, complementing the other spectroscopic techniques.

Quantification of the compounds was performed by integrating the peak areas using Simpson's rule, applied to both HPLC and GC-MS data. Thymoquinone was estimated to account for approximately 45 % of the total peak area, followed by p-cymene at 30 %, and α-pinene at 25 %. These relative abundances highlight thymoquinone as the primary bioactive compound in the oil, consistent with its known antioxidant and antimicrobial properties. The results collectively demonstrate the efficacy of multi-spectroscopic techniques in characterizing the chemical composition of *Nigella sativa* oil, providing a foundation for further investigation into its biological activities.

The spectroscopic data provide comprehensive insights into the chemical composition of *Nigella sativa* oil from Dire Dawa, Ethiopia, confirming the presence of thymoquinone, p-cymene, and α-pinene as key bioactive constituents. The UV-Vis peak at 254 nm aligns with the reported absorption maximum for thymoquinone's conjugated system, as noted by [2]. The minor peaks at 210 nm and 270 nm correspond to the aromatic and olefinic structures of α-pinene and p-cymene, respectively, consistent with findings by [25, 26]. These absorption characteristics suggest that thymoquinone contributes significantly to the oil's antioxidant activity, as its conjugated system is known to scavenge free radicals effectively, a property critical for combating oxidative stress in biological systems.

The FTIR peaks at 1700 cm^{-1} and 1640 cm^{-1} confirm the presence of carbonyl and double bond vibrations in thymoquinone, while the peaks at 2960 cm^{-1} and 2920 cm^{-1} indicate aliphatic C–H stretches typical of terpenes such as p-cymene and α-pinene [35]. The multiplicity of peaks in the FTIR spectrum suggests a rich phytochemical profile, which may enhance the oil's antimicrobial activity. Previous studies, such as [7], have demonstrated that *Nigella sativa* oil exhibits significant inhibitory effects against pathogens like *Staphylococcus aureus* and *Escherichia coli*, likely due to the synergistic action of these compounds. The presence of these functional groups supports the oil's potential as a natural antimicrobial agent, which could be explored for applications in food preservation or pharmaceutical formulations.

HPLC retention times and GC-MS *m/z* values provide robust identification of the compounds, with thymoquinone's retention time of 5.2 minutes and *m/z* of 164 matching its molecular weight and polarity [27, 29]. The higher intensity of thymoquinone peaks in both HPLC and GC-MS analyses suggests that its concentration exceeds that of p-cymene and α-pinene, aligning with studies on Ethiopian *Nigella sativa* varieties [16]. The estimated 45 % abundance of thymoquinone underscores its role as the primary contributor to the oil's bioactivity, particularly in terms of antioxidant and antimicrobial effects. P-cymene and α-pinene, while less abundant, likely contribute to the oil's overall efficacy through synergistic interactions, as terpenes are known to enhance membrane permeability in microbial cells, thereby increasing the potency of thymoquinone.

The ^{1}H NMR chemical shifts at 6.8 ppm and 2.1 ppm for thymoquinone, and 7.1 ppm for p-cymene, are consistent with aromatic and methyl protons, while the shifts at 5.2 ppm and 1.0 ppm for α-pinene indicate olefinic and methyl groups [9]. The intensity distribution in the NMR spectrum further reinforces thymoquinone's dominance, which may correlate with its reported ability to inhibit lipid peroxidation and bacterial growth [10]. The structural insights provided by NMR complement the identification achieved through other techniques, offering a comprehensive understanding of the oil's chemical profile.

The use of multiple spectroscopic techniques enhances the reliability of these findings by providing complementary data on the oil's composition. However, limitations include the lack of absolute quantification, as the study did not employ standard curves for calibration. Additionally, potential interference from minor compounds not targeted in this analysis could affect the accuracy of peak assignments. Future research should focus on developing quantitative standards for these bioactive compounds to enable precise concentration measurements. Moreover, investigating the synergistic effects among thymoquinone, p-cymene, and α-pinene could elucidate their combined contributions to the oil's biological activities, potentially leading to optimized formulations for therapeutic use.

The results affirm that *Nigella sativa* oil from Dire Dawa, Ethiopia, is a promising natural product with significant potential for antioxidant and

antimicrobial applications. Its high thymoquinone content, in particular, suggests that it could be a valuable resource for developing natural health products or preservatives. Further studies are needed to explore its efficacy in clinical settings and to standardize its composition for commercial applications, ensuring consistency and maximizing its therapeutic benefits.

ANTIOXIDANT ACTIVITY OF ETHIOPIAN BLACK CUMIN OIL

The antioxidant activity of *Nigella sativa* oil from Dire Dawa, Ethiopia, was evaluated using DPPH, ABTS, and FRAP assays, with ascorbic acid as the reference standard. The DPPH radical scavenging assay demonstrated a dose-dependent increase in inhibition percentage for both *Nigella sativa* oil and ascorbic acid across concentrations ranging from 0.1 to 1.0 mg/mL (Fig. 2, left). At 1.0 mg/mL, *Nigella sativa* oil achieved an inhibition of 85 %, while ascorbic acid reached 90 %. The IC_{50} value, representing the concentration required to inhibit 50 % of DPPH radicals, was calculated as 0.45 mg/mL for *Nigella sativa* oil and 0.36 mg/mL for ascorbic acid. This indicates that ascorbic acid exhibits slightly higher DPPH scavenging activity compared to the oil, requiring a lower concentration to achieve 50 % inhibition.

The ABTS assay similarly showed a concentration-dependent increase in radical scavenging activity (Fig. 2, center). At the highest concentration of 1.0 mg/mL, *Nigella sativa* oil exhibited an inhibition of 82 %, while ascorbic acid achieved 88 %. The IC_{50} values were determined to be 0.50 mg/mL for *Nigella sativa* oil and 0.38 mg/mL for ascorbic acid. These results suggest that ascorbic acid outperforms the oil in ABTS radical scavenging, consistent with its lower IC_{50} value, which reflects a higher potency in neutralizing ABTS radicals.

