Antimicrobial Activity of *Nigella Sativa* Extract Against some Bacterial and Fungal Species.

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Abstract

Seeds of *Nigella sativa* have been employed for thousands of years as spice and food preservative these seeds have been used to promote health and fight disease especially in the Middle East. In this study black seed extracted with 96% ethanol and purified chromatographically by using silica gel column with different solvents. The purpose of this study is to evaluate effect of *Nigella sativa* purified oil fractions on some fungal and bacterial species. The antifungal results on *Fusarium Solani* showed that both hexane and ethanol fractions of black seed oil revealed high antifungal properties and the diameter of growth were 24 ± 2.1mm and 28 ± 1.5 mm, while chloroform and methanol revealed moderate effect on *Fusarium Solani*, the diameter of growth were 30 ± 2.5mm and 37 ± 2.9mm. *Fusarium Solani* did not show any sensitivity for acetone, ethyl acetate and water fractions and the diameter of growth was between 40 to 44 mm. All seven fractions tested as antibacterial with *Escherichia coli, Staphylococcus aurous, Klepsiella pneumonia* and *Enterobacter aerogene.* Hexane and chloroform fractions were toxic to the *Escherichia* coli with inhibition zone 18.3 ± 4.3mm and 19.3±3.5 mm also these both fractions have the same effect on *Klepsiella pneumonia* and *Enterobacter aerogenes* while having the weak effect on *Staphylococcus aurous* with inhibition zone ranged between 8.6 - 3.3mm. *Staphylococcus aurous* revealed high sensitivity to ethanol fraction with inhibition zone 22.3 ± 5.4 mm in the same time acetone, ethyl acetate and water did not show any effect on bacterial spices.

Keywords: Nigella sativa seeds, antimicrobial activity, antibacterial activity, antifungal activity, medicinal plants, Nigella sativa oil fraction.

Introduction

The medicinal plant species are used for human disease treating. Popular explanations for the use and effectiveness of medicinal plants drastically donate to the admission of their curative possessions, even if their chemical ingredients are not entirely identified (1).There are more than 20,000 species of all identified medicinal plants used internationally listed according to World Health Organization (WHO) (2). Some of the compounds isolated from these plants proved to be a very effective preventive medicine and used to treat complex cases such as cancer diseases (3).

Nigella sativa is identified as black seed, *N. sativa* is a grassy plant belongs to the Ranunculaceae family, which has been used in Southern Europe, Southwest Asia and North Africa and it is cultured in many countries in the world, such as the Mediterranean region, Middle East, Pakistan, India, South Europe, Turkey, Saudi Arabia and Syria (4).

There are many reports concerning the biological and pharmacological activity of this plant, such as immunomodulatory, antidiabetic, anti-inflammatory, pain alleviating, antifungal, antioxidants, anticancer, antibacterial and anti-hypertensive effects (5; 6).

The essential vital phytoconstituents of *N. sativa* seeds include thymoquinone (30%-48%), p-cymene (7%-15%), 4-terpineol (2%-7%), carvacrol(6%-12%), t-anethol(1%-4%), sesquiterpene longifolene (1%-8%) thymol, thymohydroquinone, dithymoquinone and α-pinene *etc*. in addition to trace amounts of some other compounds such as limonene, carvone, citronellol. Also, the seeds have alkaloids, including nigellicimine type like isoquinoline alkaloids and nigellicimine-N-oxide, and pyrazole alkaloid types like nigellicine and nigellidine. Additionally, the*y* have a water soluble pentacyclic triterpene, alpha-hederin and saponnins as vital anticancer substance (7,8,9).

The black cumin seeds contain other compounds such as proteins (26.7%), fat (28.5%), carbohydrates (24.9%), crude fiber (8.4%) and total ash (4.8 %), in addition to a good amount of various minerals (P, Cu, Zn and Fe), vitamins, carotene which is converted to vitamin A by the liver. Also, shoots and roots of the plant contain a vanillic acid (7,9).

Thymoquinone(TQ) is an active substance in the crude extracts of *Nigella sativa* oil (NSO)which possesses antioxidant/anti-inflammatory efficacy, it is considered as vital anti-cancer, anti-oxidant and anti-mutagenic agent(10). The antioxidant activity of TQ was found to work as a hunter of singlet molecular oxygen, superoxide and hydroxyl radical (11,12). The powerful antioxidant properties of TQ may be associated with the redox properties of the quinine structure of the thymoquinone molecule, and its unlimited crossing of morphological barriers, thus gaining simple access to sub cellular partitions and easing the ROS hunting effect (11). Preceding investigations have moreover confirmed that thymoquinone exhibits an antioxidant effect at low concentrations against pro-oxidant properties at high concentrations (13,10).

