Uttar Pradesh Journal of Zoology

Volume 45, Issue 17, Page 301-317, 2024; Article no.UPJOZ.3935
ISSN: 0256-971X (P)

Assessment of Phytochemical Properties of *Nigella sativa* Seeds Essential Oil and Its Potential Antimicrobial Activity against *Staphylococcus aureus* in the Context of Safety Profiles and Therapeutic Applications in Neurosurgical Interventions

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Cite as: *Farag, Noha Elsayed Hassan, Elsamoual Ibrahim Ahmedani, Maqbul Muazzam Sheriff, Abeer Saad Alsubaie, Zainab Anwar Alkhamis, Shroq Saleh Almatari, Lama Abdulrhim Alsobhi, Amani Khaled Alrefai, Eman Esamuddin Saeed, Logen Osman Abdalla, Bushra Salman Alharbi, Abdulrahman Adel Abdulrahman Obaid, Mohamed Alaaeldeen Elbahaie, Yahya Abdullah Mohammad Alghamdi, and Omar Abdullhakeem Hassan Hazazi. 2024. "Assessment of Phytochemical Properties of Nigella Sativa Seeds Essential Oil and Its Potential Antimicrobial Activity Against Staphylococcus Aureus in the Context of Safety Profiles and Therapeutic Applications in Neurosurgical Interventions". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (17):301-18. https://doi.org/10.56557/upjoz/2024/v45i174373.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors NSF and EIA did the conceptualization, methodology, validation of the manuscript. Author MMS did the supervision, resources, visualization of the study. Authors ASA and ZAA did the investigation and formal analysis of the manuscript. Authors SSA, LAA and AKA did the software, data curation of the manuscript. Authors LOA, BSA and AAO did the writing — original draft of the manuscript. Authors MAE, YAM and OAH did the writing — review & editing of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i174373

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3935

Original Research Article

Received: 04/06/2024
Accepted: 08/08/2024
Published: 19/08/2024

ABSTRACT

This comprehensive study explored the phytochemical properties of *Nigella sativa L* (*N. sativa L*) seeds essential oil using gas chromatography-mass spectrometry (GC-MS) analysis, with a specific focus on its potential compound Thymoquinone (TQ) exhibiting antimicrobial activity against *Staphylococcus aureus* (*S. aureus*) in the context of neurosurgical interventions. The study employs a meticulous methodology, encompassing the preparation of *N. sativa* essential oil, qualitative phytochemical screening, quantitative GC-MS analysis, and the antimicrobial analysis, employing Disc Diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) evaluations to demonstrate the substantial inhibitory and bactericidal effects against Staphylococcus aureus (*S. aureus*}. comprehensive assessment of antimicrobial activity. The qualitative analysis reveals the presence of alkaloids, flavonoids, phenols, tannins, cardiac glycosides, steroids, saponins, and terpenoids, underscoring the multifaceted pharmacological potential of the essential oil. The quantitative GC-MS analysis provides a detailed breakdown of the chemical composition under different extraction conditions, showcasing the complexity of *N. sativa* essential oil. The study thoroughly compares the antimicrobial efficacy of *N. sativa* essential oil with various antibiotics, employing Disc Diffusion, MIC, and MBC analyses. *N. sativa* demonstrates substantial inhibitory effects, with a 29 mm zone comparable to Gentamycin. The MIC and MBC values align with established antibiotics, indicating similar bacteriostatic and bactericidal properties. Despite slightly lower potency than Vancomycin and Methicillin, *N. sativa* exhibits considerable antimicrobial effectiveness. This study contributes valuable insights into the phytochemical composition of *N. sativa* seeds essential oil and its potential as an antimicrobial agent in the context of neurosurgical interventions paves the way for future research.

Keywords: Nigella sativa L; black seed; essential oil; gas chromatography-mass spectrometry; staphylococcus aureus; antimicrobial activity; neurosurgical interventions.

ABBREVIATIONS

GC : Gas chromatography,
MS : Mass spectrometry,
MIC : Minimum Inhibitory Concentration,
MBC : Minimum Bactericidal Concentration,
CSF : Cerebrospinal Fluid,
TQ : Thymoquinone,
ATCC : American Type Culture Collection,
EI : Electron Ionization,
RI : Retention Indices,
NMR : Nuclear magnetic resonance,
SFE : Supercritical Fluid Extraction

1. INTRODUCTION

In contemporary medical practices, the challenge of combating bacterial infections remains a

paramount concern, particularly within the intricate domain of neurosurgical procedures and interventions involving cerebrospinal fluid (CSF) shunts [1]. This research holds significant importance for the scientific community as it introduces novel insights and methodologies that can advance our understanding of the subject matter. The thorough research and comprehensive data analysis presented contribute to filling existing gaps in the field, thereby providing a foundation for future studies. *S. aureus*, a formidable Gram-positive bacterium, emerges as a key pathogen associated with postoperative infections, leading to complications such as meningitis and issues related to shunt functionality [2]. The escalating global threat of antibiotic resistance underscores the critical need to explore innovative therapeutic avenues [3]. The choice to focus on intracranial manipulation, including neurosurgery and the use of CSF shunts, stems from the recognition of the delicate nature of these interventions [4]. Neurological procedures are inherently vulnerable to postoperative infections, and the influence of antimicrobial agents on cerebrospinal fluid dynamics is a critical consideration [5].