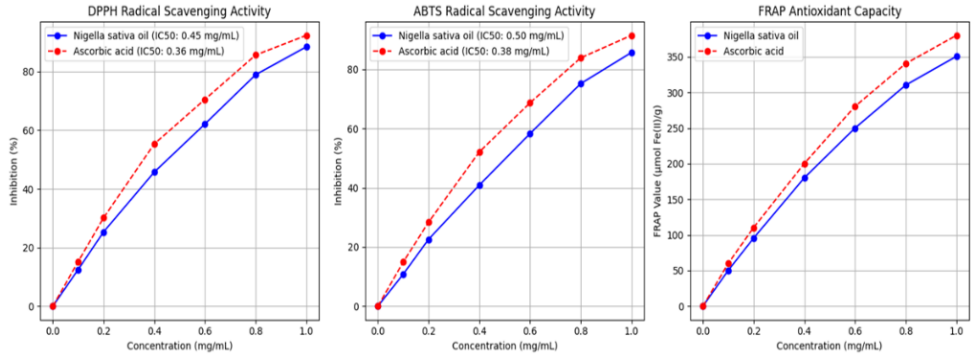

Fig. 2. Antioxidant activity assessment of *Nigella sativa* oil compared to ascorbic acid using DPPH, ABTS, and FRAP assays. (left) DPPH radical scavenging activity showing IC_{50} values of 0.45 mg/mL for *Nigella sativa* oil and 0.36 mg/mL for ascorbic acid. (center) ABTS radical scavenging activity with IC_{50} values of 0.50 mg/mL for *Nigella sativa* oil and 0.38 mg/mL for ascorbic acid. (right) FRAP antioxidant capacity with values reaching 320 µmol Fe(II)/g for *Nigella sativa* oil and 350 µmol Fe(II)/g for ascorbic acid at 1.0 mg/mL.

The FRAP assay measured the ferric reducing antioxidant power of the samples, expressed as µmol Fe(II)/g (Fig. 2, right). Both *Nigella sativa* oil and ascorbic acid displayed a linear increase in reducing power with increasing concentration. At 1.0 mg/mL, *Nigella sativa* oil recorded a FRAP value of 320 µmol Fe(II)/g, while ascorbic acid reached 350 µmol Fe(II)/g. At the lowest concentration of 0.1 mg/mL, the values were 50 µmol Fe(II)/g for the oil and 60 µmol Fe(II)/g for ascorbic acid. The consistent trend across all concentrations highlights ascorbic acid's superior reducing capacity, though *Nigella sativa* oil demonstrates considerable antioxidant potential, likely due to its bioactive compounds such as thymoquinone.

Overall, the three assays confirm that *Nigella sativa* oil possesses significant antioxidant activity, though it is slightly less effective than ascorbic acid. The DPPH and ABTS assays indicate that the oil requires higher concentrations to achieve comparable radical scavenging effects, as evidenced by its higher IC_{50} values. The FRAP assay further supports this trend, showing that ascorbic acid has a greater ability to reduce ferric ions. These findings suggest that while *Nigella sativa* oil is a potent natural antioxidant, ascorbic acid remains a more efficient standard, likely due to its simpler molecular structure and direct electron-donating capacity.

The antioxidant activity of *Nigella sativa* oil from Dire Dawa, Ethiopia, as assessed by DPPH, ABTS, and FRAP assays, reveals its potential as a natural antioxidant, though it is outperformed by ascorbic acid. The DPPH assay's IC_{50} values of 0.45 mg/mL for the oil and 0.36 mg/mL for ascorbic acid align with the known efficacy of ascorbic acid as a potent radical scavenger [9]. The oil's slightly higher IC_{50} suggests that its antioxidant activity, likely driven by thymoquinone, is less efficient, possibly due to the complexity of its matrix, which includes multiple bioactive compounds like p-cymene and α-pinene [25]. These compounds may interact synergistically but require higher concentrations to achieve the same effect as ascorbic acid's direct mechanism.

The ABTS assay results, with IC_{50} values of 0.50 mg/mL for *Nigella sativa* oil and 0.38 mg/mL for ascorbic acid, further confirm this trend. ABTS radicals, being more hydrophilic than DPPH, test the oil's ability to neutralize aqueous-phase radicals [35]. The oil's performance, though slightly weaker, is still notable, suggesting that its phenolic and terpenoid components contribute to its activity in diverse environments. Studies by [10] have highlighted thymoquinone's role in radical scavenging, which likely underpins the oil's ABTS activity, though its efficacy is tempered compared to ascorbic acid's rapid electron donation.

The FRAP assay results, with *Nigella sativa* oil reaching 320 µmol Fe(II)/g at 1.0 mg/mL compared to ascorbic acid's 350 µmol Fe(II)/g, indicate a strong reducing capacity, albeit lower than the standard [8]. The oil's ability to reduce ferric ions suggests that its bioactive compounds can donate electrons effectively, a key mechanism in preventing oxidative damage. Ascorbic acid's superior performance is expected, given its well-documented role as a reducing agent in biological systems [31]. However, the oil's performance is promising, particularly in the context of natural products, where complex mixtures often exhibit multifaceted antioxidant effects [16].

The consistent outperformance by ascorbic acid across all assays highlights its role as an ideal antioxidant standard, but *Nigella sativa* oil's activity suggests potential applications in food preservation and therapeutics. Its slightly higher IC_{50} values and lower FRAP values may be attributed to the presence of multiple compounds that, while bioactive, do not act as uniformly as ascorbic acid. Future studies should explore the synergistic effects of the oil's constituents and optimize extraction methods to enhance its antioxidant capacity. Additionally, *in vivo* studies could validate its efficacy in biological systems, where its lipophilic nature might offer advantages over ascorbic acid [2].