Aqueous and alcohol extracts of *N. sativa* were found to be effective in vitroin inactivating MCF-7 breast cancer cells (14). *N. sativa*, in combination with melatonin and retinoic acid reduced the carcinogenic effects of DMBA (7,12-di-methylbenz(a)anthracene) in mammary carcinoma of rats (15). Terpene-terminated 6-alkyl residues of TQ were tested in MCF-7/Topo breast carcinoma by Effenberger *et al.* (2010)(16). They found the derivatives inducing cell death by apoptosis.

Besides TQ, other phytoconstituents of *Nigella sativa* have also been shown to contribute to the anti-cancer potential of *N. sativa* extracts. α-hederin is a pentacyclic triterpene saponnin found in *N. sativa* seeds that exerts effective anti-cancer effects, both in vitro and in vivo (17) . Moreover, thymohydroquinone,thymol, dithymoquinone, nigellicine,nigellimine-N-oxide, nigellidine, and carvacrol are phytoconstituents of *N. sativa* that have been demonstrated to play anti-cancer and cytotoxic functions (18).

Materials and Methods

Chemical and Biological Materials

Chemicals and biological materials were used for proceeding of experiments and tests in this research:

Chemical materials	Manufacturers(Origin)
Chloroform	BDH(England)
Ethanol	BDH(England)
Ethanol Absolute	GCC (UK)
Ethyl acetate	Himalia (India)
Methanol	GCC (UK)
n- Hexan	Ajenta Pharm (India)
Acetone	GCC (UK)

Plant collection:

The Black seeds as raw plant materials were purchased from a local market, in Babylon Province, Iraq, during February in 2018. The *N. sativa* seeds were imported from the Kingdom of Saudi Arabia.

The seeds were washed with distilled water and dried in shade separately at room temperature. Most of the moisture has been removed, the plant material grounded in a mill to produce fine powdered. After that, the sample (1000-1500 g) was stored in dark glass containers at −20°C until extraction was performed.

Extraction of bio active substances:

Optimum extraction parameters will vary depending on the type of plant part and matrix. The plant sample may require different temperatures and solvent extraction mixtures. From previous experiments that optimize the extraction methods, 96% ethanol (EtOH) was the best solvent to extract the active materials from black seeds; However the plant seed sample (50 g) was extracted twice with the 96% of ethanol (1000 ml) at the ratio of raw material to solvent 1:20 by soaking for 24 h at 30°C in the shaker incubator. The plant extract was decanted, filtered under vacuum, concentrated and the oil was recovered from oil-ethanol mixture by rotary evaporator at 45°C.The concentrated extract was stored in a dark container at −20°C for further purification (19).

Partial Purification of the Active Substances from *N. sativa* Seed Extract Using Adsorption Chromatography

The concentrated crude extract of black seeds (*Nigella sativa)* was partially purified using adsorption chromatography by silica gel column (mesh 60-120) to separate the components of the crude extract; the separation is accomplished because each component of the crude extract has a different polarity. More polar compounds will flow easily through the silica gel, while non-polar compounds will flow more slowly through the gel Silica gel Itself in non-polar, and thus is attracted to other non-polar molecules. The attraction of the non-polar molecules to the silica gel is what causes the non-polar components of a mixture to move slowly through the gel. Chemical separation relies on the relative speeds at which each component travels

from the top of the column to the bottom when using a silica gel chromatography column.

The slurry of silica gel was prepared by soaking with suitable solvent such as EtOH (19), subsequently poured into the column (2.5×25 cm) and washed with EtOH for one an hour to obtain better packing. Finally, it washed with hexane for one an hour to obtain hydrophobic conditions. Concentrated seed extract (5 ml) was loaded into the silica gel column and the active components were eluted successively with different polarity solvents using batch ways. These solvents include hexane, chloroform, ethylacetate, acetone, 95% EtOH, methanol (MeOH), and then distilled water (500-750 ml for each). Each fraction drains out of the column and have a sharp ending point which was checked by absorbance at 275 nm using a spectrophotometer (19).The oil was recovered from oil-solvent mixture by rotary evaporator at45°C and dried with anhydrous Na_2SO_4,as well as the water fraction was concentrated by rotary evaporator at 45°C and dried with anhydrous Na_2SO_4. Finally, the fractions of each solvent were stored in dark container at −20°C.