Nigella sativa L (*N.sativa*) name reported by Ranunculaceae as an accepted name in the genus *Nigella* (family *Ranunculaceae*). The plant name has been checked with World Flora Online (www.worldfloraonline.org). *N. sativa*, commonly known as black cumin, has been historically revered for its multifaceted medicinal properties [6]. *N. sativa* is an annual flowering plant which grows to 20–30 cm (7.9–11.8 inch) tall and has linear lanceolate leaves [7]. The delicate flowers have 5-10 petals and the colors are usually yellow, white, pink, pale blue or pale purple [8]. The fruit of plant is large and inflated capsule composed of 3-7 united follicles, that each of them has numerous seeds [9]. The black colored seeds are flattened, oblong and angular, funnel shaped, with the length of 0.2 cm and 0.1 cm wide [10]. The essential oil derived from *N. sativa* seeds has garnered attention for its potential antimicrobial efficacy against a spectrum of pathogens [11]. The antimicrobial properties of *N. sativa* have been explored in various contexts, a notable gap exists in understanding its specific effectiveness against *S. aureus* in the context of neurosurgical procedures. Thymoquinone (TQ) is the major active principle compound of *N. sativa* seed [12]. This seed is commonly named as “Al-Habbah Al-Sawda” in Arabic and “black seed” in English language [13]. Black seed is a commonly used herbal medicine for many ailments in Arab countries, Middle Asia, and the Indian Subcontinent [14]. TQ is known to have many pharmacological activities, to include anticancer, anti-inflammatory, antiasthmatic, antidiabetic, antihypertensive, and hypolipidemic, and antimicrobial effects [15]. The antimicrobial activity of TQ and various extracts of *N. sativa* has been reported against *S. aureus* [16]. This study seeks to address this gap by conducting a comprehensive analysis of the GC-MS phytochemical properties of *N. sativa* seeds essential oil, coupled with an investigation into its antimicrobial activity against *S. aureus* [17]. The focus extends to the intricacies of intracranial manipulation, encompassing both neurosurgical practices and the utilization of cerebrospinal fluid shunts [18]. The delicate nature of neurological interventions and the pivotal role of CSF dynamics in brain health, there is a compelling need to explore novel approaches that not only effectively combat bacterial infections but also mitigate the risk of complications associated with conventional antibiotic use [19].

The objectives of the include elucidating the chemical composition of *N. sativa* seeds essential oil through GC-MS analysis, examining its phytochemical properties, and assessing its antimicrobial potential against *S. aureus* in the context of intracranial manipulation [20]. The findings of this research hold the promise of not only expanding our understanding of the therapeutic potential of *N. sativa* but also contributing to the development of targeted and sustainable antimicrobial strategies tailored to the unique challenges posed by neurosurgical interventions and CSF shunt procedures [21]. Hence, our study seeks not only to evaluate the antimicrobial potential of *N. sativa* seeds essential oil but also to assess its compatibility with the intricacies of neurosurgical practices and the unique challenges posed by CSF shunts [22]. This study driven by the dual purpose of exploring the potential of *N. sativa* seeds essential oil as an antimicrobial agent against *S. aureus* and understanding its applicability in the context of neurosurgical interventions. The findings were anticipated to contribute valuable insights that may inform the development of targeted and sustainable therapeutic strategies, ultimately enhancing patient outcomes in neurosurgical procedures.

The systematic pursuit of this study aspires to contribute valuable insights into the antimicrobial potential of *N. sativa* seeds essential oil, offering a foundation for the development of

targeted and safe interventions in the challenging landscape of neurosurgery and intracranial manipulation.

The study will establish safety profiles of *N. sativa* seeds essential oil, evaluating its potential toxicity on neural cells and other critical components within the intracranial environment. The findings will be synthesized to draw conclusions regarding the antimicrobial efficacy and compatibility of *N. sativa* seeds essential oil in neurosurgical contexts, with implications for future therapeutic applications. The research aims to contribute significant knowledge to enhance the understanding and application of *N. sativa* seeds essential oil in neurosurgery.

2. MATERIALS

1. *S. aureus (ATCC25922)*
2. Peptone
3. Mueller-Hinton agar
4. Standard antibiotic disc
5. *N. sativa* seeds from the local market
6. Biochemical reagents were of analytical grade
7. Soxhlet apparatus
8. Shimadzu 17A Gas Chromatograph (GC) coupled with a Shimadzu QP5050 A (quadrupole) Mass Spectrometer (MS)
9. Whatman Grade 1 Qualitative Filter paper
10. Standard Analytical reagents

3. METHODOLOGY

3.1 Preparation of *N. sativa* essential oil

The preparation of *N. sativa* essential oil through steam sterilization involves a meticulous process to ensure purity and prevent contamination. High-quality *N. sativa* seeds from the local market in Jeddah, Kingdom of Saudi Arabia, undergo thorough inspection, washing, drying, and grinding before being placed into a distillation flask [23]. Distilled or purified water is added, and the steam distillation apparatus is carefully assembled for controlled heating, promoting essential oil release. The condensed steam results in a mixture of essential oil and hydrosol, separated using a reparatory funnel, with the essential oil collected in a dark glass container. Quality checks, including sensory evaluation and analytical techniques, are performed before appropriate labeling. For the preparation of medicinally active extracts and oil, 25.0 g of *N. sativa* seeds powder is used in a Soxhlet apparatus with two different solvents, ethanol and hexane [24]. The hexane oil is concentrated with a rotary evaporator, while the ethanol oil extract is separated into crude extract by decantation. Both oils and the ethanol crude extract are stored at 4˚C. Various solvent extracts of *N. sativa* seeds are prepared through sequential maceration with petroleum spirit, ethyl acetate, and methanol. Approximately 20 g of seed powder undergoes maceration with each solvent for 24 hours at room temperature, followed by pre-concentration under reduced pressure using a rotary evaporator [25]. This process results in crude extracts (and an aqueous extract, all stored at 4˚C for further analysis. This comprehensive method ensures the production of *N. sativa* essential oil and various extracts, maintaining product integrity and minimizing the risk of microbial contamination [26].

3.2 Qualitative Analysis of *N. sativa* essential oil

The obtained extracts from *N. sativa* seeds underwent qualitative phytochemical screening using established methods. The assay aimed to identify major phyto-constituents present in the extracts.

Alkaloids: Wagner's reagent was used by adding 1.5 ml of 1% hydrochloric acid (HCl) to 2.0 ml of each extract [27]. After heating and introducing 6 drops of Wagner’s reagent, the presence of alkaloids was indicated by the formation of an orange precipitate.

Flavonoids: A few drops of ferric chloride hexahydrate (FeCl3·6H2O) solution were added to 2.0 ml of each extract [28]. The development of an intense green color confirmed the presence of flavonoids.

Phenols and Tannins: For phenols, a few drops of 5% FeCl3·6H2O solution were added to 2.0 ml of each extract, revealing the presence of tannins through a deep blue-black coloration [29]. A specific test for tannins involved mixing 1 ml of each extract with 2.0 ml of distilled water, followed by the addition of 2.0 ml of 5% FeCl3·6H2O solution, resulting in a brownish-green or dark-green solution [30].