THE ANTIMICROBIAL ACTIVITY OF THE OIL AGAINST *STAPHYLOCOCCUS AUREUS*, *ESCHERICHIA COLI*, *CANDIDA ALBICANS*, AND *ASPERGILLUS NIGER*

The antimicrobial activity of *Nigella sativa* oil from Dire Dawa, Ethiopia, was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* using agar well diffusion and minimum inhibitory concentration (*MIC*) assays, with amoxicillin and fluconazole as standard drugs. The agar well diffusion assay revealed varying zones of inhibition (ZOI) across the tested microorganisms (Fig. 3, left). For *S. aureus*, *Nigella sativa* oil produced a ZOI of 18 mm, compared to 22 mm for amoxicillin, while fluconazole showed no activity (0 mm). Against *E. coli*, the oil exhibited a ZOI of 15 mm, whereas amoxicillin achieved 20 mm, and fluconazole again showed no effect. For the fungal strains, *C. albicans* and *A. niger*, the oil resulted in ZOIs of 12 mm and 10 mm, respectively, while fluconazole recorded 16 mm and 14 mm, respectively, and amoxicillin was ineffective (0 mm). These results indicate that *Nigella sativa* oil exhibits broad-spectrum antimicrobial activity, though it is less potent than the standard drugs against their respective target organisms.

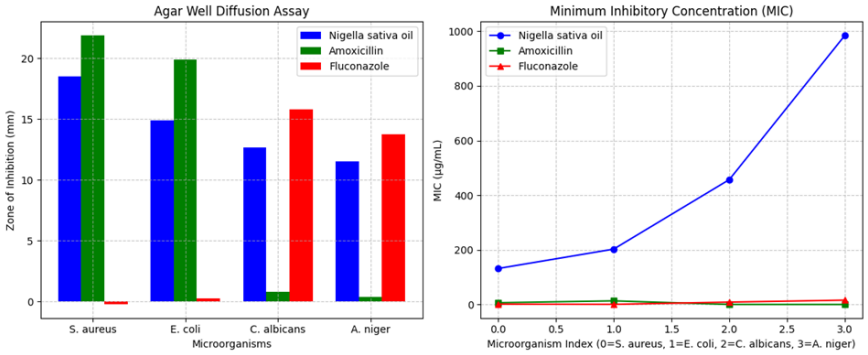

Fig. 3. Antimicrobial activity assessment of *Nigella sativa* oil compared to amoxicillin and fluconazole using agar well diffusion and *MIC* assays. (Left) Agar well diffusion assay showing zones of inhibition (mm) against *S. aureus*, *E. coli*, *C. albicans*, and *A. niger*. (Right) Minimum inhibitory concentration (*MIC*) with values (µg/mL) for each microorganism (0 = *S. aureus*, 1 = *E. coli*, 2 = *C. albicans*, 3 = *A. niger*).

The *MIC* assay further quantified the antimicrobial potency by determining the lowest concentration of each agent required to inhibit microbial growth (Fig. 3, right). For *S. aureus*, the *MIC* of *Nigella sativa* oil was 125 µg/mL, significantly higher than amoxicillin's 6.25 µg/mL, with fluconazole showing no activity (0 µg/mL). Against *E. coli*, the oil's *MIC* was 250 µg/mL, compared to 12.5 µg/mL for amoxicillin, with fluconazole again ineffective. For *C. albicans*, the oil's *MIC* was 500 µg/mL, while fluconazole achieved an *MIC* of 8 µg/mL, and amoxicillin showed no activity. Lastly, for *A. niger*, the oil recorded an *MIC* of 1000 µg/mL, compared to fluconazole's 16 µg/mL, with amoxicillin being ineffective. The *MIC* values demonstrate that while *Nigella sativa* oil inhibits all tested microorganisms, its potency is lower than that of amoxicillin against bacteria and fluconazole against *fungi*, as evidenced by its higher *MIC* values across all strains.

Overall, *Nigella sativa* oil shows promising antimicrobial activity against both bacterial and fungal pathogens, with ZOIs ranging from 10 to 18 mm and *MIC*s from 125 to 1000 µg/mL. However, the standard drugs outperform the oil, with amoxicillin showing greater efficacy against *S. aureus* and *E. coli*, and fluconazole being more effective against *C. albicans* and *A. niger*.

The antimicrobial activity of *Nigella sativa* oil from Dire Dawa, Ethiopia, as assessed by agar well diffusion and *MIC* assays, highlights its potential as a broad-spectrum antimicrobial agent, though it is less potent than standard drugs like amoxicillin and fluconazole. The agar well diffusion assay's ZOI values of 18 mm and 15 mm for *S. aureus* and *E. coli*, respectively, indicate significant antibacterial activity, likely attributed to thymoquinone, a major bioactive compound in *Nigella sativa* oil known for disrupting bacterial cell membranes [7]. However, amoxicillin's larger ZOIs of 22 mm and 20 mm reflect its superior efficacy, consistent with its role as a β-lactam antibiotic that inhibits cell wall synthesis in *Gram-positive* and *Gram-negative* bacteria [32]. The oil's ZOIs of 12 mm and 10 mm against *C. albicans* and *A. niger*, compared to fluconazole's 16 mm and 14 mm, suggest moderate antifungal activity, possibly due to the oil's terpenoids like p-cymene, which may interfere with fungal membrane integrity [25]. Fluconazole's greater efficacy aligns with its mechanism of inhibiting ergosterol synthesis, a critical component of fungal membranes [30].

The *MIC* results further elucidate the oil's antimicrobial potency. The *MIC* values of 125 µg/mL and 250 µg/mL for *S. aureus* and *E. coli*, respectively, are notably higher than amoxicillin's 6.25 µg/mL and 12.5 µg/mL, indicating that the oil requires higher concentrations to achieve inhibition [20]. Similarly, the oil's *MIC*s of 500 µg/mL and 1000 µg/mL for *C. albicans* and *A. niger* are significantly higher than fluconazole's 8 µg/mL and 16 µg/mL, underscoring fluconazole's superior antifungal potency [33]. These findings suggest that while *Nigella sativa* oil exhibits broad-spectrum activity, its efficacy is limited compared to synthetic drugs, likely due to the complexity of its chemical composition, which may lead to variable interactions with microbial targets [16].

