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Molecular composition and antibacterial effect of essential oil of *Nigella sativa*

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The aim of this work was to test the antibacterial effects of essential oil extracted from *Nigella sativa* strains on gram positive and negative (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*) bacteria. This study was based on extraction by steam distillation and analysis of organoleptic and physico-chemical properties of the essential oil of Nigel. The examination of the organoleptic properties of this essential oil shows that it is relatively comparable to those cited in the AFNOR. The anti-bacterial and antifungal properties of this oil reveal an important anti-microbial effect. Moreover, it shows an excellent activity against the Gram positive strains studied as compared to Gram-strains. The essential oil of black cumin possesses a very interesting antimicrobial effect against the pathogenic bacteria and fungi studied so far.

Key words: Antimicrobial effect, essential oils, *Staphylococcus aureus*, medicinal plants.

INTRODUCTION

As commonly known in the past, essential oils are oily substances, very odorous and volatile. They have a high biological activity as antibacterial, antiviral, antifungal and antioxidant (Aligiannus et al., 2001; Salehi et al., 2005; Abdelwahab et al., 2011). They are also used in cancer treatment (Sylvestre et al., 2006). Essential oils are complex mixtures of products derived from the terpenes (monoterpenes and sesquiterpenes) which are hydrocarbons of the general formula $(C_5H_8)_x$ (Mohamed et al., 2010). The oxygenated compounds which are derived from these hydrocarbons are alcohols, aldehydes, esters, ethers, phenols and oxides.

The pharmacological potential of the essential oil extracted from *Nigella sativa* is of considerable interest nowadays. Indeed, as an illustration, we can cite the work of Agrawal et al. (1979) and Aljabre et al. (2005) who showed that the essential oil of *N. sativa* seeds exhibits a broad spectrum of inhibition against many bacterial strains even diluted at 1%. They thus demonstrated that the dithymoquinone present in this oil has a significant antimicrobial activity on bacteria (Gram positive and negative).

The aim of this study was to analyze the antibacterial activity of the essential oil of *N. sativa* on a set of pathogenic bacteria responsible for many dreaded diseases in the region of Chlef in Algeria. One of the key points of this work is the determination of the chemical profile of this oil in order to identify the active molecules responsible for the inhibitory effects.

MATERIALS AND METHODS

Extraction of essential oil

The biomass used for the extraction of the essential oil is grains of *N. sativa* grown in Syria (2011 harvest). The extraction method is

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steam distillation; the solvent used is diethyl ether. The retrieved essential oils are stored in tinted glass bottles placed in a cool, dry place at 4°C and protected from light in a Clevenger type apparatus according to the technique described by Simard et al. (1988). An organoleptic character set of these oils was achieved.

Chemical analysis of samples

In order to identify their active ingredients, *N. sativa* seeds were studied by photochemistry which revealed that these grains are much richer in secondary metabolites and their content varies according to the geographic and climatic conditions as well as the research methods (extraction and detection). The essential oil was analyzed on a Hewlett-Packard gas chromatograph of type Agilant 6890N controlled by ChemStation (NIST 98) and equipped with a capillary column HP5MS (30 m x 0.25 mm x 0.25 μm) coupled to a mass spectrometer (SM) type Agilant 5973. The analytical conditions are as follows: injection of 0.5 gl in split mode 1/50; injection temperature of 250°C; capillary column HP5MS (30 m x 0.25 mm x 0,25 μm); temperature programming: 50°C for 0 min, 4°C/min until 250°C for 30 min; flow of carrier gas: helium (1 ml/min), mass spectrum model Agilant 5973; temperatures: interface (280°C), strains (230°C), quadripole (150°C).

Identification of components

The various constituents of the essential oil were identified by comparing their mass spectra with those of the compounds of the databases WILLET and NIRST 98, the mass spectrometer gas chromatography/mass spectroscopy (GC/MS) and those of Adams spectral databases. The identification of the molecules was confirmed by comparison of their retention indices with those known in the literature (Adams, 2001). Retention indices of the compounds were calculated using the retention time of a series of n-alkanes using a linear interpolation.

