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Investigation of *Nigella sativa* seed ethanolic extract using gas chromatography-mass spectrometry and study of its antibacterial activity against different bacterial species

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Abstract

Medicinal plants are one of the valuable natural resources that developed countries today consider to be safe alternative medicines for humans. The current study focused on Gas chromatography-mass spectrometry (GC-MS) investigations and the antibacterial properties of *Nigella sativa* (*N. sativa*) seed extract. The maceration process, the soxhlet method and suitable extraction solvents have all been used to create various extracts from *N. sativa seeds*. The GC-MS analyses identified around 25 chemical compounds with known bioactivities and/or uses that are crucial for the treatment of illnesses that pose a serious hazard to life. Using the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and Well diffusion methods, the seed extract demonstrated antibacterial activity against Gram-positive (*Staphylococcus aureus, Bacillus cereus*) and Gram-negative (*Escherichia coli, Salmonella typhimurium*) bacteria with varying degrees of efficacy. The antibacterial activity of the extract was dose-dependent and more potent against Gram-positive bacteria than Gram-negative bacteria. Considering the findings of this study, *N. sativa* seed extracts contain a variety of chemical elements associated with their antibacterial capabilities.

Keywords

antibacterial activity; GC-MASS; minimum inhibitory concentration; *Nigella sativa*

Introduction

Plants generally span a wide range of biological activities that are desired for therapeutic purposes in both conventional and modern therapies; these natural substances are frequently utilized to improve people's health with little to no negative effects (1). The usage of *N. sativa* seeds-also identified as black seed or black cumin-in herbal medicine and food preparation has grown around the world, particularly in Iraq (2). Numerous studies have shown that *N. sativa* plant extract has a high level of biological and pharmacological activities, making it suitable for treating a wide range of human conditions, including those that are antipyretic, antidiabetic, anti-inflammatory, pain-relieving, antifungal, immunomodulatory, antioxidant, anticancer, antibacterial and anti-hypertensive (3-6). *Nigella Sativa* seed has different active compounds such as alkaloids, saponins, amino acids, fixed oils and essential oils (7, 8). Thymoquinone, a significant component of Nigella saliva's essential oil, has been credited with much of the seeds' biological activity, including

antihypertensive, analgesic, antipyretic, antibacterial and antineoplastic activities (9, 10). The oil extracted from the seeds of the *N. sativa* plant has been investigated in clinical trials for cough and bronchial asthma (11). The seeds are used to treat moderate cases of puerperal fever and are used as carminative stimulants, diuretics, emmenagogues and sedatives (12).

Phytomedicines derived from plant materials such as seeds, fruits, flowers, roots, leaves and bark, as well as many of the bioactive elements of plants, have been found and furt her described using a range of conventional analytical techni ques (13). The most recent research concentrated on the active components derived from medicinal plants like *Nigella sativa* and their biomedical uses after being functionalized with other materials using electrospinning (14). The significant resistant pathogens commonly isolated from clinical specimens in hospitals, such as *S. aureus, B. cereus, E. Coli and S. typhimurium,* are multi-resistant to antibiotics and can be present on the mucous membranes and in the stools of patients. Antibiotic resistance requires a concerted effort to promote antimicrobial drugs active against pathogenic bacteria that are resistant to currently available antibiotics.

The logical localization of bioactive phytochemicals is one potential strategy for achieving this goal. The current study aimed to use GC-MS for phytochemical analysis and to investigate the antibacterial efficacy of *Nigella sativa L.* against Gram-positive and Gram-negative multidrug-resistant bacterial strains.

Materials and Methods

Materials

All materials used in the current study were summarized in Table 1.

Plant collection

N. sativa seeds were procured at a local market in Amarah City, Iraq, in February 2022 and verified at the Agriculture College, University of Misan, Maysan, southern Iraq. The seeds were meticulously rinsed with distilled water and subsequently dried in a cool, shaded environment. Most of the moisture has been removed and a mill has been employed to pulverize the plant material into a fine powder. The materials were thereafter preserved in a dark, deep-frozen container until extraction was finalized.