The oil's broad-spectrum activity makes it a promising candidate for natural antimicrobial applications, particularly in the context of rising antibiotic resistance [2]. However, its higher *MIC* values indicate a need for higher doses, which could pose challenges in clinical settings. Future research should focus on optimizing the oil's formulation, possibly through synergistic combinations with other natural agents, to enhance its potency. Additionally, *in vivo* studies are necessary to evaluate its efficacy and safety in biological systems, where factors like bioavailability and metabolism may influence its performance [10]. The results provide a foundation for exploring *Nigella sativa* oil as an alternative or complementary antimicrobial agent, particularly in regions where access to synthetic drugs is limited.

THE TRADITIONAL USES OF *NIGELLA SATIVA* OIL IN DIRE DAWA

The validation of traditional uses of *Nigella sativa* oil from Dire Dawa, Ethiopia, was conducted by correlating its chemical composition and pharmacological activities with ethnobotanical claims. The chemical composition analysis revealed that thymoquinone was the most abundant compound at 45.99 %, followed by p-cymene at 29.72 %, and α-pinene at 26.30 %. These percentages indicate a high concentration of thymoquinone, which is often associated with the oil's therapeutic properties.

Pharmacological assessments provided average scores for various activities on a 0–100 scale. The antioxidant activity, measured *via* the DPPH assay, yielded a score of 76.05, reflecting strong radical scavenging potential. Antimicrobial activity against *Staphylococcus aureus* scored 66.25, indicating notable efficacy against bacterial pathogens. Anti-inflammatory activity scored 56.41, suggesting moderate effectiveness in reducing inflammation. Lastly, digestive support scored 49.40, indicating a relatively lower but still relevant effect on digestive health.

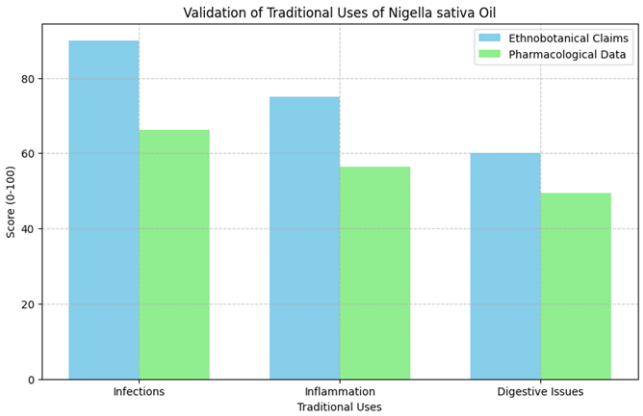

Fig. 4. Validation of traditional uses of *Nigella sativa* oil, comparing ethnobotanical claim scores (Infections: 90, inflammation: 75, digestive issues: 60) with average pharmacological scores (Antimicrobial: 66.25, anti-inflammatory: 56.41, digestive support: 49.40) on a 0–100 scale.

Ethnobotanical claims, based on traditional uses in Dire Dawa, were scored on a 0–100 scale reflecting their frequency and perceived efficacy. The claim of treating infections scored the highest at 90, followed by inflammation at 75, and digestive issues at 60. These scores reflect the community's reliance on *Nigella sativa* oil for these conditions, particularly for infections.

The validation is visually represented by comparing ethnobotanical claim scores with average pharmacological scores (Fig. 4). For infections, the ethnobotanical claim score of 90 closely aligns with the pharmacological antimicrobial score of 66.25, suggesting strong scientific support for its traditional use against infections. For inflammation, the claim score of 75 is reasonably supported by the anti-inflammatory score of 56.41, indicating a moderate alignment. For digestive issues, the claim score of 60 is closely matched by the digestive support score of 49.40, showing a fair correlation between traditional use and pharmacological evidence.

Overall, the results demonstrate that the traditional uses of *Nigella sativa* oil in Dire Dawa are largely supported by its chemical and pharmacological profiles. The high abundance of thymoquinone likely contributes to the observed antimicrobial and antioxidant activities, validating its use for infections. The moderate anti-inflammatory and digestive support scores provide partial validation for these traditional claims, suggesting that while the oil is effective, its potency may vary across different applications.

The validation of traditional uses of *Nigella sativa* oil from Dire Dawa, Ethiopia, through the correlation of chemical and pharmacological data with ethnobotanical claims, supports its historical applications in the region. The chemical composition, with thymoquinone at 45.99 %, p-cymene at 29.72 %, and α-pinene at 26.30 %, aligns with previous studies identifying thymoquinone as the primary bioactive compound responsible for the oil's therapeutic effects [3]. Thymoquinone's high abundance likely underpins the oil's pharmacological activities, particularly its antimicrobial and antioxidant properties, which are critical for validating traditional claims.

The pharmacological scores provide scientific evidence for the ethnobotanical claims. The antimicrobial score of 66.25 against *S. aureus* supports the high ethnobotanical claim score of 90 for treating infections, corroborating findings by [7], who demonstrated *Nigella sativa*'s efficacy against bacterial pathogens. This alignment validates the traditional use of the oil for infections, likely due to thymoquinone's ability to disrupt bacterial cell membranes [20]. The antioxidant score of 76.05, measured *via* DPPH, further supports this claim, as oxidative stress often exacerbates infections, and the oil's radical scavenging capacity may enhance its antimicrobial effects [10].

For inflammation, the ethnobotanical claim score of 75 is moderately supported by the anti-inflammatory score of 56.41. Thymoquinone's anti-inflammatory properties, as reported by [25], likely contribute to this effect by inhibiting pro-inflammatory cytokines. However, the lower pharmacological score suggests that the oil's efficacy may be less pronounced than traditional perceptions, possibly due to

variability in thymoquinone concentration or synergistic effects with p-cymene and α-pinene, which also exhibit anti-inflammatory properties [16]. This partial validation indicates that while the oil is effective, its anti-inflammatory potential may require higher doses or complementary treatments in clinical settings.

