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Antibacterial Screening of Essential Oil from Seeds of *Nigella sativa* and *Nigella orientalis*

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Abstract: The antioxidant activity of essential oil extracted from the dry seeds of *Nigella sativa* and *Nigella orientalis* were evaluated by the scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and the reducing power assay, total phenolic and flavonoids are also detected. The essential oil extracted from *Nigella sativa* and *Nigella orientalis* were screened for antimicrobial activities against some pathogens viz., *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus, Acinetobacter* sp. *and Klebsiella pneumonia*. The result obtained from the *in vitro* antioxidant screened showed that oils from *Nigella sativa* and *Nigella orientalis* have considerable amounts of polyphenolic and flavonoids compounds which are responsible for the antioxidant properties. And also they give the higher reductive potential due to reducing capacity and DPPH free radical scavenging activity which serves as strong indicator of antioxidant activities. The essential oils from *Nigella sativa* and *Nigella orientalis* were found to produce significant inhibition against all the pathogens. In conclusion, the present study suggests that oil extracted from *Nigella sativa* and *Nigella orientalis* have potential activity as source of natural antioxidant.

Key words: *Nigella sativa* · *Nigella orientalis* · Antioxidant activity · DPPH · Free radical and Antibacterial

INTRODUCTION

Nigella sativa (NS) and *Nigella Orientalis* (NO) has been used as a natural healing aid for thousands of years by various cultures and civilizations around the world, as well as a supplement to help maintaining good health. It is most famous for the saying of the holy prophet Muhammad P.B.U.H. "Hold on to use of the Black seed, for it has a remedy for every illness except death." The word "hold on to" indicates a long term use. The seeds of NS (*Ranunculaceae*) are commonly used in folk (herbal) medicine all over the world for treatment and prevention of a number of skin diseases and conditions [1, 2]. The seeds contain both fixed and essential oils, proteins, alkaloids and saponins [3, 4]. Much of the biological activities of the seeds have been shown to be due to the thymoquinone, in which the major component of the essential oils and volatile oil, that have been reported include protection against neuro and hepatotoxicity induced by either disease or chemicals [1]. It would appear that the beneficial effects of the use of seeds and thymoquinone might be related to their cytoprotective and antioxidant actions and to their effect on some mediator of inflammation [5].

El-Kadi and Kandil [6] investigated the effect of NS on immune system and reported that the administration of 1 g twice daily in human volunteers enhanced immune functions as manifested by improve helper T cells and natural killer cell activity. However, Fararh [7] demonstrated that NS seeds and its purified proteins enhanced the production of interleukin-3 by human lymphocytes and have an effect on macrophages as well. In other study Hanafi [8] observed that NS promote stimulatory and suppressive effects depending upon the donor and concentration used. In the same root of study, it was reported that the ethanolic extract of NS has been shown to potentiate cellular immune responses [9].

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Many plant extracts have antibacterial effect on *Staphylococcus aureus, Streptococcus pyogenes* and Candida albicans by inhibiting both proliferation of bacteria and inflammation caused by antigen. Furthermore, it was suggested that the use of such extracts as medicinal drugs, which affectively moisten (Tran epidermal water), prevent and heal dermatitis [10, 11], reported that NS extracts had antibacterial effect on gram positive (*Staphylococcus aureus*) and gram Negative (*Pseudomonas aerogenosa* and *Escherichia coli*) in concentration dependent manner. Crude extracts of NS were also found to have a promising effect on multi antibiotic resistant organisms [12]. NS and some of its active principles, thymoquinone, had been shown to possess protective effect against hematological, hepatic, renal and other toxicities induced by anticancer drugs and some toxins [13]. The moquinones of the fixed oils were also inhibit non enzymatic peroxidation in brain phospholipid lysosomes [14, 15]. Burits [3] found the essential oil and its constituents to have antioxidant effect on different chemical assays and OH radical scavenging property for non-enzymatic lipid peroxidation in liposomes and the deox-ribose degradation. Mabrouk *et al*. [16] studied the protective effect of bee honey and NS seeds on the oxidative stress and carcinogenesis induced by methylnitrosourea (MNU) in rats. The results suggested that supplementation of diet with honey and NS protects from such stresses. Regarding the mode of action of NSO, it was found that such oil modulated the fibrolytic potential of fibrosarcoma cell-line and elucidated that the oil might be used as anti-tumor and implies the inhibition of local tumor invasion and metastasis [17]. The main aim of this study was to determine the antimicrobial and antioxidant activity of essential oil from seeds of *Nigella Sativa* and *Nigella orientalis.*

MATERIALS AND METHODS

Plant Material: The seeds of *Nigella sativa* and *Nigella orientalis* of good quality were obtained from a local market, Benghazi, Libya, 2013.