Extraction of crude

The extraction was conducted with minor modifications to the procedure described by Al-Ameedy et al. (15). 100 g of *N. sativa* seed powder were homogenized for two min in a Waring blender with 100 mL of ethanol, thereafter, kept at room temperature and protected from light for 72 h. The resulting liquid was filtered under moderate suction using a Buchner funnel and a Whatman No. 1 filter. The residue was further crushed to guarantee the total recovery of the filtrate. The suspensions were subjected to filtration using sterile Whatman-1 filter paper and subsequently centrifuged at 4000 rpm for 15 min. Sterile water and commercial antibiotics were utilized as positive and negative controls, in conjunction with final concentrations of 100 mg/mL of different extracts.

GC-MS examination

The ethanolic extract of N. sativa was analyzed using a Perkin -Elmer GC/MS QP2 system equipped with a Restek5 RT×R (30m×0.215mm) capillary column. The extract was reconstituted in methanol at a concentration of 1 mg/mL and injected in a volume of 1 µL, utilizing 99.9 % helium gas as the carrier gas with a split ratio of 20:1. The process started at 60 ° C for 5 min, with the injector warmed to 250 °C, followed by an increase in the oven temperature to 280 °C over a duration of 10 min. Chemicals were identified utilizing a mass spectral database (NIST and WILEY library) connected to a GCMS to get spectral configurations.

Antibacterial activity:

To investigate effectiveness against MRD bacteria, the Well diffusion technique was used. This is the accepted technique for determining whether active substances have antibacterial capabilities (16, 17). For this, 100 mL of Mueller-Hinton agar medium was diluted with 1 mL of a standard stock suspension of microorganisms comprising 106 CFU/mL and the mixture was maintained at 45 °C. Mueller-Hinton agar media was split into 20 mL aliquots, put on previously sterilized plates and let to set at Rt. Then, using a sterilized cork borer (No. 4), three chambers of 10 mm diameter were made in each of these plates. The agar discs' pores were filled with varying amounts of ethanol extract (50 g/mL), which were then left at room temperature unattended for 2 h to diffuse. The next day, the dishes were incubated at 37 °C. The plates were then examined for signs of bacterial growth prior to determining the diameter of the inhibitory zone (mm). Erythromycin and distilled water were used as positive and negative controls, respectively.

Table 1. Identification and source of materials utilized in the investigation

Step/Method	Materials Used	Origin and Place
Sample Acquisition	*Nigella sativa* seeds	Amarah City, Iraq (Local market)
Washing and Drying	Distilled water	Sigma-Aldrich
Grinding	Mill	Laboratory
Extraction	100 g *Nigella sativa* seed powder	Amarah City, Iraq (local Market)
	100 mL ethanol	Sigma-Aldrich
Filtration	Bucher funnel	Sigma-Aldrich
	Whatman No. 1 filter	Sigma-Aldrich
Centrifugation	Centrifuge machine	Laboratory
GC-MS Examination	Perkin-Elmer-GC/MS-QP2 system	Laboratory
	Restek5, RT×R- (30m×0.215mm) capillary column	Laboratory
Antibacterial Activity	Mueller-Hinton agar medium	Sigma-Aldrich
	Standard stock suspension of microorganisms (106 CFU/mL)	Sigma-Aldrich
	Ethanol extract	Sigma-Aldrich
Analytical Statistics	One-way study of variance ANOVA	SPSS software

Analytical Statistics

All trials were performed in triplicate, with a one-way study of variance ANOVA conducted and a P value of < 0.05. considered significant (18).

Results and Discussion

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Numerous peaks were detected in a gas chromatography-mass spectrometry analysis of an ethanolic extract of *N. sativa* seeds (Fig. 1). Established component spectra from the GC-MS library were employed to interpret the peaks in the chromatogram. Twenty-five primary components were identified in the extract by GC-MS profiling. Table 2 presents the molecular weights, chemical formulae, retention periods and peak areas (in percent) of the identified compounds. A total of 25 distinct chemicals exists, but 9 of them (ethyl ester of 9,12-octadecadienoic acid, undecanoic acid, 11-bromoundecanoic acid, methyl 1-C-hydroxymethyl) are hexopyranoside, 5,6,7,8-tetrahydro-2-naphthol, methyl ester of 3,4-octadiene and methyl ester of 2,4-Dimethylheptanoic acid.