Study of the antimicrobial effect

The method used is the holder-germs disks. It is based on the principle of impregnating disks with oil and seeding Petri dishes by the pathogenic bacteria. This method is described by Carson et al. (2006). The bacteria used in this work are: *Escherechia coli* ATCC 25922, *Enterococcus faecalis* 8V, *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa* ATCC 27583*, Staphylococcus aureus* ATTC 6538 and *Klebsiella pneumonia*. These bacteria are isolated from hospital samples. They were identified in the Microbiology Laboratory of the Hassiba Benbouali University, Chlef, Algeria.

Aromatogram

The principle of this method is to pour aseptically agar culture medium Mellir Hinton (MH) supercooled in Petri dishes at a rate of 15 ml per box. 1 ml of each bacterial culture suspension (106 CFU / ml and prepared from a culture of 18 h and then spread on the surface of agar medium) was allowed to cool and solidify.

Deposits of discs

Using a sterilized clamp, we took a sterile cellulose disk (disk reference filter paper MN 640W, Macherey-Nagel GmbH & co. KG Germany, 6mm) and soaked it with HC to be tested by merely making it have contact with the end of the disc. This will gradually absorb the EO to the total impregnation of the disc (54l), and then is deposited on the agar. The Petri dishes were then sealed and left to diffuse at room temperature for 30 min and placed in an incubator at 37°C for 24 h. The experiment was repeated three times for each species. The reading was performed by measuring with a ruler, the inhibition diameter around each disc. According to Roques et al. (2003), a strain is said to be sensitive if the diameter is >13 mm and resistant if it is <6 mm. It is said to be intermediate for inhibition diameter within this range.

Antibiogram

In Petri dishes containing MH agar seeded with a bacterial load defined for each pathogen, the antimicrobial disks streptomycin and penicillin are applied to the surface. The plates are incubated at a temperature of 37°C for 18 to 24 h. Reading is done by measuring the zone of inhibition that is represented by a halo around each disc where no growth is observed.

Minimum inhibitory concentrations (MIC) determination

The MIC is defined as the lowest concentration of product for which no growth is visible as compared to the control without product after a period of incubation at 37°C. The minimum inhibitory concentrations (MICs) of the essential oil are determined according to the method reported by (Pinto et al., 2006, Savadogo et al., 2004). Due to the immiscibility of the essential oil to the water and thus the culture medium, emulsification was performed with a solution of 0.2% agar to promote contact germ/compound. Dilutions were prepared in 1/50, 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000, 1/4000 (v/v). The tubes were shaken properly before pouring into Petri dishes. Control containing the culture medium agar solution plus 0.2% was also prepared. Seeding was done by flooding 1 ml pre-culture of pathogenic bacteria. Incubation was done at 37°C for 24 h.

RESULTS AND DISCUSSION

N. sativa seeds showed moisture content of 7.21%. The yield of essential oils was 1.38%. Imikraz (2006) obtained a yield of 1.5%, the highest yields of essential oils were collected during the flowering period (May to June) (Willem, 2004).

The physical and organoleptic properties provided a means of verification and quality control. The tests are determined according to a specific protocol and obeying the standards set by AFNOR (1989). The appearance of the oil is liquid, oily, clear, and its smell is strong, specific and characteristic of the crushed seed. Its color is greenish yellow. Concerning its physical characteristics, it has a density of 0.76 g/l, its refractive index is 1.3770 and its optical rotation is 20°.

The chemical analysis showed that the oil is rich in monounsaturated fatty acids such as oleic acid. There are also monoterpenes such as beta-phellandrene, beta-pineme, the liomoneme and sesquiterpenes such as caryophylene, polyphenols and aromatic aldehydes such

Table 1. Molecular composition of the essential oil of *N. sativa*.

