J. Mater. Environ. Sci. 5 (6) (2014) 2017-2020
ISSN : 2028-2508
CODEN: JMESCN

Chemical composition and antibacterial activity of essential oil of *Nigella sativa* seeds from Beni Mellal (Morocco): What is the most important part, Essential Oil or the rest of seeds?

T. Ainane[1]*, Z. Askaoui[1], M. Elkouali[1], M. Talbi[1], S. Lahsasni[2], I. Warad[3], T. Ben Hadda[4]

[1] *Laboratoire de Chimie Analytique et Physico-chimie des matériaux, Facultés de Sciences Ben Msik, Université Hassan II, BP 7955 Casablanca-20700, Morocco.*
[2] *Department of Chemistry, Science College, King Saud University, Riyadh, Kingdom of Saudi Arabia.*
[3] *Department of Chemistry, Science College, An-Najah National University, P.O. Box 7, Nablus 0092, Palestine.*
[4] *Laboratoire Chimie Matériaux, Faculté des Sciences, Université Mohammed Premier, Oujda-60000, Morocco.*

Received 1 Aug 2014, Revised 7 Sept 2014, Accepted 8 Sept 2014
**Corresponding Author. E-mail: ainane@gmail.com; Tel: +212 (0) 6.60.39.30.90*

Abstract
Essential oils and their components are becoming increasingly popular as naturally occurring antimicrobial agents. In this work the chemical composition and the antibacterial properties of *Nigella sativa* essential oils (EO) and of their main components were determined. The essential oil components were identified by GC-MS analysis. The antibacterial activity of the essential oil was determined against a panel of strains bacteria, using a broth microdilution method. The GC-MS analysis showed that the major constituents of the oil were monoterpene hydrocarbons and phenolic monoterpenes, and results of antibacterial activity confirmed the possibility of using *Nigella sativa* essential oils or some of their components in biology and pharmaceutic preparations.

Keywords: *Nigella sativa*, essential oil (EO), GC-MS-analysis, antibacterial activity.

1. Introduction
The genus *Nigella* belongs to the Ranunculaceae family and comprises all species in morocco. *Nigella sativa* is one of these species, which is naturally distributed in different parts of the country. In addition, it is extensively cultivated in various regions of morocco. Its seeds have been widely used in moroccan traditional medicine as a natural rem- edy for a long time [1-2]. In the other hand *N. sativa* seeds are used to cure gastro-intestinal disorders as well as skin or respiratory ailments [3-4]. Several other pharmacological properties have been traditionally attributed to *N. sativa* seeds, simply as a crushed powder, or as an extract. Purified or as a mixture, metabolites of *N. sativa* seeds would present a potent and therapeutically interesting activity on the cardiovascular, respiratory, immune, and endocrine systems [5-6]. Additional properties are frequently discovered [7]. Most of these activities have already been attributed to thymoquinone, a major component of the essential oil of the seeds [8]. Additionally, in its native range and far beyond, *N. sativa* seeds are also frequently used as spice and condiment in various recipes due to their characteristic aroma and bitter and peppery taste [9]. Finally, *N. sativa* seeds are used to prepare highly prized nutritive oil. Although on the world scale *Nigella* seed oil does not really have a significant economic market share, yet, it nevertheless constitutes a niche market whose size is constantly growing due to its alleged pharmacological properties, and to spirituous reasons resulting from its mention in sacred texts and reports of the presence of *Nigella* seeds in Tutankhamen tomb [10]. Some of these activities have been predominantly attributed to the volatile and fixed oils. To the best of our knowledge chemical composition of the fixed and volatile oils obtained from the seeds of *N. sativa* has not been the subject of much study [11-12].
The aim of this study is to describe the detailed chemical composition of essential oil of N. sativa seeds from Beni Mellal (morocco). This investigation will be useful to identify the bioactive compounds of the oils, which may be responsible for the therapeutic properties of the seeds.