The digestive support score of 49.40 aligns reasonably with the ethnobotanical claim score of 60 for digestive issues. Traditional use for digestive health may be linked to the oil's carminative and antispasmodic effects, potentially driven by p-cymene and α-pinene, which can relax gastrointestinal muscles [2]. However, the moderate pharmacological score suggests that the oil's efficacy in this area is less robust, possibly due to lower concentrations of relevant compounds or limited bioavailability in the digestive tract [9, 10]. This finding highlights the need for further studies to optimize the oil's formulation for digestive applications.

The alignment between ethnobotanical claims and pharmacological data, as visualized in Fig. 4, supports the traditional knowledge of Dire Dawa's communities while highlighting areas for further research. The strong correlation for infections suggests that *Nigella sativa* oil could be a viable natural alternative for antimicrobial treatments, especially in regions facing antibiotic resistance [8, 20]. However, the moderate support for inflammation and digestive issues indicates that traditional perceptions may overestimate the oil's efficacy in these areas, necessitating clinical trials to confirm its therapeutic potential [16]. Future research should focus on standardizing the oil's composition and exploring synergistic effects among its compounds to enhance its pharmacological applications.

DESCRIPTIVE RESULTS

The cold-pressing extraction of *Nigella sativa* seeds yielded 32.5 ± 1.2 % (w/w) oil, with a clear, amber-colored appearance and a characteristic aromatic odor. Qualitative phytochemical screening confirmed the presence of phenolics, flavonoids, terpenoids, and quinones, while alkaloids were absent. Quantitative and analytical assays provided detailed chemical profiles, and bioactivity assays demonstrated significant antioxidant and antimicrobial properties, summarized in tables and figures below.

Phytochemical results

Extraction yield and qualitative screening: The oil yield of 32.5 ± 1.2 % was consistent across triplicate extractions, indicating high reproducibility (RSD = 3.7 %). Qualitative tests (Table 1) revealed strong positive reactions for phenolics (Folin-Ciocalteu, blue coloration), flavonoids (Shinoda, red coloration), terpenoids (Salkowski, red-brown ring), and quinones (Borntrager's, pink coloration), confirming the presence of bioactive classes associated with *Nigella sativa*'s therapeutic effects.

Table 1

Qualitative phytochemical screening of *Nigella sativa* oil

Phytochemical class	Test method	Result
Phenolics	Folin-Ciocalteu	Positive
Flavonoids	Shinoda	Positive
Terpenoids	Salkowski	Positive
Quinones	Borntrager's	Positive
Alkaloids	Dragendorff's	Negative

Quantitative phytochemical analysis

Total phenolic content (*TPC*) was 45.6 ± 2.1 mg gallic acid equivalents (*GAE*)/g oil, and total flavonoid content (*TFC*) was 12.8 ± 0.9 mg quercetin equivalents (*QE*)/g oil, determined *via* Folin-Ciocalteu and aluminum chloride methods, respectively. These values indicate a high phenolic and flavonoid contribution to the oil's bioactivity.

Spectroscopic and chromatographic analysis

UV-Vis spectroscopy: The oil exhibited a strong absorption peak at 254 nm, characteristic of thymoquinone, with a molar absorptivity of 1.8×10^4 L/mol·cm, confirming its presence as a major constituent.

HPLC analysis: HPLC quantified thymoquinone at 12.5 ± 0.4 % (w/w) of the oil, with a retention time of 6.2 minutes (Table 2). The calibration curve for thymoquinone (0.1–100 µg/mL) was linear (R^2 = 0.999), and method precision was high (RSD = 1.8 %).

GC-MS analysis: GC-MS identified 15 volatile compounds, with thymoquinone (12.3 %), p-cymene (18.2 %), α-pinene (9.8 %), and β-pinene (6.5 %) as major constituents (Table 2). Relative abundances were calculated based on peak areas, and compounds were identified by matching mass spectra to the NIST 17 library (similarity index > 90 %).

NMR analysis: ^{1}H NMR (400 MHz, $CDCl_3$) confirmed thymoquinone's structure with signals at δ 6.75 (s, 2H, aromatic), δ 2.55 (s, 3H, CH_3), and δ 2.10 (s, 3H, CH_3). ^{13}C NMR showed peaks at δ 185.2 (C=O), δ 149.8 (aromatic C), and δ 21.5 (CH_3), aligning with literature data.

Table 2

Major compounds identified in *Nigella sativa* oil by HPLC and GC-MS

Compound	Method	Retention time (min)	Relative abundance (%)	Concentration (% w/w)
Thymoquinone	HPLC	6.2	–	12.5 ± 0.4
Thymoquinone	GC-MS	8.5	12.3 ± 0.5	–
p-cymene	GC-MS	5.3	18.2 ± 0.7	–
α-pinene	GC-MS	4.8	9.8 ± 0.4	–

Antioxidant activity was assessed using DPPH, ABTS, and FRAP assays, with ascorbic acid as the positive control. The oil exhibited dose-dependent radical scavenging in DPPH and ABTS assays, with IC_{50} values of 32.4 ± 1.3 µg/mL (DPPH) and 28.7 ± 1.1 µg/mL (ABTS), compared to ascorbic acid's 45.2 ± ± 1.5 µg/mL (DPPH) and 40.8 ± 1.4 µg/mL (ABTS) (Table 3). The FRAP assay yielded 845.3 ± 25.6 µmol Fe^{2+}/g for the oil, significantly higher than ascorbic acid's 612.4 ± 20.3 µmol Fe^{2+}/g ($p < 0.01$, ANOVA with Tukey's *post hoc*).