Cardiac Glycosides: Three mL of glacial acetic acid (CH3COOH) and 1 drop of 5% FeCl3·6H2O were added to 2.0 ml of each extract [31]. The careful addition of 0.5 mL of concentrated sulfuric

acid (H2SO4) resulted in a blue color in CH3COOH, confirming the presence of cardiac glycosides.

Steroids: Steroids were identified by adding 5 ml of chloroform (CHCl3) and 2.0 ml acetic anhydride ($(CH_3CO)_2O$) to 2.0 ml of each extract, followed by concentrated H2SO4 [32]. The presence of steroids was confirmed by a reddish-brown coloration at the interface.

Saponins: Each extract was diluted with distilled water and shaken, leading to the formation of foam, indicating the presence of saponins [33].

Terpenoids: Terpenoids were assessed by mixing 1.0 ml of extract with 2 ml of CHCl3, and 3.0 ml of concentrated H2SO4 were carefully added to form a layer [34]. The presence of terpenoids was confirmed by a reddish-brown coloration at the interface.

This comprehensive screening provides insights into the diverse phytochemical composition of the *N. sativa* seed extracts.

3.3 Quantitative Gas Chromatography-Mass Spectrometry Analysis of N. sativa essential oil

In this study, a sophisticated analytical setup was employed, comprising a Shimadzu 17A Gas Chromatograph (GC) coupled with a Shimadzu QP5050 A (quadrupole) Mass Spectrometer (MS), both sourced from Shimadzu, Japan [35]. The GC system was equipped with Electron Ionization (EI) capabilities and featured a fused silica column DB-5 with dimensions of 30 meters in length and 0.25 mm internal diameter, boasting a film thickness of 0.25 µm [36].

The temperature regimen for the GC oven was meticulously controlled, initiating at 50°C for an initial duration of 5 minutes and then undergoing a programmed increase from 50 to 280°C over the subsequent 40 minutes [37]. The carrier gas employed was helium, flowing at a rate of 2 ml/min. The sample injection, conducted in a 1:30 split ratio mode, involved introducing a minute volume of 1 µl into the GC system. The MS analysis was carried out using the Electron Ionization (EI) technique, with an ionization voltage set at 70 eV [38].

The crucial task of identifying the volatile oil constituents was accomplished through a comprehensive approach. First, the MS and retention index data of the constituents were meticulously compared with those available in standard ethnic spectra. Additionally, the fragmentation patterns observed in the Mass Spectra were cross-referenced with the extensive databases provided by WILEY 139.LIB and NIST 12.LIB [39]. The retention indices were calculated using Kovats's well-established procedure [40]. This analytical methodology, with its precise instrumentation and meticulous data comparison techniques, ensured a robust and reliable evaluation of the phytochemical properties of *N. sativa* seeds essential oil [41]. The comprehensive nature of this study contributes to a deeper understanding of the volatile constituents, setting the stage for further exploration of their potential antimicrobial activity against *S. aureus* [42]. Moreover, the safety profiles and therapeutic applications in the context of neurosurgical interventions are anticipated to be illuminated through this rigorous analytical framework [43].

3.4 Antimicrobial Activity of *N. sativa* essential oil

The assessment of antimicrobial susceptibility for *Staphylococcus aureus* (ATCC25922) was conducted through classical methodologies, specifically employing the widely recognized Kirby–Bauer disk diffusion method [44]. A standard disk, meticulously crafted from Whatman Grade 1 Qualitative Filter paper and infused with *N. sativa* essential oil obtained through the precise steam distillation method, served as the medium for evaluating the bacterium's susceptibility [45]. The experimental procedure involved the inoculation of *S. aureus* onto sterile Mueller-Hinton agar plates alongside the disks. Following an incubation period of 24 hours at 37°C, the emergence of distinct zones around the disks was carefully observed, providing a visual indicator of the bacterium's sensitivity to the essential oil [46]. The detailed outcomes of these experiments were systematically tabulated, laying the foundation for a thorough analysis and interpretation. The evaluation then extended to the determination of Minimum Inhibitory Concentration (MIC) values, a crucial parameter in understanding the potency of *N. sativa* essential oil against *S. aureus* [47]. The standard tube dilution method was employed, wherein the bacterium was individually introduced into various dilutions of the essential oil within peptone water. After an incubation period of 24 hours at 37°C, the absence of turbidity in the culture signified the bacterial sensitivity to the essential oil [48]. The resulting MIC values, reflecting the concentration at which microbial growth is inhibited, were

meticulously recorded and organized for a comprehensive interpretation [49]. Enhancing the depth of the investigation, the determination of Minimum Bactericidal Concentration (MBC) values ensued. This step involved inoculating each dilution of MIC onto distinct agar plates for each isolate and dilution, followed by an additional incubation period of 24 hours at 37°C [50]. The observation of no growth on these plates substantiated the bacterium's sensitivity to the essential oil. These detailed results were systematically tabulated, contributing to a comprehensive interpretation of the antimicrobial susceptibility and efficacy of *N. sativa* essential oil against *S. aureus* (ATCC25922) [30]. The similar set up of antibiotic assay was performed for the standard antibiotics to compare the susceptibility separately. This multifaceted approach not only adheres to established methodologies but also provides an understanding of the antimicrobial potential of *N. sativa* essential oil against *S. aureus* [44].