2. Materials and methods

2.1. Plant material and extraction of essential oils

Seeds *Nigella sativa* were harvested in June 2013 in the agricultural province of Beni Mellal (Morocco). After harvest, the seeds were stored at 4 °C until processed.

The extraction of essential oils was carried out by the Clevenger-type apparatus. The seeds of *Nigella sativa* were washed and dried. The plant material thus prepared was weighed and added to the flask. A volume of water representing the two thirds of the volume of the flask is then added before the beginning of the distillation, it lasts about four hours. The recovered essential oil is recovered in the separator at the end of the distillation, and then a focus using a rotary evaporator and finally stored in a refrigerator at 4 °C before analysis or use.

2.2. Essential oil (EO) analysis

The qualitative analysis of essential oils is done by gas chromatography coupled to mass spectrometry (GC-MS: Hewlett Packard 5971A). Determining the relative proportions of various molecules is obtained by gas chromatography coupled with flame ionization (GC-FID: Hewlett Packard 5890A). Analysis by GC-MS and GC-FID are made under identical conditions. GC-MS was performed on a DB-5 column (5% phenyl methyl siloxane) whose dimensions are: length: 30 m; diameter: 250 μm; film thickness 0.32 microns. The applied temperature program was 40 °C for 5 min, 40 to 200 °C at 3 °C/min then held at 200 °C for 5 min. The carrier gas was helium (pressure: 49.9 kPa, flows: 1mL/min). The source of the mass spectrometer to a temperature of 230 °C and the mass range is scanned from 50 to 350 amu.

2.3. Antibacterial activity

All the bacterial strains used in this study were from Pasteur Institute of Casablanca (Morocco), the bacteria include the following: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Salmonella enterica*. In vitro antibacterial activity was determined by the broth microdilution method using 96-well microtitre plates. Two-fold dilutions of each extract were prepared in appropriate broth media (mueller hinton) in concentrations from 10 mg/mL to 1.25 mg/mL. Each well was inoculated with 5l of bacterial suspension at a density of 10^7 CFU/mL. The microtitre plates were incubated at 37 °C for 24 h. The growth of bacteria was observed as turbidity determined by the UV–VIS spectrophotometer microplate reader (*Asys* UVM 340) at 600 nm. Minimum inhibitory con-centrations (MICs) were calculated based on the density of the growth control and were the lowest extract concentrations that resulted in 100% reduction in growth compared with that of the extract-free growth control. The solution of DMSO (5%, v/v) was assayed as the negative control, simultaneously. All samples were tested in triplicate [13].

3. Results and discussion

After distillation of *Nigella sativa* seeds in the Clevenger apparatus, it has been found essential oil a yield of the order of 0.832 ± 0.025%. Qualitative analysis of essential oil of *Nigella sativa* by gas chromatography coupled to mass spectrometry GC / MS are shown in Table 1. These results confirm the presence of p-Cymene (60.5%) as the compound majority of essential oil of *Nigella sativa* of Moroccan origin, and interesting percentages of other components: α-Thujene (6.9%), Thymoquinone (3%), Carvacrol (2.4%) and β-Pinene (2.4%). Other constituents present in low yield.

The results of the antibacterial activity of essential oil of *Nigella sativa* vis-à-vis the bacteria *Escherichia coli*, *Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter cloacae* and *Salmonella enterica* are described in Table 2. Note that the *Enterobacter cloacae* appears be more resistant to the essential oil of *Nigella sativa*. This strain presented a MIC of 1.236 mg/mL, essential oil, followed by *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella enterica* they give CMI respectively 1.059 mg/mL, 0.964 mg/mL and 0.815 mg/mL, thus a remarkable activity on *Escherichia coli* strain which has a MIC of about 0.676 mg/mL.