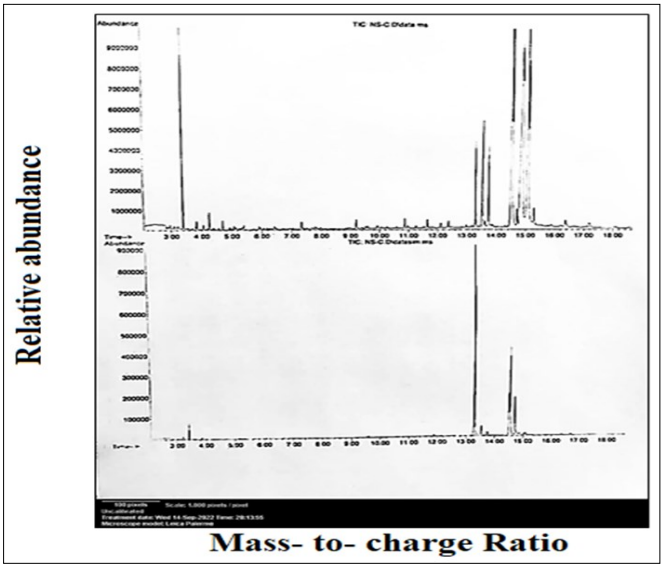

Fig. 1. The GC-MS analysis of the ethanol extract of *Nigella sativa* seeds.

Numerous plant glycosides contain the polyunsaturated fatty acid known as 9,12-octadecadienoic acid. It is a necessary fatty acid for mammalian diets because it is used in the production of prostaglandins and the creation of cell membranes. Human malignant melanoma, colon and breast cancer cell proliferation is suppressed *in vitro* by 9,12-octadecadienoic acid (19) and it also has anti-atherogenic effects. In addition, undecanoic acid is a saturated fatty acid found in human tissues and blood. Humans metabolize it and it has antifungal effects (20). The small amino acid 11-bromoundecanoic acid exhibited moderate to good antibacterial activity (21). Hexadecanoic acid is an example of a saturated fatty acid. The antibacterial and antifungal effects of several fatty acids are well established (22). Condensation of the carboxy group of tetradecanoic acid (myristic acid) with methanol yields the methyl ester ethyl 13-methyl-tetradecanoate. Plants produce it as a secondary metabolite and it has culinary and aromatic uses. It is produced by synthesizing tetradecanoic acid and exhibits anti-cancer, anti-cancer, anti-hypercholesterolemic and nematicide properties (23). The methyl ester ethyl 13-methyl-tetradecanoate is made by combining the carboxy group of tetradecanoic acid (myristic acid) with methanol. Plants produce it as a secondary metabolite and it has culinary and aromatic uses. It is produced by synthesizing tetradecanoic acid and exhibits anti-cancer, anti-cancer, anti-hypercholesterolemic and nematicide properties (24).

Antibacterial activity

S. aureus, E. coli, Bacillus cereus and *S. typhimurium* were used to test how well *N. sativa* seed extract killed bacteria. Table 3 displays the findings regarding the extract's antibacterial activity. The diameter of the inhibitory zone for *N. sativa* seed extract against *S. aureus, E. coli, B. cereus* and