Bacteria Used: Bacteria were taken from the laboratory of microbiology in Banghazi Medical centre, which are known as multi drug resistant bacteria. The bacteria used were *Escherichia coli* (MDR) ATCC, *Staphylococcus aureus* (MDR) ATCC, *Pseudomonas aeruginosa* (MDR) ATCC, *Klebsiella pneumonia* (MDR) and *Acinetobacter sp*. (MDR). Other bacteria were not multi drag resistant such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The organisms were isolated and identified by standard methods and identification confirmed by using phonex. The organisms were then sub cultured and maintained on nutrient agar slants.

Chemicals: 1, 1-Diphenylpicrylhydrazyl (DPPH•) was supplied from Sigma. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, anhydrous sodium sulfate and pyrogallol were obtained from the biochemistry laboratory of chemistry department-Benghazi University.

Extraction of Volatile Oil: The dry powdered seeds of *Nigella sativa* and *Nigella orientalis* (200g) were subjected to hydrodistillation using Clevenger apparatus. The isolation of volatile oils was complete within 6 hours [18].

Antioxidant Activities Assays and Quantitative Analysis: All of this experimental work has been conducted in biochemistry laboratory at Benghazi University.

Total Phenolic Contents (TPC): Total concentration of phenolic compound in the essential oils obtained from *Nigella sativa* and *Nigella orientalis* were estimated using the colorimetric method based on Folin-Ciocalteu reagent [19]. Quantification was done with respect to standard calibration curve of Pyrogallol the results were expressed as pyrogallol (μg/ml).

Total Flavonoids Contents (TFC): Aluminum chloride colorimetric method was used for determination [20]. The calibration curve was obtained by preparing different quercetin solutions in methanol at concentrations of 100 to 500 μg/ml.

Reducing Power Assay (RPA): The reducing power was determined according to the [21]. Quantification was done with respect to standard calibration curve of ascorbic acid the results were expressed as ascorbic acid (μg/ml).

Potassium ferricyanide + ferric chloride $\xrightarrow{\text{antioxidant}}$ potassium ferricyanide + ferrous chloride.

DPPH Free Radical Scavenging Activity (RSA): The antioxidant activity of the essential oils was measured in terms of hydrogen donating or radical-scavenging ability

using the stable DPPH• method as modified by [22]. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:

% DPPH "RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] × 100

Antibacterial Activities of Essential Oils Extracted from the Seeds of *Nigella sativa* and *Nigella orientalis*: In this study diluted essential oils extracted were used. The diluted different extracts were prepared by using Dimethyl Sulfoxide (DMSO) to obtain 60% (v/v) concentration. DMSO was used as negative control. A screening assay using well diffusion [23]. Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100ì L^{-1} bacterial suspensions on plates (overnight cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation 9 mm diameter wells were cut into the surface of the agar using a sterile cork borer. The concentrations (60 %) were added to the wells. Plates were incubated at 37°C for 24 h. Control wells contained solvent DMSO. Zones of inhibition were measured by using ruler. The diameter of zones was recorded. Each assay was carried out in triplicate. The antibacterial assay plates were incubated at 37°C for 24hr. The effect of essential oils extracted on the tested bacteria was compared with the sensitivity of the same bacteria to five antibiotics Colisti sulphate, Amicacin, Amoxycillin, gentamicin and sulphamethoxazole trimethoprim (60µg/ml) [23, 24, 25, 26].

RESULTS AND DISCUSSION

Table 1 shows the results obtained from the essential oils extracted from *Nigella sativa* and *Nigella orientalis*, where the results showed that essential oil from the *Nigella orientalis* having higher total phenolic contents. The essential oil extracted from *Nigella orientalis* contain highly significant amount of flavonoids compounds as compared with the qurecetin which is used as standard as shown in Table 2.

The reducing power assay of essential oils extracted from *N. sativa* and *N. orientalis,* exhibit higher reducing activity than the ascorbic acid (Table 3). The result of the DPPHÿ radical scavenging activity of essential oils extracted from *N. sativa* and *N. orientalis* are shown in Table 4, this result compared with the well-known antioxidant ascorbic.