Hypothesis on origin of bioactivity of essential oil of Nigella sativa seeds

By using more sophisticated analytic material, Mohamed A. Farag (2014) recently reported a wonderful and more judicious analysis of contents of 6 species of *Nigella sativa* [14]. It appears that *Nigella sativa* contains numerous phytochemicals including terpenoids, saponins, flavonoids, alkaloids (Figure 1). Under optimized conditions of ultra-sophisticated analysis by UPLC-MS, it was possible to annotate 52 metabolites including 8 saponins, 10 flavonoids, 6 phenolics, 10 alkaloids, and 18 fatty acids. Major peaks in UPLC–MS spectra contributing to the discrimination among species were assigned as kaempferol glycosidic conjugates, with kaempferol-3-O-[glucopyranosyl-(1→2)-galactopyranosyl-(1→2)-glucopyranoside, identifyed as potential taxonomic marker *for N. sativa*. Compared with GC–MS, UPLC–MS was found much more efficient in *Nigella* sample classification based on genetic and geographical origin. Nevertheless, both GC–MS and UPLC–MS support the remote position of *Nigella nigellastrum* in relation to the other taxa [14]. It is important to point here that all the extracts in different solvents and essential oil showed varying degree of inhibition [15].

Table 1: Percentages of chemical compositions of the essential oil *Nigella Sativa*.

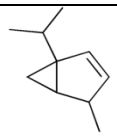
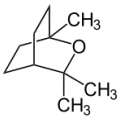
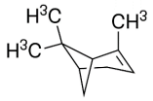
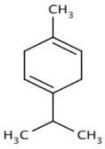
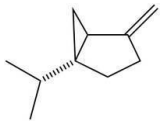
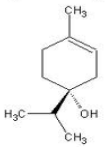
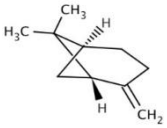
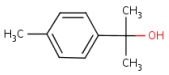
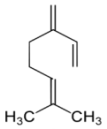
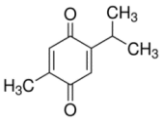
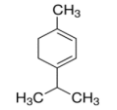
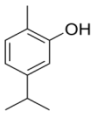
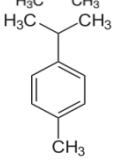
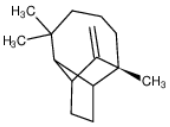
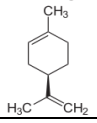
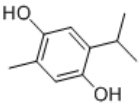
Compd.	Structure	Percentage	Compd.	Structure	Percentage
1		α-Thujen 6.9%	**9**		1,8-Cineol 0.1%
2		α-Pinen 1.7%	**10**		γ-Terpinen 3.5%
3		Sabinen 0.9%	**11**		Terpinen-4-ol 2.1%
4		β-Pinen 2.4%	**12**		p-Cymen-8-ol 0.2%
5		Myrcen 0.1%	**13**		Thymoquinon 3.0%
6		α-Terpinen 1.0%	**14**		Carvacrol 2.4%
7		p-Cymen 60.5%	**15**		Longifolen 0.9%
8		Limonen 1.4%	**16**		Thymohydroq-uinon 0.4%

Table 2: MIC (mg/mL) of the essential oil to the bacteria tested.

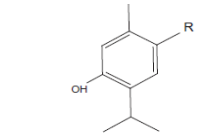
Sample	Bacterial strain ; MIC (µg/mL)				
	Escherichia coli	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Enterobacter cloacae*	*Salmonella enterica*
EO	676 ± 45	964 ± 21	1059 ± 76	1236 ± 72	815 ± 54
SD-1	22 ± 5	31 ± 7	24 ± 5	15 ± 6	19 ± 4
SD-2	25 ± 4	28 ± 6	25 ± 6	23 ± 5	23 ± 4

SD-1: Standard Drug-1 is Penicillin G; **SD-2**: Standard Drug-2 is Tetracyclin.

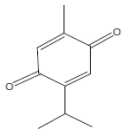
Conclusion

The work we have presented in order to enhance the seeds of *Nigella sativa* medicinal plant in the region Beni Mellal (Morocco), for the determination of the chemical composition of the essential oil and the extent of power of the antibacterial activity. The chemical composition of the essential oil showed the presence of tens of molecules that have very interesting especially p-cymene molecule yields the majority. Antibacterial tests essential oil showed that it possesses antibacterial activities vis-à-vis the used bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Salmonella enterica*.