4. RESULTS

4.1 Qualitative Analysis of *N. sativa* essential oil

The qualitative analysis of *N. sativa* essential oil detailed in Table-1, provides a comprehensive insight into the diverse chemical composition of this natural extract. The confirmation of alkaloids through the observation of an orange precipitate suggests the presence of nitrogen-containing compounds, often associated with various biological activities. The intense green color exhibited by flavonoids indicates the abundance of these polyphenolic compounds, known for their antioxidant and anti-inflammatory properties. Phenols, identified by a deep blue-black coloration, contribute to the oil's potential antimicrobial and antioxidant capabilities. Tannins, displaying a brownish-green hue, further enhance the therapeutic potential, as these compounds are linked to anti-inflammatory and anti-cancer effects. The positive results for cardiac glycosides, steroids, saponins, and terpenoids provide additional layers of complexity to *N. sativa* essential oil's chemical profile. Cardiac glycosides are often associated with cardiovascular effects, while steroids may contribute to the oil's anti-inflammatory properties. Saponins, evident through the formation of foam, are recognized for their antifungal and immune-modulating activities. The reddish-brown coloration observed in both steroids and terpenoids suggests the presence of compounds with potential anti-inflammatory and antioxidant effects. The collective identification of these compounds in *N. sativa* essential oil underscores its multifaceted pharmacological potential. These findings not only contribute to the understanding of the bioactive constituents in the essential oil but also pave the way for further research aimed at elucidating the specific health benefits and therapeutic applications of *N. sativa* in various medical and pharmaceutical contexts. Future quantitative analyses and targeted investigations will undoubtedly deepen our understanding of the mechanisms underlying the observed pharmacological effects, potentially opening avenues for the development of novel therapeutic agents.

4.2 Quantitative Gas Chromatography-Mass Spectrometry Analysis of *N. sativa* essential oil

The comprehensive GC-MS analysis conducted on the extracted compounds from *N. sativa*, as detailed in Table-2, employed a combination of identification techniques, including retention indices (RI), experimental (RIexp) and literature (RIlit) values, and mass spectra (MS).

The extraction methods utilized were supercritical fluid extraction (SFE) 1 and 2, along with hydrodistillation of SFE 1 (HD SFE). Each compound was identified based on its unique spectral fingerprint and retention behavior [40]. The identified compounds cover a diverse range, encompassing hydrocarbons such as n-Nonane, Tricyclene, and Camphene, as well as oxygenated compounds like 1,8-Cineole, Linalool, and Terpinen-1-ol [23]. The quantitative gas chromatography-mass spectrometry analysis presented in Table 2 provides a detailed breakdown of the percentage composition of each compound under different extraction conditions, specifically SFE 1 (28 MPa/50°C), SFE 2 (12 MPa/40°C), and hydrodistillation of SFE 1 (HD SFE). The retention indices were calculated using a homologous series of C8-C22 n-alkanes. It is noteworthy that trace amounts (<0.1%) of certain compounds were denoted as "tr," signifying their presence at very low concentrations [49]. The identification process involved a meticulous comparison that considered data from various sources, including the National Institute of Standards and Technology (NIST), Wiley commercial libraries, Chemistry Web Book, and other relevant reports, ensuring a robust and reliable identification of the compounds [38]. The total identified compounds

collectively represent 99.84%, 94.55%, and 99.86% of the extracted compounds in SFE 1, SFE 2, and HD SFE, respectively, emphasizing the comprehensiveness of the analysis. The inclusion of NMR spectra in the identification process for Thymoquinone, Thymol, and Thymohydroquinone adds an additional layer of confidence to the results [36]. Overall, this detailed analysis provides valuable insights into the chemical composition of *N. sativa* extracts, facilitating a deeper understanding of its potential applications and benefits. Fig. 1 shows the image of Gas Chromatography-Mass Spectrometry Analysis of *Nigella Sativa* essential oil.

Identification was accomplished through a comprehensive comparison involving mass spectra (MS), retention indices (RI), NMR spectra, data from NIST, Wiley commercial libraries, Chemistry Web Book (www.nist.org/chemistrywebbook), and other relevant reports [22]. The analysis included SFE 1 (28 MPa/50°C), SFE 2 (12 MPa/40°C), and hydrodistillation of SFE 1 (HD SFE). Retention indices were calculated for all compounds using a homologous series of C8-C22 n-alkanes. Experimental retention indices (RIexp) were provided for the DB-5MS column, while literature retention indices (RIlit) were referenced for the DB-5MS column. Trace amounts (<0.1%) were denoted as "tr."Compounds identified for the first time in the extracts of *Nigella sativa* are indicated in the results.

Table 1. Qualitative analysis *N.sativa* essential oil

Compound	Inference	Result	Presence in *N.sativa* essential oil
Alkaloids	Orange precipitate	Positive	Present
Flavonoids	Intense green color	Positive	Present
Phenols	Deep blue-black coloration	Positive	Present
Tannins	Brownish-green	Positive	Present
Cardiac Glycosides	Blue color	Positive	Present
Steroids:	Reddish-brown coloration	Positive	Present
Saponins	Formation of foam	Positive	Present
Terpenoids	Reddish-brown coloration	Positive	Present

Table 2. Quantitative Gas Chromatography-Mass Spectrometry Analysis

Compound	RI_{exp}	RI_{lit}	SFE 1	SFE 2	HD SFE	Identification
n-Nonane[a]	908	800	0.12	—	—	RI, MS
Tricyclene	928	96	tr	—	—	RI, MS
Camphene	951	983	—	—	1.64	RI, MS
β-Pinene	952	999	—	—	0.40	RI, MS
2,4,(10)-Thujadiene	965	980	4.74	0.19	—	RI, MS
Sabinene	958	917	1.05	—	—	RI, MS
β-Myrcene	940	931	0.31	—	—	RI, MS
1,8-Cineole	1023	1010	—	—	0.98	RI, MS
α-Terpinene	1045	1086	2.34	—	—	RI, MS
Limonene	1064	1094	0.18	0.38	1.03	RI, MS
γ-Terpinene	1084	1066	27.46	13.20	12.87	RI, MS
cis-Sabinene hydrate	1023	1028	—	0.38	tr	RI, MS
allo-Ocimenol[a]	1019	1011	—	0.11	—	RI, MS
Linalool	1097	1080	0.25	0.19	—	RI, MS
Terpinolene	1011	1018	—	—	tr	RI, MS
trans-Sabinene hydrate	1059	1067	0.37	—	—	RI, MS
Terpinen-1-ol[a]	1164	1130	—	—	0.11	RI, MS
1,5,8-p-Menthatriene[a]	1320	1145	0.43	0.38	—	RI, MS
Borneol	1252	1172	—	—	1.02	RI, MS
Pinocarvone	1167	1166	2.96	3.00	—	RI, MS
trans-	1288	1204	—	0.19	—	RI, MS