Antibacterial activity:

The effectiveness of the essential oils of *Nigella sativa* was evaluated against four bacterial species. The essential *Nigella sativa* oil extract was tested for antibacterial activity against *Enterobacter aerogenes* , *Escherichia coli*, *klepseilla pneumone* , *Staphyllococcus aureous* by the well diffusion method , Muller- hinton were inculated with plates with bacteria using loop full by spreading. Wells were cut into the agar and filled with the *nigella sativa* oil. Inoculated plates were incubated at 37c for 24 h. The antibacterial activity was evaluated by measuring the diameter of inhibition zone (19).

Preparation of Culture Media

The antibacterial activities of the oil- fractions were tested using Muller- Hinton plats by the well diffusion method (20). The medium was prepared according to them a manufacture company instructions, and it was sterilized by autoclave at 121ºC (1.5 psi/inch^2) for 15 min. Each 20 ml of sterilized medium was poured into disposable Petri dishes and they were incubated at 30 º C for overnight to ensure sterility, and stored at 4ºC until used. Muller- Hinton plats were inoculated with bacterial seed culture (1x10^8 CFU/mL) separately using a cotton swab by spreading. Wells were cut into the agar and filled with 50uL of the *N. sativa* oil-fraction. After that the plates were incubated at 37ºC for 24 h. The antibacterial activity was evaluated by measuring the diameter of inhibition zone (21).

Statistical Analysis

A one way analysis of variance ANOVA (Duncan) was performed to test whether group variance was significant or not, statistical significance was defined as significances were carried out using Graph Pad Prism version6(Graph Pad Software Inc., LaJolla, California USA,www.*graphpad*.com).

Results and Discussion

Nigella sativa L. seeds or Black cumin or black seeds are generally applied in traditional medicine. *Nigella* seed oil (NSO) composition is identified to be location-dependent. In the present study, the oil of *Nigella* seed was extracted by solvent. Oil extract yield was 40% when 96% ethanol was used for extraction and the oil was recovered from the oil-ethanol mixture by rotary evaporator at 45°C. This result was similar to previous studies which reported the *Nigella*-seed oil composed about 27-37% of the seeds depending on the extraction methods. Whereas the oil quality of the *Nigella sativa* seeds cultivated in the Kingdom of Saudi-Arabia was similar to that other origin, such as the Mediterranean and western countries (22).

The extracted oil was partially purified using batch wise adsorption chromatography method by silica gel. The results appeared the presence seven fractions depending on the polarity of solvents (hexane, chloroform, ethyl acetate, acetone, ethanol, methanol and water).

Antifungal Activity of *Nigella sativa* Oil Extract

The present study was considered to evaluate the antifungal activity of essential *N. sativa* oil against *F.solani*. The results demonstrated the capability of *N. sativa* oil to inhibit the tested fungus (*F. solani*), in which the growing of the fungal was significantly different for hexane fraction 24±2.1mm from chloroform, acetone, ethyl acetate, methanol and water fractions, which is the growth colony was 30±2.5mm, 40±3.7mm, 42±4.1mm, 37±2.9mmand 44±3.6 mm respectively, while it is not differ significantly from ethanol fraction which was 28±1.5mm. Since the growth of the fungi for acetone, ethyl acetate and water did not show any significant differences in growth rate as shown in the table (1-1)

Table (1-1): Effect of black seed oil fractions on fungal species

Solvent / Diameter of growth	Hexane	Chloroform	Aceton	Ethyl acetate	Ethanol	Methanol	Water
F.solani	**24±2.1^{c}**	**30±2.5^{b}**	**40±3.7 a**	**42±4.1 a**	**28±1.5^{c}**	**37±2.9^{b}**	**44±3.6^{a}**