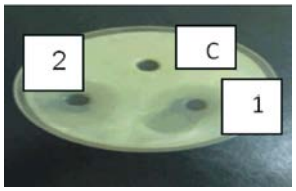

Fig. 1: Antibacterial activity of essential oils extracted of *Nigella sativa* and *Nigella orientalis* against *Klebsiella. pneumonia* bacteria at 60 % concentrations. 1=*Nigella sativa*, 2 = *Nigella orientalis*, C=control.

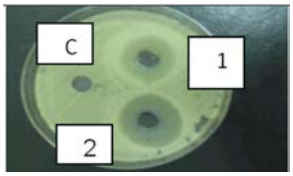

Fig. 2: Antibacterial activity of essential oils extracted of *Nigella sativa* and *Nigella orientalis* against *E. coli* bacteria at 60 % concentrations. 1=*Nigella sativa*, 2 = *Nigella orientalis*, C = control.

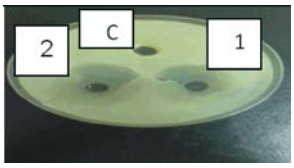

Fig. 3: Antibacterial activity of essential oils extracted of *Nigella sativa* and *Nigella orientalis* against *Staphylococcus aureus* bacteria at 60 % concentrations 1=*Nigella sativa*, 2 = *Nigella orientalis*, C= control.

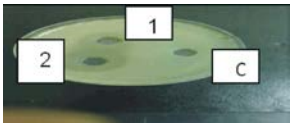

Fig. 4: Antibacterial activity of essential oils extracted of *Nigella sativa* and *Nigella orientalis* against *Pseudomonas aeruginosa* bacteria at 60 % concentrations. 1=*Nigella sativa*, 2 = *Nigella orientalis*, C = control.

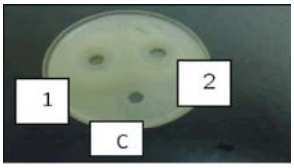

Fig. 5: Antibacterial activity of essential oils extracted of *Nigella sativa* and *Nigella orientalis* against Acinetobacter sp. Bacteria at 60 % concentrations. 1= *Nigella sativa*, 2 = *Nigella orientalis*, C = control.

Antibacterial Activity Assay: The results of the well diffusion test revealed that the essential oils extracted from the seeds of *Nigella sativa* and *Nigella orientalis* showed different degrees of growth inhibition, depending upon the bacterial strains (Table 5 and Figs. 1, 2, 3, 4 & 5) essential oil extracted of the *Nigella orientalis* was observed to be more active than *Nigella sativa* oil extract as compared to the antibiotic of standard (Table 6).

This studied that the oils rich in phenolic constituent such as α-pinene, Longifolene and 4-terpineol have the highest antioxidant activity against bacteria. [26] reported that the α-pinene and Pcymene have an antioxidant property, in fact, α-pinene found in these oils, act on the cell membrane increasing its permeability. [29] reported the antibacterial effects of hydrous, methanolic and ethanolic extracts of *Nigella sativa* on Gram-positive and

Table 1: Total phenolic content (TPC) of essential oil extracted from *Nigella sativa* and *Nigella orientalis.*

Concentration of Pyrogallol (µg/ml)	Mean ± Standard Deviation	Concentration of essential oil extracted from *Nigella sativa* (µg/ml)	Mean ± Standard Deviation	Concentration of essential oil extracted from *Nigella orientalis* (µg/ml)	Mean ± Standard Deviation
100	1.17± 0.032	100	0.863 ±0.025	100	1.526± 0.011
200	1.18 ± 0.022	200	0.957 ±0.037	200	1.810± 0.038
300	1.520 ± 0.0045	300	1.034 ± 0.028	300	1.824± 0.029
400	1.828 ± 0.0117	400	1.22 ±0.031	400	1.916± 0.019
500	2.105 ± 0.0225	500	1.314 ± 0.044	500	1.970± 0.034

Table 2: Total flavonoids content of essential oil extracted from *Nigella orientalis* and *Nigella sativa*.

Concentration of qurecetin "µg/ml"	Mean ± Standard Deviation	Concentration of essential oil extracted from *Nigella sativa* "µg/ml"	Mean ± Standard Deviation	Concentration of essential oil extracted from *Nigella orientalis* "µg/ml"	Mean ± Standard Deviation
100	0.6440± 0.092	100	0.263 ± 0.001	100	0.894± 0.088
200	0.666 ± 0.035	200	0.403 ± 0.09	200	0.956 ± 0.032
300	0.834 ± 0.003	300	0.611 ± 0.003	300	1.102 ± 0.090
400	1.066 ± 0.009	400	0.821 ± 0.02	400	1.178 ± 0.052
500	1.300 ± 0.006	500	1.00 ± 0.01	500	1.268 ± 0.011

Table 3: Reducing power assay of essential oil extracted from *Nigella sativa* and *Nigella orientalis*.