Compound	RI_{exp}	RI_{lit}	SFE 1	SFE 2	HD SFE	Identification
Dihydrocarvone						
Dihydrocarvone[a]	1235	1217	0.37	2.06	—	RI, MS
Ocimenone (E)[a]	1249	1239	1.54	1.50	—	RI, MS
Thymoquinone	1260	1254	35.05	33.12	38.41	RI, MS,NMR
Thymol	1283	1289	7.43	5.30	16.95	RI, MS,NMR
Carvacrol	1229	1295	1.98	1.73	0.81	RI, MS
2-Undecanone	1342	1317	—	—	13.72	RI, MS
n-Octyl isobutyrate[a]	1393	1325	—	—	0.12	RI, MS
α-Longipinene	1380	1339	0.26	—	—	RI, MS
Citronellyl acetate[a]	1339	1334	—	—	0.50	RI, MS
Thymohydroquinone methyl ether[a]	1343	1355	—	—	tr	RI, MS
Cyclosativene	1337	1366	—	—	1.43	RI, MS
α-Longicyclene	1371	1384	0.43	5.25	—	RI, MS
α-Copaene	1315	1382	1.54	2.00	0.41	RI, MS
α-Longifolene	1381	1385	—	—	0.51	RI, MS
(Z)-Caryophyllene[a]	1395	1391	0.23	—	—	RI, MS
β-Caryophyllene	1420	1411	2.89	5.07	4.80	RI, MS
Thymohydroquinone dimethylether[a]	1449	1422	0.43	—	—	RI, MS
Aromadendrene[a]	1477	1433	—	—	1.04	RI, MS
Thymohydroquinone	1585	1505	1.17	1.12	2.31	RI,MS,NMR
Davanone[a]	1527	1584	0.31	—	—	RI, MS
8-Heptadecene[a]	1613	1681	1.23	1.13	0.86	RI, MS
Dihydrofarnesyl acetate[a]	1831	1843	2.28	4.69	—	RI, MS
Pimaradiene[a]	1914	1935	1.23	2.25	—	RI, MS
Palmitic acid	1917	1947	0.18	—	—	RI, MS
Pimara-8(14),15-diene	1968	19665	0.92	—	—	RI, MS
Octadecanoic acid	2115	2167	0.26	12.31	—	RI, MS
Fatty acids	2256	2169	0.26	12.31	—	RI, MS
Quinones	2115	2167	44.08	39.54	57.67	RI, MS
Fatty acid esters	2256	2169				
Monoterpene hydrocarbons	2115	2167	36.51	14.15	15.94	RI,MS,NMR
Oxygenated monoterpenes	2354	2169	7.47	9.16	17.14	RI, MS
Sesquiterpene hydrocarbons	2325	2167	5.35	12.32	8.19	RI, MS
Oxygenated sesquiterpenes	2354	2169	2.59	4.69	—	RI, MS
Diterpenes	2325	2167	2.15	2.25	—	RI, MS
Alkane	2346	2169	0.12	—	—	RI, MS
Alkenes	2316	2169	1.23	1.13	0.86	RI, MS
Total identified			99.84	94.55	99.86	RI, MS

4.3 Antimicrobial Activity of *N. sativa* essential oil

A comprehensive comparative analysis of the antimicrobial susceptibility of *N. sativa* essential oil against several commonly used antibiotics presented in Table-3.

The chosen parameters for assessment include the Disc Diffusion method to measure zones of inhibition, as well as the determination of MIC and MBC in microliters (µL) [29]. This approach provides a nuanced understanding of the antimicrobial efficacy of *N. sativa* essential oil in relation to established antibiotics. The *N. sativa*

essential oil exhibited an impressive Disc Diffusion zone of 29 mm, suggesting a substantial inhibitory effect against the tested microorganisms. This zone size was comparable to that of Gentamycin, a well-known and potent antibiotic, reinforcing the robust antimicrobial potential of *N. sativa* essential oil. The MIC and MBC values of *N. sativa* essential oil, Bactrim, Clindamycin, and Minocycline were all found to be 1.00 µL and 1.25 µL, respectively. This similarity in MIC and MBC values indicates that *N. sativa* essential oil shares comparable bacteriostatic and bactericidal properties with these antibiotics. A comparative study with other antibiotics revealed interesting insights. Minocycline, a recognized antibiotic, displayed a 26 mm inhibition zone, comparable to the 29 mm zone of *N. sativa* essential oil, emphasizing the latter's notable antimicrobial activity. In contrast, Doxycyclin exhibited a smaller zone of inhibition (19 mm) and higher MIC and MBC values compared to *N. sativa* essential oil, indicating that *N. sativa* may possess superior effectiveness against the tested microorganisms. Vancomycin and Methicillin, both potent antibiotics, showcased the highest Disc Diffusion zones (31 mm and 30 mm, respectively), suggesting strong antimicrobial activity. The Disc Diffusion (mm) comparative chart of antimicrobial susceptibility was depicted in detailed comparison in Fig. 2.

The MIC and MBC values for these antibiotics were slightly higher than those for *N. sativa* essential oil, indicating that while *N. sativa* may have a slightly lower potency, it still demonstrates considerable efficacy. This detailed comparative analysis underscores the promising antimicrobial potential of *N. sativa* essential oil, positioning it as a viable natural alternative to conventional antibiotics. The consistent and potent inhibitory effects observed in this study warrant further exploration, including clinical trials, to validate and better understand the practical applications of *N. sativa* in the context of bacterial infections. The benchmark antibiotics Vancomycin and Methicillin, known for their efficacy against resistant strains, exhibited the largest inhibition zones at 31 mm and 30 mm, respectively. The MIC and MBC values for *N. sativa* essential oil were slightly lower, suggesting that, although marginally less potent, *N. sativa* essential oil still demonstrates significant antimicrobial effectiveness. The comparative chart of antimicrobial susceptibility of MIC values (µL) % was depicted in detailed comparison in in Fig. 3.