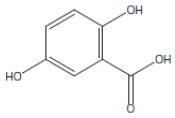
Figure 1: Structure of most inspected bioactive constituents of *Nigella sativa*.



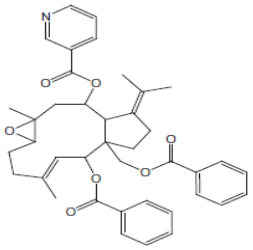
Thymol (R = H)
Thymoquinol (R = OH)



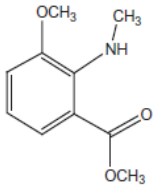
Thymoquinone



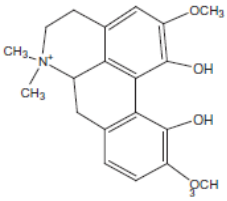
Gentisic acid



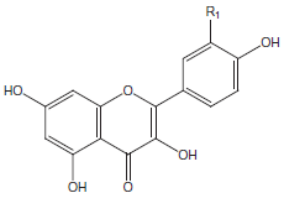
Nigellamine



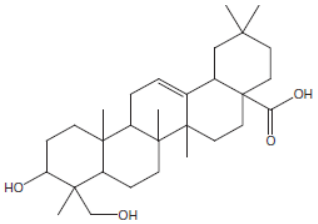
Damascenine



Magnoflorine



Kaempferol (R = H)
Quercetin (R) OH)



Hederagenin

Aknoweldgements -The authors would like to extend their sincere appreciation to the Deanship of Scientific at King Saud University for its funding of this research through the Research Group Project no RGP-VPP-222.

References

1. Mouhajir F., Pedersen J.A, Rejdali M., Towers G.H.N., *Pharm. boil.* 37 (1999) 391.
2. Le P.M., Benhaddou-Andaloussi A., Elimadi A., Settaf A., Cherrah Y., Haddad P.S., *J. Ethnopharmacol.* 94 (2004) 251.
3. Riaz M., Syed M., Chaudhary F.M., *Hamdard Medicus* 39 (1996) 40.
4. Ali B.H., Blunden G., *Phytother. Res.* 17 (2003) 299.
5. Gilani A.U.H., Jabeen Q., Khan M.A.U., *Pak. J. Biol. Sci.* 7 (2004) 441.
6. Hussein El-Tahir K.E.D., Bakeet D.M., *J. Taibah Univ. Med. Sci.* 1 (2006) 1.
7. Al-Okbi S.Y., Mohamed D.A., Hamed T.E., Edris A.E., *Eur. J. Lipid Sci. Tech.* 115 (2013) 774.
8. Hosseinzadeh H., Parvardeh S., *Phytomedicine* 11 (2004) 56.
9. Hedrick U.P., Sturtevant's edible plants of the world, New York, (1972) 686.
10. Padhye S., Banerjee S., Ahmad A., Mohammad R., Sarkar F.H., *Cancer therapy* 6 (2008) 495.
11. D'Antuono L.F., Moretti A., Lovato A.F., *Ind. Crop Prod.* 15 (2002) 59.
12. Ashraf M., Ali Q., Iqbal Z., *J. Sci. Food Agric.* 86 (2006) 871.
13. Cosentino S., Tuberoso C.I.G., Pisano B., Satta M., Mascia V., Arzedi E., Palmas F., *Lett. Appl. Microbiol.* 29 (1999) 130.
14. Farag M.A., Gad H.A., Heiss A.G., Wessjohann L.A., *Food Chem.* 151 (2014) 333.
15. Benlafya K., Karrouchi K., Charkaoui Y., Karbane M.E, Ramli Y., *J Chem Pharm Res* 6 (2014) 9.