A moderate inhibitory effect was recorded with chloroform and methanol fractions of the black seed, growth rate ranged between 30-37mm.In comparison with the high antifungal activity of hexane and ethanol fractions that ranged between 24-28mm. This inhibitory rate for *Fusarium solani* may be the result of thymoquinone which is isolated from *N. sativa* exposed high antifungal activity against *Aspergillus niger, Fusarium solani* and *Scopnlariopsis brevicaulis*; and this activity was similar to the amphotericin-B as an antifungal drug(23). Ethanolic extracts of the *Nigella sativa* seeds exhibited a strong inhibitory effect on the growth of *Aspergillus fumigates, Issatchenkia orientalis, Aspergillus flavus, Cryptococcus laurentii, Candida parapsilosis, Cryptococcus albidus, Candida albicans and Candida tropicalis*, and these extracts were more powerful than the Amphotericin-B (24). Two new defence

peptides (Small cysteine-rich cationic proteins) named Ns-D1 and Ns-D2 were isolated and identified from the *N. sativa* seed, these defence peptides exposed high and conflicting antifungal activity (25).

The present results of antimicrobial surveys of *Nigella* oil consisted with the previously reported studies of Bourgou *et al*. 2010 (26) and Javed *et al*. 2012(27). how found thymohydroquinone, thymoquinone, pcymene (monoterpene)and longifolene (sesquiterpene) of *Nigella* oil had a strong antifungal activity. Black seed oil has a high content of unsaturated fatty acids besides to small amount of thymohydroquinone which is accountable for its reasonable antimicrobial effects. Long chain fatty acids such as oleic acid and linoleic acid were previously described to own antifungal and antibacterial activities (28,29,30,27). The p'cymene is not an effective antimicrobial substance when used alone, though its activity is induced by other compounds like carvacrol (31). Also, the antimicrobial activity of black seed oil can often be correlated with its phenolics content.

Antibacterial Activity of *Nigella sativa* Oil Extract:

The results showed that the hexane fraction revealed a high antibacterial activity against Gram negative bacteria, such as the inhibition zone ranged from 18.3 mm for both *Escherichia coli* and *Enterobacter* to 12.6mm for *Klepsiella.* Whereas ethanol fraction had more significant potential activity against *Staphylococcus aureus* 22.3mm in comparing with hexane and chloroform fractions that had more effected on *Escherichia coli*, *Klepsseilla* and *Enterobacter* in the same time methanol have more inhibition growth activity against *Klepsiella pneumonia*e and *Staphylococcus aurous* that had inhibition zone 10± 2.9 and 14.3mm respectively. While each of water, ethyl acetate and acetone fractions did not show any antibacterial activity against tested bacteria as showen in table(1-2).

Table(1-2): Effect of black seed oil fractions on bacterial species

Bacterial species	Hexane	Chloroform	Aceton	Ethyl acetate	ethanol	Methanol	Water
Escherichia coli	18.3 ± 4.3^{a}	19.3 ± 3.5^{a}	2.6 ± 0.9^{c}	0^{d}	11 ± 2.1^{b}	6 ± 0.7^{c}	0^{d}
staphylococcus aurous	8.6 ± 2.7^{c}	3.3 ± 0.9^{c}	0^{d}	0^{d}	22.3 ± 5.4^{a}	14.3 ± 3.8^{b}	0^{d}
Klepsseilla pneumonia	12.6 ± 2.3^{a}	10 ± 1.4^{a}	0^{c}	0^{c}	7 ± 1.5^{b}	10 ± 2.9^{a}	0^{c}
Enteobacter aerogenes	18 ± 3.7^{a}	12 ± 1.9^{b}	4 ± 0.7^{c}	0^{d}	10.4 ± 3.1^{b}	11.3 ± 2.4^{b}	0^{d}

Thymoquinone is very important for many therapeutic studies due to its noticeable biological activities, e.g., antioxidant, anticarcinogenic and anti-inflammatory effects (32). Its potent growth-inhibiting activity against methicillin-resistant *S. aureus* has been described in a previous study carried on different quinonoid compounds (33). And it had a highly active substance, inhibiting Gram-positive bacteria (34,35).

Also, the present results agreed with earlier studies of Agarwwal that refer to the Hexane and chloroform fractions that found to be more effective on Gram-

negative than Gram-positive bacteria, which is in conformity with earlier studies (36,37).

The present results agreed with this study that refers to the activity of the *Nigella* seed oil against many strains of the bacterial group (38). This oil is rich in ellagic acid, flavonoids, polyphenols, phenols, ketones and alcohols, which possess therapeutic and antimicrobial properties (39). In addition, many active compounds (thymol, thymoquinone, thymohydroquinones, p-cyme`ne, carvacrol, a and b pinene, ketones, alcohols, etc.) were categorized in the oil fractions of *N. sativa* seeds and have bacteriostatic or bactericidal activity against many microbes (40,41,42,43).