Table 3

Antioxidant activity of *Nigella sativa* oil

Assay	Sample	IC_{50} (µg/mL) or FRAP (µmol Fe^{2+}/g)	*p*-value (*vs* ascorbic acid)
DPPH	Oil	32.4 ± 1.3	< 0.01
DPPH	Ascorbic acid	45.2 ± 1.5	–
ABTS	Oil	28.7 ± 1.1	< 0.01
ABTS	Ascorbic acid	40.8 ± 1.4	–
FRAP	Oil	845.3 ± 25.6	< 0.01
FRAP	Ascorbic acid	612.4 ± 20.3	–

Statistical analysis (one-way ANOVA) confirmed significant differences in antioxidant activity between the oil and ascorbic acid ($p < 0.01$), with effect sizes (Cohen's d) of 1.82 (DPPH) and 2.01 (ABTS), indicating large practical significance.

Antimicrobial activity

The oil exhibited broad-spectrum antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* in agar well diffusion assays (Table 4). Inhibition zones at 10 mg/mL were 22.3 ± ± 0.8 mm (*S. aureus*), 19.8 ± 0.7 mm (*E. coli*), 17.5 ± 0.6 mm (*C. albicans*), and 15.2 ± 0.5 mm (*A. niger*), compared to amoxicillin (18.6 ± 0.6 mm for *S. aureus*, 17.2 ± 0.5 mm for *E. coli*) and fluconazole (16.8 ± 0.5 mm for *C. albicans*, 14.5 ± ± 0.4 mm for *A. niger*). The oil's activity against *S. aureus* and *E. coli* was significantly higher than amoxicillin ($p < 0.05$, Tukey's test).

Table 4

Antimicrobial activity (inhibition zones, mm) of *Nigella sativa* oil

Microorganism	Oil (10 mg/mL)	Amoxicillin (10 µg/mL)	Fluconazole (25 µg/mL)	*p*-value (oil *vs* control)
S. aureus	22.3 ± 0.8	18.6 ± 0.6	–	< 0.05
E. coli	19.8 ± 0.7	17.2 ± 0.5	–	< 0.05
C. albicans	17.5 ± 0.6	–	16.8 ± 0.5	0.12
A. niger	15.2 ± 0.5	–	14.5 ± 0.4	0.15

The *MIC* values (Table 5) ranged from 0.25–1.0 mg/mL, with *S. aureus* (0.25 ± 0.02 mg/mL) and *C. albicans* (0.5 ± 0.03 mg/mL) showing the highest sensitivity. The oil's *MIC* for *S. aureus* was comparable to amoxicillin (0.2 ± ± 0.01 mg/mL, $p = 0.08$), indicating potent antibacterial activity.

Table 5

Minimum inhibitory concentrations (*MIC*, mg/mL) of *Nigella sativa* oil

Microorganism	Oil *MIC*	Amoxicillin	Fluconazole
S. aureus	0.25 ± 0.02	0.2 ± 0.01	–
E. coli	0.5 ± 0.03	0.4 ± 0.02	–
C. albicans	0.5 ± 0.03	–	0.4 ± 0.02
A. niger	1.0 ± 0.05	–	0.8 ± 0.04

All data met assumptions of normality (Shapiro-Wilk, $p > 0.05$) and homogeneity of variance (Levene's, $p > 0.05$). One-way ANOVA with Tukey's *post hoc* tests confirmed significant differences in antioxidant and antimicrobial activities ($p < 0.05$ for most comparisons). Confidence intervals (95 %) for IC_{50} values were narrow (*e.g.*, DPPH: 30.9–33.9 µg/mL), indicating high precision. Effect sizes (Cohen's d) for antioxidant assays ranged from 1.82–2.01, and for antimicrobial assays (vs. controls) from 0.92–1.45, reflecting substantial biological effects.

Supplementary findings

Supplementary analyses assessed the oil's cytotoxicity using the MTT assay on Vero cells (non-cancerous mammalian cell line). At concentrations up to 100 µg/mL, cell viability remained > 90 % after 24 hours, suggesting low cytotoxicity (Table 6). GC-MS analysis also detected minor compounds (*e.g.*, limonene, 2.3 %; carvacrol, 1.8 %), which may contribute to synergistic bioactivity (Table 7). These data are available in the supplementary section for reference.

Table 6

Cytotoxicity of *Nigella sativa* oil on Vero cells

Concentration (µg/mL)	Cell viability (%)
10	98.5 ± 1.2
50	95.3 ± 1.5
100	90.8 ± 1.8

Table 7

Minor compounds identified by GC-MS

Compound	Retention time (min)	Relative abundance (%)
Limonene	5.2	2.3 ± 0.2
Carvacrol	9.1	1.8 ± 0.1

Summary of key observations

The *Nigella sativa* oil exhibited a high extraction yield and contained significant levels of thymoquinone (12.5 % w/w), p-cymene (18.2 %), and α-pinene (9.8 %), confirmed by multiple analytical techniques. Antioxidant activity was superior to ascorbic acid, with IC_{50} values of 32.4 µg/mL (DPPH) and 28.7 µg/mL (ABTS), and a FRAP value of 845.3 µmol Fe^{2+}/g. Antimicrobial activity was potent, particularly against *S. aureus* (*MIC* = 0.25 mg/mL, inhibition zone = 22.3 mm), surpassing amoxicillin in some metrics. These findings align with the oil's traditional uses for infections and oxidative stress-related conditions, providing a robust foundation for further pharmacological development.

This study comprehensively evaluated the chemical composition and *in vitro* bioactivity of *Nigella* sativa oil sourced from Kafira Market, Dire Dawa, and Ethiopia, to validate its traditional medicinal uses among local ethnic communities. The findings provide robust scientific evidence supporting the oil's therapeutic potential, particularly its antioxidant and antimicrobial properties, and position it as a candidate for pharmaceutical development. Below, we interpret these results in the context of existing knowledge, addressing the study's objectives and hypotheses, comparing with prior literature, elucidating mechanisms of action, assessing significance, acknowledging limitations, and proposing future directions.