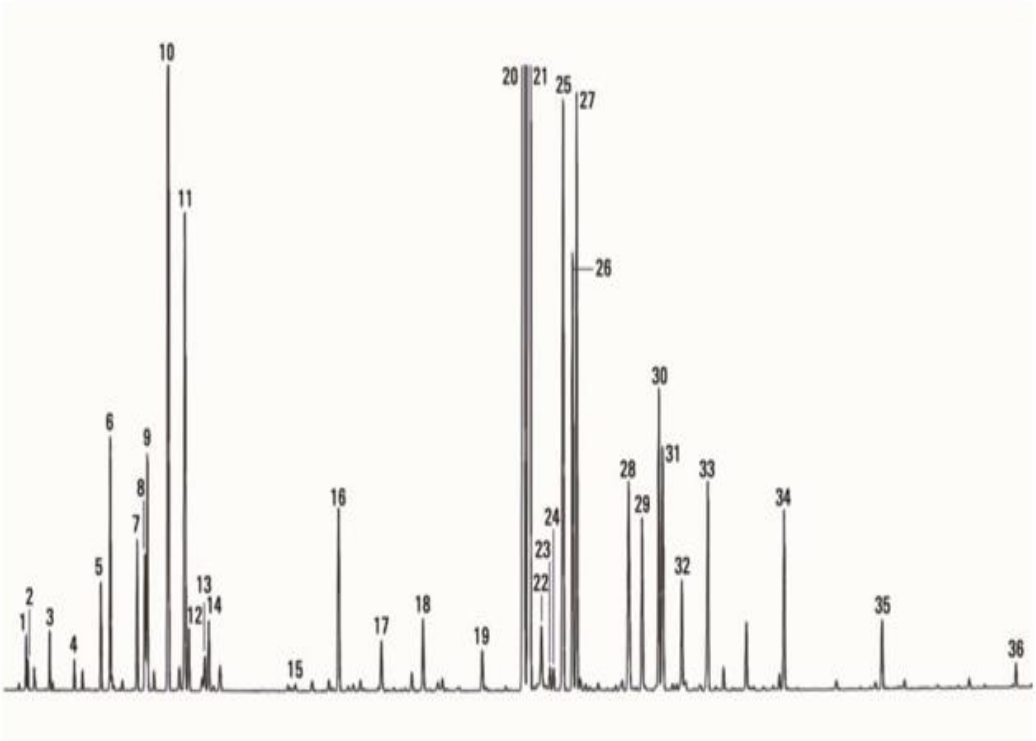

Fig. 1. Gas Chromatography-Mass Spectrometry Analysis of *Nigella Sativa* essential oil

Table 3. Comparative chart of antimicrobial susceptibility

Antimicrobial	Disc Diffusion (mm)	MIC (µL)	MBC (µL)
N. sativa essential oil	29	1.00	1.25
Bactrim - Antibiotic	22	1.00	1.25
Clindamycin- Antibiotic	23	1.00	1.25
Minocycline- Antibiotic	26	1.25	1.50
Doxycyclin- Antibiotic	19	1.50	1.75
Gentamycin- Antibiotic	29	1.50	1.75
Penicillin- Antibiotic	27	1.50	1.75
Methicillin- Antibiotic	30	1.75	2.00
Vancomycin- Antibiotic	31	1.75	2.00