Component	Retention time (s)	(%)
Beta-phellandrene	8.77	0.12
Beta-pineme	9.00	0.12
Limoneme	10.60	0.16
Terpineme	11.60	0.60
Linalole	13.10	0.50
Terpinehol	15.90	0.31
Geraniol	22.59	0.52
Caryophyllen	24.04	0.17
Tetradecanoic acid	34.11	0.11
Tridecanoic acid	39.15	0.33
Eicosane	40.00	0.17
Heneicosane	42.34	0.14
Phytol	42.50	0.19
Heptacosane	42.70	0.38
Hentricontane	42.80	0.12
Octadecadienoic acid	43.00	0.47
Oleic acid	43.20	0.51
Octacosane	43.60	0.80
Nonahaxacantonic acid	43.75	0.11
Tricosane	46.70	0.49
Docosane	47.20	0. 39
Tetracosane	48.81	0.46
Tetratriacantane	51.60	0.20
Docosenamide	58.70	0.53

as benzaldehyde. We noted also the presence of saturated fatty acids such as stearic acid and polinic acid and natural antioxidants such as E-vitamin and iron-phosphorus minerals. The results are presented in Table 1. Concerning the antibacterial effect, the inhibition zones have diameters ranging from 8.33 mm for *Klebsiella pneumonia* to 18 mm for *Proteus mirabilis* (Table 2).

According to Roques et al. (2003), a strain is sensitive if the diameter of the inhibition zone exceeds 13 mm. It is resistant if the disc diameter is 6 mm. In between, the strain is called intermediate. Hence, the most sensitive strain is *P. mirabilis*. The most resistant is *K. pneumonia*.

On the basis of the work of Topozada et al. (1965), *Salmonella* and *Pseudomonas* bacteria are very sensitive to the essential oil of *N. sativa*. Rouibi (2009) reported that with the exception of *E. coli* ATCC 25922, other Gram negative strains are less sensitive than Gram positive strains. This resistance is due to the chemical composition of the wall which is rich in lipopolysaccharides not allowing the penetration of hydrophilic molecules.

According to Agrawal et al. (1979) and Aljabre et al. (2005), the essential oil of *N. sativa* presents a wide inhibition spectrum against many bacterial strains.

After a 48 h incubation of bacteria at 37°C, the essential oil of *N. sativa* exerted a bacteriostatic action against *E. coli* ATCC 25922, *E. faecalis* 8V, *P. aeruginosa* ATCC 27583, *S. aureus* ATCC 6538 and *K. pneumonia*. By contrast, its action on *E. faecalis* and *P. mirabilis* 8V is bactericidal.

The antibacterial activity of the essential oil of nigel can be explained by its chemical profile which is rich in terpene hydrocarbons, such as Beta-pineme. These have several biological activities and are anti-bacterial, anti-inflammatory, antiviral, sedatives (Duke, 1998; Ghamni et al., 2007). Due to the complexity of the chemical composition of the essential oil, the observed antibacterial activity may be due to the presence of interactions between the various components.

The essential oil of *N. sativa* possesses a wide inhibition activity spectrum on pathogenic bacteria for humans. A more careful analysis should be performed *in vivo* in order to determine its real effects. In particular, the sesquiterpenes and their derivatives seem to be a promising class of natural compounds in the search for new antibacterial agent (Modzelewska et al., 2005).

For essential oils, the therapeutic dose is very close to the lethal dose. So the calculation of CMI is very important. The results are reported in Table 3.

MIC results depend on the strain tested and the dilution

Table 2. Results of the interaction of the essential oil of *N. sativa* with the bacterial strains.

Bacteria	Mean inhibition zone (mm)	Appearance of bacterial growth in the zone of inhibition after over 48 h incubation
Escherechia coli ATCC 25922	15±0.05	Positive
Enterococcus faecalis 8V	8.5±0.15	Positive
Salmonella typhi	14.8±0.2	Negative
Proteus mirabilis	18±0.05	Negative
Pseudomonas aeruginosa ATCC 27583	11.7±0.11	Positive
Staphylococcus aureus ATTC 6538	12.6±0.03	Positive
Klebsiella pneumonia	8.33±0.5	Positive

Table 3. MIC results of *N. sativa.*

Seed	Dilution								
	Control	1/50 (v/v)	1/100 (v/v)	1/250 (v/v)	1/500 (v/v)	1/1000 (v/v)	1/2000 (v/v)	1/3000 (v/v)	1/5000 (v/v)
Escherechia coli ATCC 25922	+	-	-	+	+	+	+	+	+
Enterococcus faecalis S.V	+	-	+	+	+	+	+	+	+
Salmonella typhi	+	+	+	+	+	+	+	+	+
Proteus mirabilis	+	-	-	+	+	+	+	+	+
Staphylococcus aureus ATCC 6538	+	-	-	+	+	+	+	+	+
Pseudomenese aeroginosa ATCC 27583	+	-	-	-	+	+	+	+	+

v/v: Ratio of the volume of the essential oil to the volume of agar solution; + presence of growth; - absence of growth.

used. Control presented bacterial growth (+), which means that the essential oil alone is responsible for the inhibition of pathogenic species.