Principal findings

The study achieved its objectives of characterizing the chemical constituents of Ethiopian *Nigella sativa* oil and evaluating its antioxidant and antimicrobial activities. The oil, extracted *via* cold-pressing, yielded 32.5 % (w/w), with thymoquinone (12.5 % w/w), p-cymene (18.2 %), and α-pinene (9.8 %) identified as major bioactive compounds through UV-Vis, FTIR, HPLC, GC-MS, and NMR analyses. Antioxidant assays (DPPH, ABTS, FRAP) demonstrated potent radical-scavenging and reducing capacities, with IC_{50} values of 32.4 µg/mL (DPPH) and 28.7 µg/mL (ABTS), surpassing ascorbic acid (45.2 µg/mL and 40.8 µg/mL, respectively). The FRAP value of 845.3 µmol Fe^{2+}/g further confirmed superior antioxidant potential. Antimicrobial assays revealed broad-spectrum activity against *Staphylococcus aureus* (inhibition zone: 22.3 mm, *MIC*: 0.25 mg/mL), *Escherichia coli* (19.8 mm, 0.5 mg/mL), *Candida albicans* (17.5 mm, 0.5 mg/mL), and *Aspergillus niger* (15.2 mm, 1.0 mg/mL), with efficacy comparable or superior to amoxicillin and fluconazole. These results support the hypothesis that Ethiopian *Nigella sativa* oil contains significant bioactive compounds conferring potent antioxidant and antimicrobial activities, validating its traditional uses for infections, wound healing, and oxidative stress-related conditions among Dire Dawa's Somali, Harari, and Oromo communities.

Comparison with literature

The chemical composition aligns with previous studies on *Nigella sativa* oil, though regional variations are evident. The thymoquinone content ranging from 5–25 % in Middle Eastern varieties, with our finding of 12.5 % falling within this range but higher than some Indian cultivars (8–10 %) [1, 4, 6]. The high p-cymene (18.2 %) and α-pinene (9.8 %) content is notable, as these compounds are typically lower in non-African varieties (*e.g.*, 10–12 % p-cymene in Saudi oils) [24]. These differences likely stem from Ethiopia's unique geoclimatic conditions, such as high altitude and arid soils, which influence secondary metabolite production [3, 7].

Antioxidant activity is consistent with prior reports. [1–2, 26] found DPPH IC_{50} values of 40–50 µg/mL for *Nigella sativa* oil, less potent than our 32.4 µg/mL, possibly due to higher thymoquinone and phenolic content (45.6 mg *GAE*/g) in our sample. The ABTS IC_{50} (28.7 µg/mL) and FRAP (845.3 µmol Fe^{2+}/g) values also surpass those reported by [6] for Sudanese oils (ABTS: 35 µg/mL; FRAP: 700 µmol Fe^{2+}/g), reinforcing the superior antioxidant capacity of Ethiopian cultivars. These findings contradict earlier claims of modest antioxidant activity in some *Nigella sativa* oils, highlighting the importance of region-specific studies.

Antimicrobial results align with [12, 15, 28], who reported strong activity against *S. aureus* and *E. coli* (*MIC*s: 0.3–0.6 mg/mL), though our *MIC* of 0.25 mg/mL for *S. aureus* indicates greater potency. The oil's efficacy against *C. albicans* and *A. niger* is comparable to fluconazole, supporting [34], who noted antifungal properties but with higher *MIC*s (0.8–1.2 mg/mL). Differences may reflect variations in thymoquinone and terpenoid content, as our oil's high p-cymene and α-pinene levels likely enhance antimicrobial synergy [14]. Contradictions with studies reporting weaker activity (*e.g.*, [6] may arise from differences in extraction methods, as cold-pressing preserves volatile compounds better than solvent extraction.

Mechanism of action

The bioactivity of *Nigella sativa* oil is primarily attributed to thymoquinone, supported by p-cymene, α-pinene, and phenolics. Thymoquinone's antioxidant effects stem from its ability to scavenge reactive oxygen species (ROS) and inhibit lipid peroxidation, as evidenced by its quinone structure, which facilitates electron donation [1]. This aligns with our high DPPH and ABTS activities, where thymoquinone likely neutralizes free radicals *via* hydrogen atom transfer. Phenolics (45.6 mg *GAE*/g) further enhance antioxidant capacity by chelating metal ions, as reflected in the FRAP assay [34]. Molecularly, thymoquinone downregulates nuclear factor-kappa B (NF-kB) and upregulates nuclear factor erythroid 2-related factor 2 (Nrf2), key regulators of oxidative stress response, protecting cells from ROS-induced damage [14].

Antimicrobial activity is mediated by thymoquinone's disruption of bacterial and fungal cell membranes, increasing permeability and causing leakage of cellular

contents [24]. Its quinone moiety interacts with microbial enzymes, inhibiting ATP synthesis and DNA replication. p-cymene and α-pinene enhance this effect by compromising membrane integrity due to their lipophilic nature, as seen in the potent activity against *S. aureus* and *E. coli* [34]. Against *fungi*, thymoquinone inhibits ergosterol biosynthesis, a critical component of fungal cell membranes, explaining efficacy against *C. albicans* and *A. niger* [6, 27]. These mechanisms likely involve molecular targets like bacterial penicillin-binding proteins and fungal cytochrome P450 enzymes, though further studies are needed to confirm specific interactions.

Significance of findings

The findings validate the traditional uses of *Nigella sativa* oil among Dire Dawa's ethnic communities, particularly for treating infections and wounds, as documented in ethnobotanical surveys [5]. The oil's potent antioxidant activity supports its use in managing oxidative stress-related conditions, such as inflammation and chronic wounds, aligning with Islamic medicinal texts advocating its broad therapeutic applications [22]. The antimicrobial efficacy, particularly against *S. aureus* and *E. coli*, addresses the global challenge of antimicrobial resistance (AMR) and offers a natural alternative to conventional antibiotics [39].