Table 2. GC-MS analysis of *Nigella sativa* seed extract

Peak No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak %
1	3.402	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.271531	5.088
2	3.811	9-cis,11-trans-Octadecadienoate	$C_{21}H_{38}O_2$	294.47	0.138
3	4.847	2-Chloroethyl linoleate	$C_{20}H_{35}ClO_2$	342	0.236
4	4.060	9,17-Octadecadienal	$C_{18}H_{32}O$	264.4	0.239
5	4.260	1-Hexadecyne	$C_{16}H_{30}$	222.41	0.448
6	4.704	3,4-Octadiene, 7-methyl-	C_8H_{14}	124	0.252
7	5.360	cis-7,cis-11-Hexadecadien-1-yl acetate	$C_{18}H_{32}O_2$	280.4455	0.110
8	5.395	1-Methyl-2-methylenecyclohexane	C_8H_{14}	110.1968	0.141
9	7.324	3. Tetradyne	$C_{38}H_{42}N_2O_6$	256	0.329
10	9.921	1,13-Tetradecadiene	$C_{14}H_{26}$	194.3562	0.168
11	10.875	9-Octadecen-1-ol, (E)	$C_{18}H_{36}O$	268.4778	0.495
12	10.974	Tetradecanoic acid,ethyl ester	$C_{16}H_{32}O_2$	256.4241	0.250
13	11.264	Pentenoic acid ethyl ester	$C_7H_{12}O_2$	128.1690	0.097
14	11.652	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340.5836	0.353
15	13.538	Undecanoic acid	$C_{11}H_{22}O_2$	186.2912	5.006
16	13.616	Dodecyl N-ethyl-N-(methoxycarbonyl) glycinate	$C_{18}H_{35}$	329.475	0.310
17	13.744	11-Bromoundecanoic acid	$C_{11}H_{21}BrO_2$	265.187	2.886
18	14.540	Methyl 1-C-(hydroxymethyl)hexopyranoside	$C_8H_{16}O$	224.208	17.665
19	14.540	n-Decanoic acid	$C_{10}H_{20}O_2$	172.265	8.402
20	14.709	Adipic acid, isohexyl methyl ester	$C_{13}H_{24}O_4$	244.327	0.850
21	14.860	5,6,7,8-Tetrahydro-2-naphthol	$C_{10}H_{12}O$	148.20	18.992
22	14.889	3,4-Octadiene, 7-methyl-	C_9H_{16}	124.22	6.890
23	15.052	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	14.952
24	15.087	Ethyl 13-methyl-tetradecanoate	$C_{17}H_{34}O_2$	270.5	7.882
25	15.279	2S,4R)-2,4-Dimethylheptanoic acid	$C_9H_{18}O$	158.238	1.372

S. typhimurium strains was 14.83 ± 0.2 mg/mL, 6.4 ± 0.3 mg/mL, 14.04 ± 0.2 mg/mL and 7.2 ± 0.2 mg/mL, respectively, while the MIC and MBC values were illustrated in Table 4.

The findings indicate that an ethanol extract of *N. sativa* possesses antibacterial activity against both Gram-positive and Gram-negative pathogens. Our results corroborate prior studies indicating that ethanolic extracts of *Eucalyptus* and *N. sativa* seeds demonstrate antibacterial efficacy against *B. subtilis*, *S. aureus* and *E. coli* (25). In accordance with prior studies indicating that plant-derived compounds have greater efficacy against Gram-positive bacteria compared to Gram-negative bacteria (26, 27). Tables 3, 4 demonstrate that the extracts exhibit greater efficacy against Gram-positive bacteria compared to Gram-negative bacteria.

Despite being a considerable barrier for hydrophilic medications, the inner membrane (IM) of Gram-negative bacteria is primarily permeable to amphiphilic medications (28). It's conceivable that this permeability barrier is to blame for the plant antimicrobial activity's apparent ineffectiveness.

According to the findings, black seed extract exhibited dose-dependent inhibition towards concentration. Herbal plants and extracts have antimicrobial properties that have been known since ancient times. The development of resistance to antibiotics by pathogens requires further efforts to find new antimicrobial agents to eliminate the infection and solve the problems of resistance and side effects associated with the antimicrobial drugs already in use (29, 30). The use of bioactive compounds derived from plant matter in biomedical research has increased significantly (31). The antibacterial effect was established by taking different concentrations of crude extract of *N. sativa* seed from the seed crude extract and applying it directly. The concentration that produced the best effect was 300 mg/mL. The comparison of the inhibition induced by the different concentrations of both preparations is shown in Fig. 2. *N. sativa,* which reached 14.83 ± 0.2 mm, had the highest inhibition observed. The negative control (D.W.) showed no inhibition (Fig. 1, 2). The positive control antibiotic Erythromycin was used to emphasize these positive effects of both preparations (Fig. 2, 3).