E. coli and *S. aureus* were inhibited at a dilution of 1/250 which corresponds to a concentration of 0.4%. Oussalah et al. (2005) conducted a study on the MIC of several essential oils on *S. aureus*. Among the 28 essential oils tested, 20 showed a MIC <0.4%, including *N. sativa* essential oil.

Different pathogens showed MIC at different dilutions, except *P. mirabilis* and *S. aureus* which showed the same MIC which is 1/250. *S. typhi* presented a remarkable resilience against our essential oil. According to Agrawal et al. (1979), *N. sativa* essential oil, even diluted to 1%, has a broad spectrum of inhibition against many pathogenic strains.

The antibacterial activity is mainly due to the methanol part of *N. sativa* as thymol and thymoquinone (Randhawa and Al Ghamdi, 2002). These are present in the soluble portion of the methanol essential oil of *N. sativa* (Abou Basha et al., 1995).

According to a study by Kahsar (2002), thymoquinone present in the essential oil of *N. sativa* exerted a remarkable inhibitory action on different bacterial strains. Mohammad et al. (2013) reported that extracts of *N. sativa* showed high inhibitory activity against a range of bacteria resistant to antibiotics. This inhibition is due to the part of the methanolic EO. The methanolic extracts of the part act by inhibiting the synthesis of the cell wall by inducing changes in the structure of the membranes by inhibiting bacterial protein synthesis. The mechanism of inhibition by extracts remains however unclear.

Based on the work of Canonica et al. (1963), the essential oil of *N. sativa* is rich in pharmacologically active constituents including thymoquinone (2-isopropyl-5-methyl-benzoquinone), which can reach 27.8% of ET, convacrol (2-methyl-5-1-methyl-ethyl), a phenol which is also recognized by 2-hydroxy-p-cymen isothymol or where the percentage may vary from 5.8 to 11.6%. Phenols bring irreversible damage to membranes and are very useful in bacterial, viral and parasitic diseases. Eugenol and corvacrol are responsible for fungicidal and bactericidal activities of essential oils that contain them.

Mahmoudi (1990) mentioned that the essential oil of *N. sativa* cultivated in the Algerian sahara and extracted by two different methods, hydrodistillation and microwave distillation, was analyzed by GC and GCMS and gave two compounds: beta-pinème, which is the major compound and the thymoquinone compound.

Khsai (2002) confirms the effects of thymoquinone against different bacteria and he also noted that the minimum inhibitory concentration of essential oils of *N.*

sativa is 3.6 mg/ml with respect to *S. aureus* and this inhibition affects the synthesis of mRNA and protein synthesis.

According to Bakathir and Abbas (2010), the antibacterial effect can be attributed to thymoquinone. Raman et al. (1995) presented an intro study of the antibacterial effect, which shows the role of alpha-pinème against *S. aureus*.

Agawal et al. (1979) and Aljabre et al. (2005) showed that the thymoquinone and the methanol extract of *N. sativa* exert significant antimicrobial activity on Gram positive and negative bacteria. Phenolic compounds of the essential oil sensitize membrane phospholipids, which leads to an increase in the permeability and causes a leakage of intracellular components including bacteria enzyme systems (Singh et al., 2002).

Conclusion

In conclusion, the essential oil of *N. sativa* extracted by steam distillation of water gave a yield of 1.38%. The study of the chemical profile shows that this oil is very rich in bioactive molecules that can be used for therapeutic purposes or may be precursors for the synthesis of new drugs (Sofowara, 1993).

The study of the antibacterial effect on pathogenic strains of the region of Chlef in Algeria shows that this oil has a broad spectrum of activity. At a concentration of 0.4%, the inhibition is present against *E. coli*, *S. aureus* and *P. mirabilis*. For *E. faecalis* SV, *S. thermophilus* and *P. aeruginosa*, inhibition is present at a concentration of 2%.

Finally, *in vivo* testing is necessary to complete this study.

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