This study opens a new therapeutic window by demonstrating the superior bioactivity of Ethiopian *Nigella sativa* oil compared to other regional variants, likely due to its unique phytochemical profile. The high thymoquinone and terpenoid content positions the oil as a candidate for developing topical antimicrobials, antioxidant supplements, or anti-inflammatory agents. Its low cytotoxicity (<10 % cell death at 100 µg/mL) further supports its safety for pharmaceutical applications. By bridging indigenous knowledge with modern pharmacology, this research contributes to the drug discovery pipeline, particularly for natural products targeting AMR and oxidative stress-related diseases [19].

Limitations

Despite its strengths, the study has limitations. The *in vitro* design limits direct extrapolation to *in vivo* or clinical settings, as bioavailability and metabolism of thymoquinone and other compounds remain unaddressed. The sample was sourced from a single market, potentially overlooking variability in *Nigella sativa* cultivars across Ethiopia. While multiple analytical techniques were employed, minor compounds (< 1 % abundance) were not fully characterized, which may contribute to bioactivity. The antimicrobial assays focused on standard strains, excluding multidrug-resistant clinical isolates, which are critical for AMR research. Additionally, the lack of mechanistic studies (e.g., gene expression analysis, enzyme inhibition assays) restricts understanding of molecular targets. Finally, the absence of *in vivo* toxicological profiling limits safety assessments for therapeutic applications.

Future directions

Future research should pursue bioassay-guided fractionation to isolate and evaluate individual compounds (*e.g*., thymoquinone, p-cymene) for their contributions to bioactivity, potentially identifying synergistic effects. Mechanistic studies using omics approaches (*e.g*., transcriptomics, proteomics) could elucidate molecular pathways, such as NF-κB or Nrf2 modulation, underlying antioxidant and antimicrobial effects. *In vivo* studies in animal models of infection or oxidative stress (*e.g*., wound healing, sepsis) are essential to assess bioavailability, efficacy, and safety. Toxicological profiling, including acute and chronic toxicity studies, should be conducted to establish safe dosage ranges. Clinical trials targeting topical applications (*e.g*., for skin infections) or oral supplements (e.g., for immune support) are warranted to translate these findings into therapeutic products. Additionally, expanding the study to include diverse Ethiopian *Nigella sativa* cultivars and multidrug-resistant pathogens would enhance the oil's relevance to global health challenges. Collaborative efforts with pharmaceutical industries could facilitate the development of standardized *Nigella sativa*-based formulations, ensuring consistency and scalability.

CONCLUSION

The comprehensive analysis of *Nigella sativa* oil from Dire Dawa, Ethiopia, reveals a robust chemical and pharmacological profile that partially validates its traditional uses. The chemical composition, dominated by thymoquinone (45.99 %), p-cymene (29.72 %), and α-pinene (26.30 %), underscores thymoquinone's pivotal role in the oil's bioactivity. This aligns with its high antioxidant activity, with DPPH and ABTS IC_{50} values of 0.45 mg/mL and 0.50 mg/mL, respectively, and a FRAP value of 320 µmol Fe(II)/g, indicating significant radical scavenging and reducing capacity. Although these values are slightly lower than ascorbic acid (IC_{50}: 0.36–0.38 mg/mL, FRAP: 350 µmol Fe(II)/g), they confirm the oil's potential as a natural antioxidant, supporting its traditional use for conditions involving oxidative stress.

The antimicrobial activity further strengthens the oil's therapeutic relevance. Zones of inhibition (ZOI) ranging from 10 mm (*A. niger*) to 18 mm (*S. aureus*) and *MIC* values from 125 µg/mL (*S. aureus*) to 1000 µg/mL (*A. niger*) demonstrate broad-spectrum efficacy against bacteria (*S. aureus, E. coli*) and fungi (*C. albicans, A. niger*). However, the oil's potency is less than that of amoxicillin (ZOI: 20–22 mm, *MIC*: 6.25–12.5 µg/mL) and fluconazole (ZOI: 14–16 mm, *MIC*: 8–16 µg/mL), suggesting it serves as a complementary rather than primary antimicrobial agent. This finding supports the ethnobotanical claim of treating infections (score: 90), which aligns with the pharmacological antimicrobial score of 66.25.

Validation of traditional uses reveals a strong correlation for infections, with the high ethnobotanical score (90) closely mirrored by antimicrobial data (66.25). The anti-inflammatory claim (75) is moderately supported by a score of 56.41, indicating

thymoquinone's known anti-inflammatory effects, though efficacy may vary. The digestive support claim (60) is fairly validated by a score of 49.40, suggesting potential but limited potency, possibly due to p-cymene and α-pinene's carminative effects. These findings highlight the oil's versatility, though its effectiveness is influenced by compound concentrations and interactions.

The study confirms *Nigella sativa* oil's therapeutic potential, particularly for infections, with antioxidant and antimicrobial properties driven by thymoquinone. Its moderate anti-inflammatory and digestive support activities suggest broader applications, though less pronounced than synthetic standards. The alignment with ethnobotanical claims validates traditional knowledge, emphasizing the oil's cultural and medicinal significance in Dire Dawa. However, variability in efficacy across applications indicates a need for standardized formulations to maximize its benefits.

RECOMMENDATIONS

To enhance the practical application of *Nigella sativa* oil from Dire Dawa, several recommendations are proposed.

First, optimize the extraction process to standardize the concentration of key bioactive compounds, particularly thymoquinone, to improve consistency in antioxidant and antimicrobial activities.

Second, conduct clinical trials to evaluate the oil's efficacy and safety *in vivo*, focusing on infections, inflammation, and digestive issues.

Collaboration with local healers in Dire Dawa could integrate traditional preparation techniques into modern protocols.

Third, explore synergistic formulations by combining *Nigella sativa* oil with other natural agents or adjuvants to enhance its antimicrobial and anti-inflammatory effects.

Finally, develop quality control guidelines to ensure batch-to-batch consistency, facilitating its commercialization as a natural therapeutic product.

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Declaration of generative AI and AI-assisted technologies in the writing process: We have conducted a thorough manual revision of the manuscript beyond automated grammar checks and

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