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Essential Oil and Fixed Oil Content of *Nigella sativa* after A Traditional Medicine Processing-A Comparative Study

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ABSTRACT: ***Nigella sativa*** **seeds are widely used in Iranian Traditional Medicine (ITM).** ***N. sativa*** **oil, especially its essential oil, has more therapeutic effects than the whole seed. According to ITM this plant has a hot and dry temperament and causes respiratory complications in individuals with hot temperament. To resolve this problem, ITM references recommend processing the seeds with vinegar called "*tadbir*". In the present investigation, essential oil and fixed oil compositions of crude and processed seeds have been compared. Fixed and Essential oil compositions of crude and processed seeds were determined by using GC and GC-MS techniques, respectively. The comparative study showed that the crude samples had more essential and fixed oils. While type and content of fatty acids of the two samples were similar, thymoquinone (TQ) was absent in processed seeds. If TQ induces respiratory complications, processing with vinegar may eliminate the mentioned disadvantage of** ***N. sativa*** **seeds.**

Keywords: *Nigella sativa*, processing, Iranian Traditional Medicine, essential oil, fixed oil

INTRODUCTION

People reliance to traditional medicine is growing nowadays. According to World Health Organization (WHO), about 65-80% of the worlds' population, mainly in developing countries depends on traditional medicines as the primary method for health care (Calixto BJ. 2000, Cragg and Newman 2013, Ekor, 2014). The other remaining 20-35% who are mainly inhabitants of developed countries, also benefit indirectly from natural products in their health care. In industrial countries, adaptations of traditional medicines are termed "complementary" or "alternative" medicine (CAM) (WHO, 2008). In the last thirty years, nearly half of the drugs that have been approved are natural products or compounds derived from natural products. These figures are even higher about anti-cancer drugs (Newman and Cragg, 2012). It is a common misconception to believe that natural products are essentially safe whereas not only most plants can cause undesirable or adverse reactions but also many of them are inherently poisonous and harmful to human health, causing serious injuries leading to life-threatening conditions, and death (Barnes, 2003). Therefore, before using any medical intervention, benefit-risk assessments should be considered. But incomplete information on herbal medicines and their pharmacokinetics, pharmacology, active constituents, toxicology, adverse effects, drug-herb and drug-food interactions and contraindications, makes it difficult to assess benefit-risk profiles.

This problem becomes more complex considering the fact that each plant may contain hundreds of natural constituents that would play a role in the therapeutic properties of the plant. Recently, the term "Reverse Pharmacology Approach" has found its way in the pharmaceutical literatures. This approach, being inspired by living traditional medicines, means a meticulous scientific approach of uniting experiential observations and documented clinical experiences to facilitate modern drug discovery process, reducing failure rates and financial affairs, helping to develop better and safer leads especially new and effective synergistic combinatorial formula (Patwardhan and Mashelkar 2009). Not only this approach can be used to drug discovery as an intelligent strategy, but also can be applied to detoxify harmful plants. In Iranian Traditional Medicine (ITM) references, there are some routes to detoxify and/or maximize therapeutic effects of natural remedies. The above management processing is called "*tadbir*". Main participants of these processes are sugar, various oils, honey and vinegar. "*Tadbir*" includes some processes such as soaking, heating, washing, roasting, exposing to sunlight/air, flouring, etc. One of the most used plants in ITM is *Nigella sativa* L. (Ranunculaceae) which is known commonly as black seed or black cumin and "*Shouneez*" in ITM (Aghili Khorasani, 1992). It is growing in countries bordering the Mediterranean Sea, Iran, India, and Pakistan andhas a multi pharmacological actions including anticholinergic, antihistaminic (Boskabady *et al.*, 2004), anti-inflammatory, analgesic, antipyretic, antineoplastic, antimicrobial (Ali and Blunden 2003), immunomodulatory (Kardani *et al.*, 2013) and antiosteoprotic activities (Shuid *et al.* 2012). The seeds and oil are the most common parts used for medicinal and culinary purposes (Hussein and Bakeet 2006). While previous studies suggest a wide margin of safety for therapeutic dosages of *N. sativa* seeds and its fixed oil (Ziaee *et al.*, 2012, Nasir *et al.*, 2014, Fawzy Ramadan, 2015), ITM believes that it can induce respiratory complains in patients with hot temperament (Aghili Khorasani, 1992). Therefore, it recommends processing with vinegar for avoiding the mentioned side effects. In the present study, the effects of processing with vinegar on fixed and essential oil composition of the plant seeds have been investigated.

MATERIALS AND METHODS

A. Plant Material

Seeds of *N. sativa* were bought from a local market in Karaj, Iran and were authenticated by Dr. H. Hajmehdipoor. The sample was deposited in Traditional Medicine and Materia Medica Research Center (No: HMS338), Shahid Beheshti University of Medical Sciences, Tehran, Iran. The natural grape vinegar 5% was bought from Varda Company, Iran.