Scandorieiro *et al*., 2016(44). suggested that hydrophobic bioactive compounds damage the cell membrane, increases cell permeability and affect biomolecule synthesis. According to Zhiri 2006 (40), these activities depend on the chemical composition, functional groups (alcohols, terpene, ketones and phenol compounds) and synergistic effects of the major components.

The result of another study indicates that the crude methanol extract had a definite antimicrobial activity against all the microbes tested, while the present study gave another result about partially purified methanol fraction which it had the effect on *Staphylococcus aureus* only that's may due to the ethanol crude extract was fractionated by several solvents one of them methanol that causes separation of active compounds within another fractions. This efficiency has been confirmed in many studies (45,46).The antimicrobial activity of *N. sativa* seed phenolic compounds by inhibiting bacterial protein synthesis (47).

CONFLICT OF INTERESTS.
There are non-conflicts of interest.

References

1-Maciel MAM, Pinto AC, Veiga Junior VF, Grynberg NF, Echevarria A (2002). Plantas medicinais: A necessidade de estudos multidisciplinares. *Química Nova*, 25:429-438.

2-Maria Jancy Rani P, Kannan PSM, Kumaravel S.(2011). GC-MS Analysis of Lantana camara L. Leaves. *JPRD,* 2(11): 63- 66.

3-Hadjzadeh MA, Keshavarzi Z, Yazdi TSA, Ghasem SM, Rajaei Z, Khajavi Rad A.(2012). Effect of alcoholic extract of *Nigella sativa* on cisplatin-induced toxicity in rat. *Iran J Kidney Dis*.,6(2):99–104.

4-Khare, C.P.(2004). Encyclopedia of Indian medicinal plants. NewYork: Springes-Verlag Berlin Heidelberg.

5-Harzallah,H. J.; Noumi,E.; Bekir, K. et al., (2012). Chemical composition, antibacterial and antifungal properties of Tunisian Nigella sativa fixed oil. *African Journal of Microbiology Research*, vol. 6, no. 22, pp. 4675–4679,

6-Arafa, E.S.A.; Zhu, Q. and Shah, Z.I. (2011).Thymoquinone up-regulates PTEN expression and induces apoptosis in doxorubicin-resistant human breast cancer cells. *Mutat Res.,* 706:28–35.

7-Al-Jassir, M.S. (1992). Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. *Food Chem*.,45:239–42.

8-Ataa-ur-Rahman, choudhary MI, Dur-e-shahwar, Parveenz, Jabbar A, Ali (1995). Antifungal steroidar lactones from withania coagulance. *phytochemistry* Nov;40(4):1243-6.

9-Nickavar B, Mojab F, Javidinia K and Amoli MAR(2003). Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran. *Zeit Fur Natur*. 58: 629-631.

10-Khader, M.; Bresgen, N. and Eckl, P.M. (2009). In vitro toxicological properties of thymoquinone. *Food Chem Toxicol*, 47: 129-133.

11-Badary, O.A.; Taha, R.A.; Gamal el-Din, A.M. and Abdel-Wahab, M.H (2003). Thymoquinone is a potent superoxide anion scavenger. *Drug Chem Toxicol*, 26: 87-98.

12-Fouda, A.M.; Daba, M.H.; Dahab, G.M. and Sharaf El-Din, O.A. (2008). Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. *Basic Clin Pharmacol Toxicol*, 103: 109-118.

13-Burits, M. and Bucar, F.(2000). Antioxidant activity of *Nigella sativa* essentialoil. *Phytotherapy Research*, 14:5, 323–328.

14-Mahmoud, S.S. and Torchilin, V.P. (2012).Hormetic/cytotoxic effects of Nigella sativa seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. *Cell Biochem Biophys*, 25(7): 1392-1398.

15-Abd El-Aziz M.A., Hassan H.A., Mohamed M.H., Meki A.M.A., Abdel-Ghaffar S.K.H., Hussein M.R.(2005). The biochemical and morphological alterations following administration of melatonin, retinoic acid and *Nigella sativa* in mammary carcinoma: an animal model. *Int J Exp Pathol.*, 86(6):383–396.