Concentration of vitamin C (µg/ml)	Mean ± Standard Deviation	Concentration of essential oil extracted from *Nigella sativa* (µg/ml)	Mean ± Standard Deviation	Concentration of essential oil extracted from *Nigella orientalis* (µg/ml)	Mean ± Standard Deviation
100	0.201 ± 0.0280	100	1.282 ±0.0280	100	1.258± 0.027
200	0.495 ± 0.0350	200	1.286 ±0.0288	200	2.620± 0.019
300	0.697 ± 0.0087	300	1.440 ± 0.0234	300	2.740± 0.020
400	0.992 ± 0.0727	400	1.544 ±0.063	400	2.860± 0.011
500	1.201 ± 0.0305	500	1.550 ± 0.0227	500	2.870± 0.023

Table 4: DPPH radical scavenging activity of essential oil extracted from *Nigella sativa* and *Nigella orientalis* according to % inhibition

Concentration of vitamin C (µg/ml)	Percent inhibition (%)	Concentration of essential oil extracted from *Nigella sativa* (µg/ml)	Present of inhibition (%)	Concentration of essential oil extracted from *Nigella orientalis* (µg/ml)	Percent inhibition (%)
100	92.3%	100	80%	100	92.7%
200	93.8%	200	83.0%	200	95.0%
300	95.1%	300	85.1%	300	96.6%
400	95.8%	400	88.9%	400	97.2%
500	96.7%	500	92.0%	500	97.4%

Table 5: Screening of antibacterial activity of essential oil extracted of *Nigella sativa* and *Nigella orientalis*.

Bacteria	Essential oils	
Escherichia coli	1	2
	++	++
Staphylococcus aureus	1	2
	+	++
Klebsiella pneumonia	1	2
	++	++
Acinetobacter sp.	1	2
	++	+++
Pseudomonas aeruginosa	1	2
	+	++

Key: ++++ =>20 mm, +++ =15-20 mm, ++ = >5 -15 mm, + = 1-5 mm presence of anti-bacterial activity, mainly Bactericidal . - = no Inhibition (absence of activity), 1= *Nigella sativa*, 2 = *Nigella orientalis*, C=control.

Table 6: Antibiotic activity of different type of bacteria.

	Zone of Inhibition (mm) ± Standard deviation				
Antibiotic	EC	SA	PA	AS	KP
Colisti sulphate	-	0±.012	3±0.01	0±.036	0±.014
Amicacin	15±0.02	0±.0213	9±0.01	-	0±.0412
Amoxycillin	-	3±0.01	-	-	0±.012
Gentamycin	10±0.03	6±0.01	5±0.02	3±0.03	0±.011
Sulphmethoxazole	3±0.12	19±0.03	-	0±.084	-

Values are expressed as Mean (X) + SD, n = 3. EC= *Escherichia coli,* PA = *Pseudomonas aeruginosa,* KP = *Klebsiella pneumonia,* SA= *Staphylococcus aureus,* AS = *Acinetobacter sp.*

Gram- negative bacteria. The results showed that of this plant had antibacterial action on methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis*, but they were weakly active against Gram-negative bacteria such as *Pseudomonas aeruginosa* and enteropathogenic *Escherichia coli* [28]. Gram-negative bacteria are more resistant to antibiotics than the Gram-positive bacteria due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [29].

Chemical constituent of *Nigella sativa* volatile oil contains in the % Range (W/W) of Thymoquinone 27.8, Carvacrol 5.8-11.6, Pcymene15.5-31.7, α-pinene 9.3, 4-terpineol 2-6.6, Longifolene 1-8, t-anethole 0.25-3, Reduction product of thymoquinone. 16. [30, 31, 32]. The seeds of *N. sativa* have been subjected to arrange of pharmacological investigations in recent years. These studies have showed a wide spectrum of activities such as antibacterial [33, 34], antitumor [35], anti-inflammatory [36, 37], CNS depressant and analgesic [38], hypoglycemic [39], smooth muscles relaxant [40, 41, 42] cytotoxic and immunostimulant [43].

CONCLUSIONS

It may be concluded from this study that *Nigella sativa* and *Nigella orientalis* seed extract has antimicrobial activity against MRSA. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

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