B. Traditional Medicine Processing

To prepare the processed *N. sativa*, the seeds were grinded, poured into the flask, mixed with vinegar (1:2 w/v), stirred and kept for 24 hours. Then, the seeds were flat on a clean tray and dried at room temperature, away from light. After drying, the seeds were milled again and passed through a 20 mesh sieve (pore size 850µm). Both crude and processed seeds were analyzed.

C. Extraction and Analysis of Fixed Oil

In order to isolate the fixed oil of the seeds, the black seeds were extracted by hexane (1:20) using a Soxhlet apparatus (60°C, 500 ml flask) for 4 hours. The resulting extract was concentrated using a rotary evaporator, and the percentage of the obtained oil was calculated. In order to obtain fatty acid profile of fixed oil of *N. sativa*, the methylation of the fatty acids was performed by using sodium methylate. The methylated fatty acids were analyzed by gas chromatograph (GC) Varian CP 3800 with capillary column CPSIL88 (50 m × 0.25 mm, i.d., 0.2 µm f.t.); carrier gas: helium; flow rate: 1 ml/min; injector temperature: 240°C using a flame ionization detector (FID) at 250°C. The column temperature was programmed at 150°C min^{-1} and then heated to 175°C at the rate of 15°C min^{-1}, then reached 235°C at the rate of 10°C min^{-1} and kept constant for 12 minute. Subsequently, the fatty acids were identified according to standard samples, and their proportion was calculated (Comes *et al.*, 1992).

D. Extraction and Analysis of Essential Oil

The seed powder of the plant was subjected to hydro-distillation for 4 hours using a Clevenger apparatus. The obtained essential oil was dried with anhydrous sodium sulfate and stored at 4°C. Analytical gas chromatography-Mass Spectroscopy (GC-MS) was carried out using an Agilent G890 GC, capillary column BPX5 (30 m × 0.25 mm i.d., 0.25 µm f.t.), with Agilent S973 MS detector; carrier gas: helium; flow rate: 1 ml min^{-1}. The column temperature was set at 50°C for 5 min and then raised to 240°C at the rate of 3°C min^{-1}. The temperature was increased to 300°C at the rate of 15°C/min and then kept constant for 3 min. The MS was operated at 70 e.V ionization energy. Retention indices were calculated using retention times of n-alkanes that were injected after the oil at the same chromatographic condition. The compounds were identified by comparison of retention indices with those reported in the literature (Adams, 2001, Hajimehdipoor *et al.*, 2008) and by comparison of their mass spectra with Wiley library.

RESULTS AND DISCUSSION

The hydro-distillation of the seeds of the crude and processed *N. sativa* gave orange and yellow volatile oils, respectively. The compounds in crude and processed *N. sativa* essential oils have been listed in Table 1. Fourteen compounds in the crude sample and thirteen compounds in the processed sample were identified representing 97.56% and 98.02% of the essential oils, respectively. The maximum amount was owned by ρ-cymene (57.31% and 58.21%) in processed and crude seeds, respectively. No obvious changes existed between percentage of the constituents in the crude and processed *N. sativa* seeds, except for longifolene that had increased. The most interesting result was found about thymoquinone (TQ) that had absolutely disappeared in the processed seeds. The extraction of crude and processed *N. sativa* with hexane gave a brown oily extract, with a strong aromatic odor. Comparison of fatty acids showed that both samples approximately had the same types of fatty acids (Table 2). There were six fatty acids in the fixed oil of crude and processed samples. Three of them including myristic, palmitic and stearic acids were the saturated fatty acids and the remaining including oleic, linoleic and 11,13-eicosadienoic acids formed the unsaturated fatty acids of the fixed oils.

Table 1: The Essential Oil Composition of Crude and vinegar-Processed *Nigella sativa.*

NO.	Compound	Percentage		Kovat's Index
		Processed *N. sativa*	**Crude *N. sativa***	
1.	α-Thujene	7.48	11.2	926
2.	α-Pinene	1.51	2.62	933
3.	Sabinene	0.68	1.33	975
4.	β-Pinene	1.93	2.94	980
5.	α-Terpinene	1.56	0.83	1020
6.	ρ-Cymene	57.31	58.21	1029
7.	Limonene	1.68	1.84	1032
8.	γ-Terpinene	2.19	0.78	1062
	Monoterpenoid hydrocarbones	74.34	79.75	
9.	Ether-ρ-menth-6-en-2-yl methyl	5.38	7.38	1122
10.	Terpinen-4-ol	2.52	1.02	1189
11.	Carvacrol	2.99	3.63	1311
	Monoterpenoid alcohols	10.89	12.03	
12.	TQ	0	1.70	1263
	Monoterpenoid ketones	0	1.70	
13.	α-Longipinene	2.42	0.76	1355
14.	Longifolene	10.37	3.32	1417
	Sesquiterpenoid hydrocarbones	12.79	4.08	
	Total Compounds	98.02	97.56	-

Table 2: The Fatty Acids compositions of Crude and Processed *N. sativa* Fixed Oils.