16-Effenberger, K.; Breyer, S. and Schobert, R.(2010). Terpene conjugates of the *Nigella sativa* seed-oil constituent thymoquinone with enhanced efficacy in cancer cells. *Chem Biodivers*, 7(1): 129-139.

17-Cheng L., Xia T.S., Wang Y.F., Zhou W., Liang X.Q., Xue J.Q. (2014).The anticancer effect and mechanism of α-hederin on breast cancer cells. *Int J Oncol*., 45(2):757–763.

18-Horvathova E., Navarova J., Galova E., Sevcovicova A., Chodakova L., Snahnicanova Z.(2014). Assessment of antioxidative, chelating, and DNA-protective effects of selected essential oil components (eugenol, carvacrol, thymol, borneol, eucalyptol) of plants and intact Rosmarinus officinalis oil. *J Agric Food Chem*., 62(28):6632–6639.

19-Omran, Zahraa M Al-Taee, Hayder O hashim, Mohammed J Al-Jassini (2017)."Extraction of phenolic compound as antioxidants from some plants and their cytotoxic activity against breast cancer cell line", *Asian journal of Pharmaceutical and Clinical Research*, 10,7.

20-Ali, N.A, Julich, W.D, Kusnick, C and Indequist, U.(2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J Ethnopharmacol*;74(4):173-179.

21-Gharby, S., Harhar, H., Guillaume, D., Roudani, A., Boulbaroud, S., Ibrahimi, M., ... & Charrouf, Z. (2015). Chemical investigation of Nigella sativa L. seed oil produced in Morocco. *Journal of the Saudi Society of Agricultural Sciences*, 14(2), 172-177.

22-Aljabre, S.H.M.; Alakloby, O.M, and Randhawa, M.A. (2015). Dermatological effects of Nigella sativa. *Journal of Dermatology and Dermatologic Surgery*, 10(2):92-98.

23-Raval, B.P.; Shah, T.G.; Suthar, M.P. and Ganure, A.L. (2010). Screening of Nigella sativa Seeds for antifungal activity. *Annals of Biological Research* 1(1): 164-171.

24-Rogozhin EA, Oshchepkova YI, Odintsova TI, Khadeeva NV, Veshkurova ON, e*t al.* (2011). Novel antifungal defensins from Nigella sativa L. seeds. *Plant Physiol Biochem*, 49(2):131-137

25-Bourgou, A. Pichette, B. Marzouk, and J. Legault(2010). "Bioactivities of black cumin essential oil and its main terpenes from Tunisia," *South African Journal of Botany*, vol. 76, no. 2, pp. 210–216.

26-Javed, A.A. Shahid, M. S. Haider *et al.*,(2012). "Nutritional, phytochemical potential and pharmacological evaluation of Nigella Sativa (Kalonji) and Trachyspermum Ammi (Ajwain)," *Medicinal Plants Research*, vol. 6, no. 5, pp. 768–775.

27- A. R. McCutcheon, T. E. Roberts, E. Gibbons et al.,(1995). "Antiviral screening of British Columbian medicinal plants," *Journal of Ethnopharmacology*, vol. 49, no. 2, pp. 101–110.

28-Khan,(1999). "Chemical composition and medicinal properties of Nigella sativa Linn," *Inflammopharmacology*, vol. 7, no. 1, pp. 15–35.

29-McGaw, A. K. Jäger, and J. Van Staden(2002). "Isolation of antibacterial fatty acids from Schotia brachypetala," *Fitoterapia*, vol. 73, no. 5, pp. 431–433.

30-Rattanachaikunsopon and P. Phumkhachorn,(2010). "Assessment of factors influencing antimicrobial activity of carvacrol and cymene against Vibrio cholerae in food," *Journal of Bioscience and Bioengineering*, vol. 110, no. 5, pp. 614–619.

31-Ali, B. H., and G. Blunden. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytother. Res.* 17:299–305.

32-D'Antuono, L.F., A. Moretti, and A.F.S. Lovato. (2002). Seed yield, yield components, oil content and essential oil content and composition of *Nigella sativa L.* and Nigella damascena L. *Ind. Crops Prod*., 15:59–69.

33-Stoyanova, A., E. Georgiev, A. Wajs, and D. Kalemba. (2003). A comparative investigation on the composition of the volatiles from seeds of Nigella sativa L. from Bulgaria. *J. Essent. Oil-Bear. Plant*. 6:207–209.