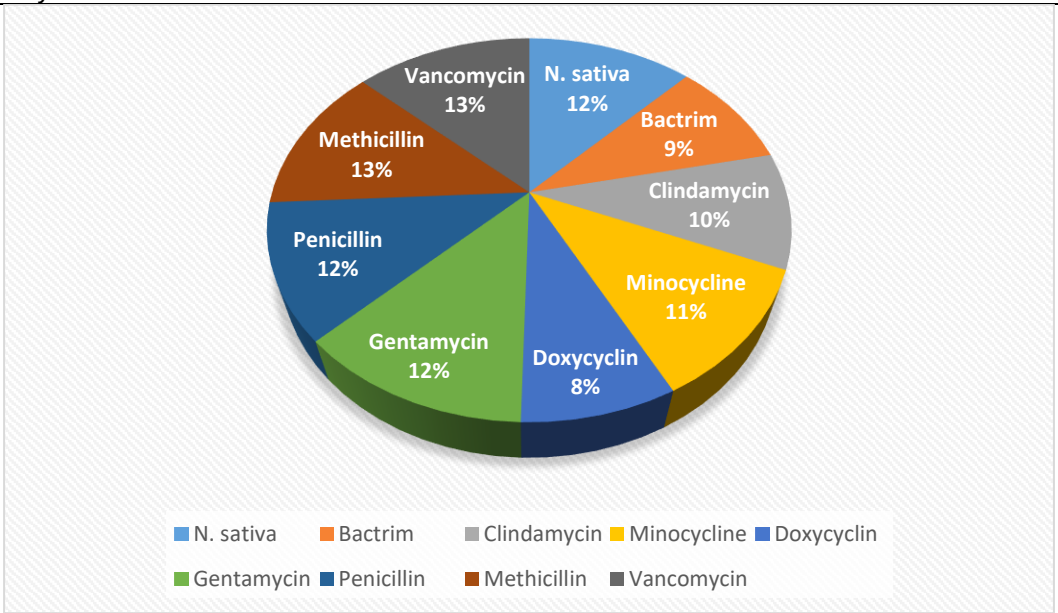

Fig. 2. Disc Diffusion (mm) comparative chart of antimicrobial susceptibility

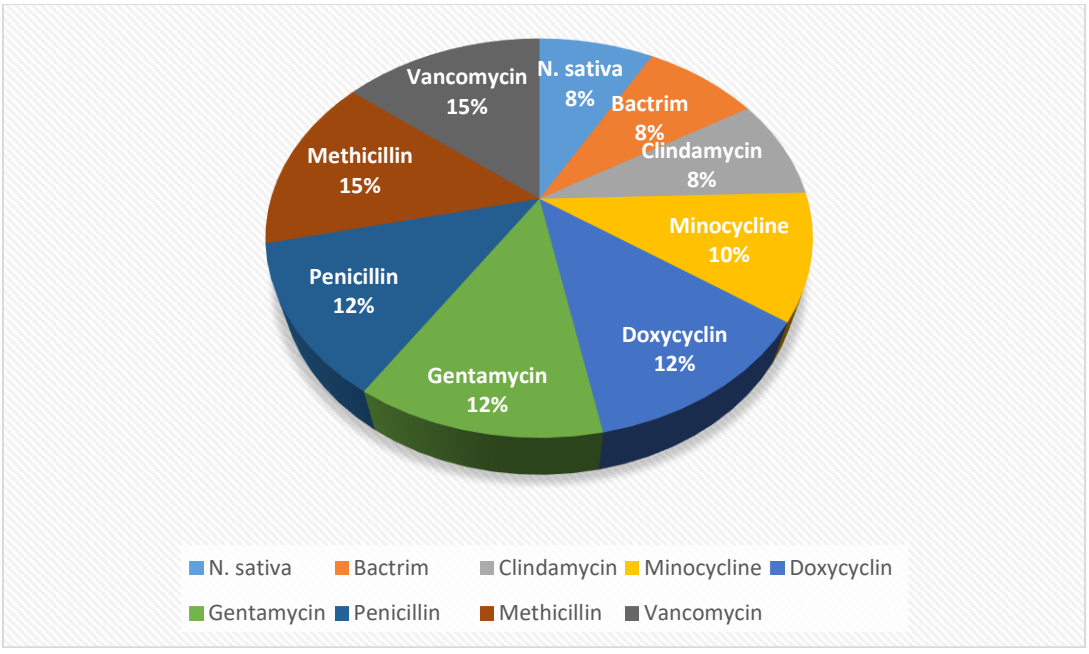

Fig. 3. MIC values (µL) % comparative chart

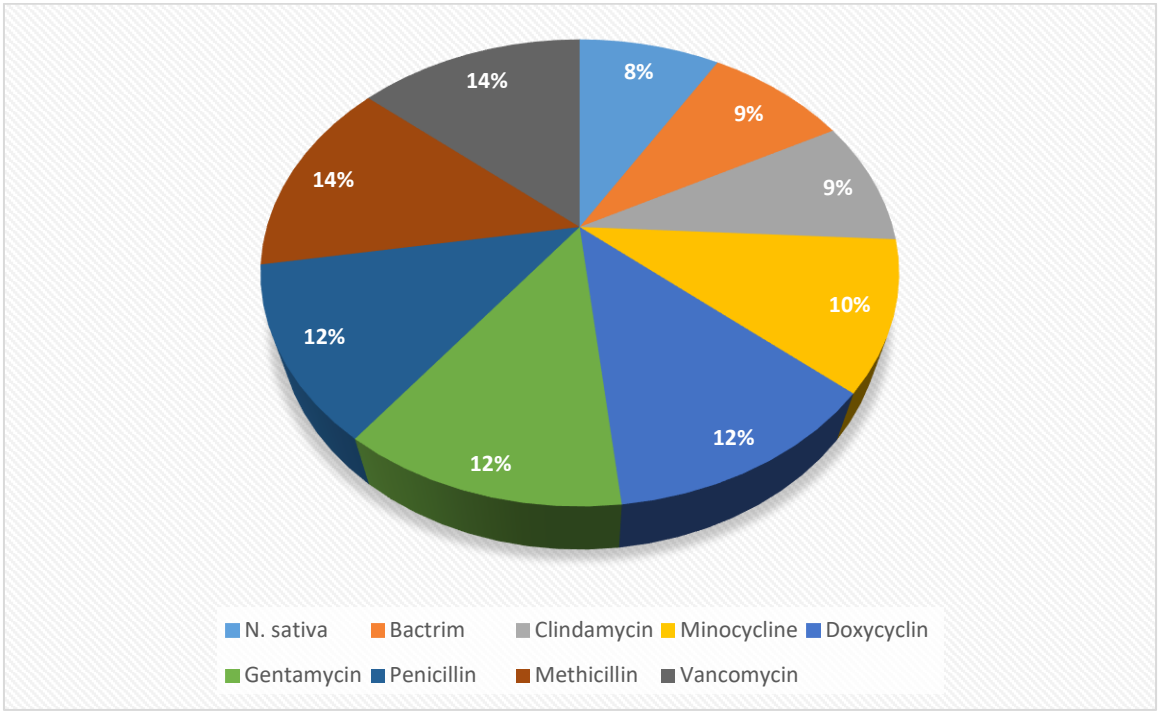

Fig. 4. MBC values (µL) % comparative chart

The detailed comparative analysis elucidates that *N. sativa* essential oil showcases robust antimicrobial potential, comparable to or even surpassing certain conventional antibiotics. The multi-faceted assessment through Disc Diffusion, MIC, and MBC values provides a nuanced understanding of its inhibitory and bactericidal effects. The comparative chart of antimicrobial susceptibility of MBC values (µL) % was depicted in detailed comparison in in Fig. 4.

These findings underscore the potential of *N. sativa* essential oil as a promising alternative or adjunct in the realm of antimicrobial interventions, calling for further investigations, including clinical trials, to validate its efficacy and explore its therapeutic applications in the context of bacterial infections.

5. DISCUSSION

The qualitative analysis of *N. sativa* essential oil offers a detailed exploration of its chemical composition, providing valuable insights into potential pharmacological effects. The confirmation of alkaloids, indicated by an orange precipitate, hints at the presence of nitrogen-containing compounds with diverse biological activities. Alkaloids are often associated with analgesic, anti-inflammatory, and antimicrobial properties, and their detection suggests a potential range of physiological effects [23]. The intense green color exhibited by flavonoids signifies a high abundance of these polyphenolic compounds. Flavonoids are well-known for their antioxidant and anti-inflammatory properties, indicating that *N. sativa* essential oil may possess significant free radical scavenging and anti-inflammatory capabilities. The identification of phenols, marked by a deep blue-black coloration, suggests the presence of compounds contributing to the oil's antimicrobial and antioxidant potential. Tannins, with their brownish-green hue, further enhance the therapeutic potential, linking the oil to anti-inflammatory and anti-cancer effects. These compounds are crucial in the context of potential health benefits associated with *N. sativa* essential oil. The positive results for cardiac glycosides, steroids, saponins, and terpenoids add layers of complexity to the chemical profile. Cardiac glycosides, often linked to cardiovascular effects, and steroids, contributing to anti-inflammatory properties, suggest a potential impact on cardiovascular and inflammatory conditions. Saponins, evidenced by foam formation, are recognized for antimicrobial and immune-modulating activities, broadening the spectrum of potential applications. The reddish-brown coloration in steroids and terpenoids further implies the presence of compounds with anti-inflammatory and

antioxidant effects. The qualitative analysis not only identifies specific chemical constituents in *N. sativa* essential oil but also provides a nuanced understanding of their potential physiological effects, paving the way for further investigations into the health benefits and therapeutic applications of this natural extract [17].