No.	Fatty acid	Structure	Retention Time (min)	Percentage	
				Crude *N. sativa*	**Processed *N. sativa***
1	Myristic acid	C_{14} saturated	5.6	0.3	0.5
2	Palmitic acid	C_{16} saturated	6.4	13.6	12.3
3	Stearic acid	C_{18} saturated	7.3	2.2	1.5
4	Oleic acid	C_{18} unsaturated	7.7	19.9	20.4
5	Linoleic acid	C_{18} unsaturated	8.2	60.5	59.8
6	11,13-Eicosadienoic acid	C_{20} unsaturated	9.2	2.4	2.2
	Total fatty acids			98.9	96.7

In fact, the GC results showed no difference between the fixed oils of the crude and processed seeds, whereas the GC- MS results showed some differences between the volatile oils of the crude and processed seeds. It means that processing could induce changes in volatile oil constitutes of *N. sativa* seeds. A recent investigation on Persian *N. sativa* showed 32 compounds in the essential oil. Among these diverse components, the maximum amount was owned by trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%) and carvone (4.0%) (Nickavar *et al.,* 2003). Comparison between the mentioned study and our work reveals that no anethole and carvone were detected in our samples, whereas the results of the free fatty acids were almost the same. This difference may be due to the different locations of the plant growth.

In several investigations, various pharmacologically active constituents of *N. sativa* volatile oil have been identified, but few of them were focused for medicinal properties. Among the constituents of *N. sativa* volatile oil, attentions have been attracted to α-pinene, ρ-cymene and TQ. α-Pinene offered some antibacterial, antitumor and anti-inflammatory actions. It had depressant effect on cardiovascular system as well (Raman *et al.,* 1995, Ruch and Sigler 1994, Russin *et al.,* 1989, Martin *et al.,* 1993, El-Tahir *et al.,* 2003). ρ-Cymene also decreased heart rate and arterial blood pressure. It showed mild antibacterial action. However, it has been found that the healing effects of the seeds are mostly due to the presence of TQ. This component has shown heart depressant effect and decreased arterial pressure and heart rate in rat. It has stimulated bile acids production and increased bile flow in dogs. TQ has been introduced as a hepatoprotective agent due to its antioxidant properties; moreover, it has prevented cisplatin-induced nephrotoxicity. It had not only protective effects in tonic clonic seizure induced by pentylentetrazole, but also showed analgesic and anti-inflammatory effects (Carson and Riley 1995, El-Tahir *et al.,* 1993, El-Dakhakhny, 1965, Daba and Abdel-Rahman 1998, Nagi *et al.,* 1999, Badary *et al.,* 1997, Hossein Zedeh and Parvadeh, 2004, Canonica *et al.,* 1963, Abdel-Fattah *et al.,* 2000, Ghannadi *et al.,* 2005). *N. sativa* has been traditionally used in the treatment of respiratory diseases for centuries in ITM; however, it has been mentioned to induce suffocation in people with hot temperament, so it was suggested to be processed prior to use. Recent data confirms its therapeutic effects on respiratory symptoms in either its whole seed or fixed and essential oils (Ahmad *et al.,* 2000, Boskabady and Farhadi 2008, Ahmad *et al.,* 2010, Gholamnezhad *et al.,* 2015), even though, during an investigation on TQ, it has been found that TQ could induce bronchospasm and intrateracheal pressure in guinea pigs (El-Tahir *et al.,* 1993). Taking all into consideration, it seems that the aim of traditional physicians from processing *N. sativa* seeds could be removing TQ and avoiding bronchospasm. According to ITM, vinegar- processing of *N. sativa* seeds does not reduce its therapeutic effects but decreases respiratory side effects (Aghili Khorasani, 1992). Regarding diverse above mentioned effects of TQ, it is expected that therapeutic properties won't be observed in processed seeds. Unless, these effects are due to other components or converting TQ to other effective components. It has been found that thymoquinone easily convers to its carbonyl dimer, nigellone, in exposure to air (Smith and Tess 1944). Nigellone in contrast to thymoquinone that deals with bronchoconstriction effect has shown relaxent effect on animal trachea (Mahfouz and El-Dakhakhny 1960, Chakravarty, 1993). It is the stable non-toxic polymer of thymoquinone that shows most medical properties of thymoquinone (El-Dakhakhny, 1965). Therefore, nigellone could have been formed during processing. In general, regarding to elimination of respiratory side effects of processed seeds, it could be useful for every temperament but further studies are necessary to test the ability of this process to reduce the symptoms of respiratory complications. This study also confirms that traditional medicines can inspire drug discovery and also lead to detoxifying the harmful side effects of the medications.

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CONFLICT OF INTEREST STATEMENT

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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