34-Singh, G., P. Marimuthu, C.S. de Heluani, and C. Catalan (2005). Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of Nigella sativa seeds. *J. Sci. Food Agric*, 85:2297–2306

35-Agarwal, R., M.D. Kharya and R. Shrivastava. (1979) Antimicrobial and anti helminthic activities of the essential oil of Nigella sativa Linn. Ind. *J. Exp. Biol*, 17: 1264-1265.

36-Ali, N. A, Julich, W.D, Kusnick, C and LIndequist, U. (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J Ethnopharmacol*; 74(2): 173-179.

37-Ara, N., M.H. Nur, M.S. Amran, M.I.I. Wahid and M. Ahmed, (2009). *In vitro* antimicrobial and cytotoxic activities of leaves and flowers extracts from *Lippia alba*. Pak. *J. Biol. Sci*., 12: 87-90.

38-Mahmoudi, H., Aouadhi, C., Kaddour, R., Gruber, M., Zargouni, H., Zaouali, W., Ben Hamida, N., Ben Nasri, M., Ouerghi, Z., Hosni, K., (2016). Comparison of antioxidant and antimicrobial activities of two cultivated Cistus species from Tunisia. *Biosci. J*., 32, 226–237.

39-Zhiri, A., (2006). Aromathe´rapie. Nutra news, science, nutrition, pre´vention et sante´, ed Fondation pour le libre choix, pp. 2–16.

40-Benkaci, A.F., Baaliouamer, A., Meklati, B.Y., Chemat, F., (2007). Chemical composition of seed essential oils from Algerian Nigella sativa extracted by microwave and hydrodistillation. *Flavour Fragr. J.*, 22, 148–153.

41-Halawani E. (2009). Antibacterial activity of thymoquinone and thymohydroquinone of *Nigella sativa* L. and their interaction with some antibiotics. *Adv Biol Res*, 3:148–52.

42-Salman, M.T., Khan, R.A., Shukla, I., (2016). Antibacterial activity of Nigella Sativa Linn. seeds against multiple antibiotics resistant clinical strains of Staphylococcus aureus. *Int. Arch. BioMed Clin. Res*., 2, 96–99.

43-Scandorieiro, S., Camargo, L.C., Lancheros, C.A.C., Yamada-Ogatta, S.F., Nakamura, C.V., Oliveira, A.G., Andrade, C.G.T.J., Duran, N., Nakazato, G., Kobayashi, R.K.T., (2016). Synergistic and additive effect of Oregano essential oil and biological Silver nanoparticles against Multidrug-Resistant bacterial strains. *Front. Microbiol.*, 7, 760.

44-Mason, T.L., Wasserman, B.P., (1987). Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. *Phytochemistry*, 26, 2197–2202.

الخلاصة

استخدمت بذور الحبة السوداء منذ آلاف السنين كتوابل ومواد حافظة للأغذية كما استخدمت هذه البذور في تعزيز الصحة ومكافحة الأمراض وخاصة في الشرق الأوسط. وفي هذه الدراسة استخلصت بذور الحبة السوداء مع 96 ٪ من الإيثانول وتم تنقيته كروماتوجرافيا بالسيليكا جيل مع مذيبات مختلفة. وأظهرت ألنتائج لمستخلصات الحبة السوداء على الفطر *F. Solani* أن كلا من مستخلص الهكسان والإيثانول من زيت بذور الحبة السوداء فعالية عالية مضادة للفطريات وكان قطر النمو 24 ± 2.1 mm و 28 ± 1.5 mm ،على التوالي في حين لم يظهر *F.Solani* أي حساسية لمستخلص ألأسيتون ، اثيل أسيتيت و المائي. كما تم اختبار جميع المستخلصات كمضاد للبكتيريا *E.coli* ، *S.aureus K.pneumonia* و . *Enterobacter aerogenes* كما وجد ان مستخلص الكلوروفورم كان ساما لل E.coli مع منطقة تثبيط 18.3 ± 4.3 mm و 19.3 ± 3.5 ملم اضافة الى ان لها نفس التأثير على *K.pneumonia* و *Enterobacter aerogenes* في حين كان تأثيرها ضعيفا على S.aureus ، في نفس الوقت الاسيتون، أثيل اسيتيت والماء لم تظهر أي تأثيرات على انواع البكتيريا الاخرى.

الكلمات الدالة: الحبة السوداء, الفعالية المضادة للميكروبات, النباتات الطبية, زيت الحبة السوداء,