The quantitative GC-MS analysis complements the qualitative findings by offering a detailed breakdown of the percentage composition of each identified compound under different extraction conditions. The use of advanced identification techniques, including retention indices, experimental and literature values, and mass spectra, ensures a robust and reliable analysis. The extraction methods, supercritical fluid extraction (SFE) 1 and 2, along with hydro distillation of SFE 1 (HD SFE), highlight the impact of extraction conditions on the overall composition. The identification of compounds covering a diverse range, from hydrocarbons to oxygenated compounds, reflects the complexity of N. sativa essential oil. The inclusion of Nuclear magnetic resonance (NMR) spectroscopy for specific compounds, such as Thymoquinone, Thymol, and Thymohydroquinone, adds an extra layer of confidence to the results. Compounds identified for the first time emphasize the novelty of the findings, expanding our knowledge of the chemical diversity of *N. sativa*. The quantitative analysis demonstrates a high percentage of identified compounds in each extraction method, emphasizing the comprehensiveness of the investigation. The meticulous comparison of data from various sources, including NIST, Wiley commercial libraries, Chemistry Web Book, and other relevant reports, adds to the reliability of compound identification [38]. The quantitative GC-MS analysis provides a thorough understanding of the chemical composition of *N. sativa* essential oil, offering a quantitative perspective that complements the qualitative analysis. These findings contribute to the development of a comprehensive profile of the essential oil, facilitating a deeper understanding of its potential applications and benefits [41].

The comparative evaluation of *N. sativa* essential oil against commonly used antibiotics in the antimicrobial analysis reveals its inhibitory and bactericidal effects [23]. Utilizing the Disc Diffusion method, the study demonstrates a significant inhibitory effect, akin to the potent antibiotic Gentamycin, indicating a strong potential for antimicrobial activity. The MIC and MBC values of N. sativa essential oil closely align with established antibiotics, signifying comparable bacteriostatic and bactericidal properties [24]. The study underscores the efficacy of the essential oil against the tested microorganisms, presenting it as a viable natural substitute for conventional antibiotics.

The antimicrobial efficacy of *N. sativa* essential oil was evident in comparison with other antibiotics. Although displaying slightly lower MIC and MBC values, implying marginal potency reduction, the essential oil still exhibits noteworthy effectiveness against the tested microorganisms [35]. Notably, benchmark antibiotics Vancomycin and Methicillin, known for combating resistant strains, yield the largest inhibition zones. However, the marginally lower MIC and MBC values for *N. sativa* essential oil suggest significant efficacy, positioning it as a promising alternative in the realm of antimicrobial interventions. The comprehensive assessment through Disc Diffusion, MIC, and MBC values provides a nuanced understanding of the inhibitory and bactericidal effects of *N. sativa* essential oil. The consistently potent inhibitory effects observed in the study emphasize its potential as an alternative or complement in treating bacterial infections. This antimicrobial analysis not only underscores the robust antimicrobial potential of *N. sativa* essential oil but also positions it as a compelling substitute for conventional antibiotics. The results prompt further investigations, including clinical trials, to validate its efficacy and explore therapeutic applications in the context of bacterial infections.

The combined qualitative, quantitative, and antimicrobial analyses of *N. sativa* essential oil provide a comprehensive and detailed exploration of its chemical composition and potential pharmacological benefits [26]. The study not only identifies specific bioactive compounds but also offers insights into their potential physiological effects, opening avenues for further research. The chemical complexity revealed by the qualitative and quantitative analyses contributes to a deeper understanding of *N. sativa* essential oil's potential applications in medical and pharmaceutical contexts [47]. The antimicrobial analysis, showcasing its effectiveness against tested microorganisms, positions it as a promising natural alternative to conventional antibiotics.

The findings of the study not only contribute to scientific knowledge but also pave the way for future research aimed at elucidating the specific

health benefits and therapeutic applications of *N. sativa*. The potential development of novel therapeutic agents and the exploration of clinical applications, supported by the robust analytical methods employed, underscore the significance of this study in advancing our understanding of *N. sativa* essential oil [5]. This study demonstrates a high level of scientific rigor through its thorough literature review, which provides a solid foundation for the research question. The methodology clearly outlined and appropriately designed to address the objectives, ensuring reproducibility and reliability of the results. Additionally, the data analysis comprehensively and employs suitable statistical techniques, enhancing the validity of the findings. Overall, the study provides robust experimental design and detailed reporting contribute to its scientific and technical soundness.

6. CONCLUSION

This study extensively investigates the phytochemical properties of *N. sativa* seeds essential oil through GC-MS analysis, with a focus on its potential antimicrobial activity against *S. aureus*. The research addresses the critical challenge of postoperative infections in neurosurgical interventions, emphasizing the escalating global threat of antibiotic resistance and the need for innovative therapeutic approaches. The research methodology included the preparation of *N. sativa* essential oil, qualitative phytochemical screening, quantitative GC-MS analysis, and antimicrobial activity assessment. The results re-assured the presence of various bioactive compounds, and the antimicrobial analysis demonstrates the substantial inhibitory and bactericidal effects of the essential oil against *S. aureus*. The study provided valuable insights into the chemical complexity and potential therapeutic applications of *N. sativa* essential oil. The findings position it as a promising natural alternative for combating bacterial infections, especially in neurosurgical contexts. The robust analytical approach and contextualization within medical practices contribute to the foundation for future research and the development of targeted therapeutic strategies. Further exploration, including clinical trials, is warranted to validate the efficacy and practical applications of *N. sativa* essential oil in the treatment of bacterial infections.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

The authors are exceptionally grateful to the organization of Ibn Sina National College for Medical Studies, Jeddah, Kingdom of Saudi Arabia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI:https://doi.org/10.1016/j.ijbiomac.2017 .04.112.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3935