Conclusion

This study verified the antibacterial activity of an ethanol extract from *N. sativa* seeds. The presence of phytochemicals was assessed using conventional techniques and the chemical composition of the ethanol extract was analyzed by GC-MS. GC-MS identified 25 phytochemicals in the ethanol extract of *N. sativa*. The extract demonstrated significant antibacterial activity, likely attributable to the presence of active chemicals. In conclusion, the antibacterial properties and phytochemical analysis of *N. sativa* seed extract indicate the potential for broader application of such plants in the food business, especially in novel health-focused products for humans. Additional research is required to isolate and characterize moieties from the seed extract, as well as from the roots and stems of this plant, for biomedical applications.

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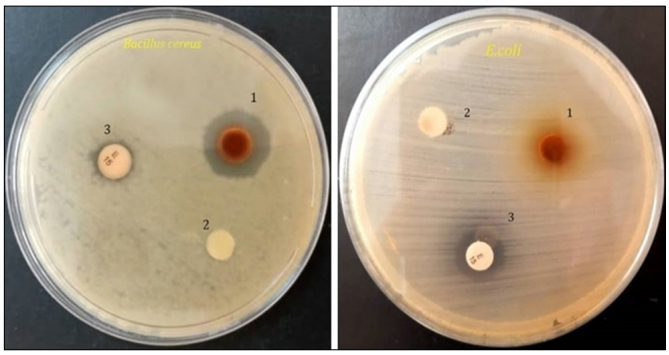

Fig. 2. The antibacterial effects (inhibition zone) of *N. sativa* seed extract, (1), D.W (2) and erythromycin (3) on Gram-negative bacteria (*B. cereus* and *E. coli*).

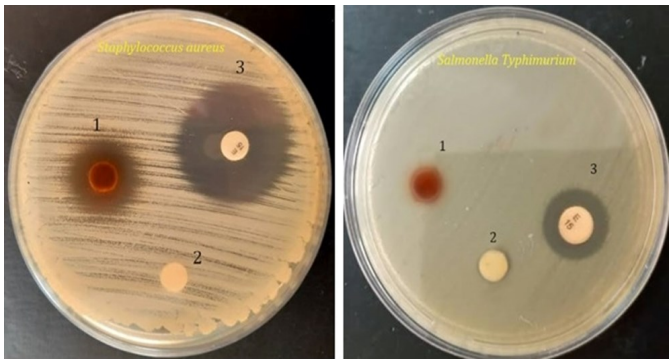

Fig. 3. The antibacterial effects (inhibition zone) of *N. sativa* seed extract, (1), D.W (2) and erythromycin (3) on Gram-positive bacteria (*S. aureus* and *S. typhimurium*).

Table 3. Mean ±SD of the inhibition zone (mm) of *N. sativa* seed extract suspension against tested bacteria

Compounds (mg/mL)	Zone of inhibition (mm)			
	S. aureus	***E. coli***	***B. cereus***	***S. typhimurium***
Distillated water	6.2 ± 0.2	6.2 ± 0.2	6.2 ± 0.2	6.2 ± 0.2
Nigella sativa	14.83 ± 0.2	6.4 ± 0.3	14.04 ± 0.2	7.2 ± 0.2
E (15)-Erythromycin	25.32 ± 0.2	11.0 ± 0.2	8.6 ± 0.3	13.81 ± 0.2

Table 4. The MIC and MBC of *N. sativa* seed extract on *S. aureus*, *E. coli*, *B. cereus* and *S. typhimurium*

Compounds (mg/mL)	Inhibition			
	S. aureus	***E. coli***	***B. cereus***	***S. typhimurium***
MBC	300	400	300	400
MIC	200	300	200	300

Authors' contributions

ASH and SMJ were focused on conceptualization. ASH, SMJ and HNA executed the approach. SMJ and HNA performed a formal analysis. ASH and HNA were accountable for the investigation, data curation and validation of the study. ASH and SMJ contributed to the visualization and the production of the initial draft. ASH, SMJ and HNA collaborated on composing reviews and editing. ASH undertook supervision duties and managed project administration. All authors granted consent for the final version of the paper.

Data Availability

The data used to verify the findings of this study can be obtained by contacting the corresponding author upon request.

Compliance with ethical standards

Conflict of interest: The authors have no competing interests to declare that they are relevant to the content of this article.

Ethics Permission and Agreement to Participate: The author stated that the current study was carried out in compliance with the appropriate norms and regulations, as well as with the approval of the University of Misan's Colleges of Science and Agriculture.

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