COMPARING THE EFFECT OF SUPERCRITICAL AND SUB-CRITICAL FLUIDS EXTRACTION WITH CONVENTIONAL EXTRACTION METHODS ON THE CHEMICAL COMPOSITION OF *NIGELLA SATIVA L. SEEDS*

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The volatile extract components of *Nigella sativa L.* seeds were extracted by supercritical carbon dioxide ($SCCO_2$) , subcritical water(SbCW), and subcritical ethanol (SbEtOH), hydrodistillation (HD), organic solvent extraction under reflux (RFE) and organic solvent extraction under ultrasonic irradiation (USE). Composition of the extracts were estimated by GC-MS. The major compounds in all six extracts were α-thujene, cymol, thymoquinone, 1,4-dimethoxy-2,3-dimethylbenzene, ledol, palmitic acid, elaidic acid, oleic acid and linoleic acid, but they differ in quantitatively. SbCW and SbEtOH extraction of *Nigella sativa L.* didn't appear literature before.

INTRODUCTION

Nigella sativa (*N. sativa*), commonly known as black seed, belongs to the botanical family of *Ranunculaceae.* It has been in use in many Middle Eastern and Far Eastern Countries as a natural remedy for over 2000 years (1,2).

The pharmaceutical studies of natural products are one of the most interesting and active research areas. The conventional methods used to prepare essential oils are steam distillation and solvent extraction. Steam distillation is also most used methods to prepare essential oils on a commercial basis. However, there are few adjustable parameters to control the selectivity of these methods. Therefore, developing alternative extraction methods and with better selectivity and efficiency are highly desirable. Recently, more efficient extraction methods, such as supercritical fluid extraction (SCFE) have been used for the isolation of organic compounds from various natural products (3,4). Because SCFE has several distinct properties, it is regarded as a promising alternative technique to conventional solvent extraction method. Most widely used supercritical fluid is (SCF) CO_2 because of its preferred critical properties, low toxicity and chemical inertness (5). It has also an advantage with its low critical constants over other SCF. This technique has recently been used for the isolation of essential oils from plants (6–8). However, CO_2 is not able to dissolve some moderately polar compounds such as alcohols, esters, aldehydes and ketons. In recent years, a continuous_and static sub-critical water extraction technique has been used for extraction of solid materials(5,10). The aim of this study was to develop an approach for the extraction methods for medicinal essential oil components determined of Turkish N. sativa and to compare sub-super critical fluids extraction methods with others based on the use of hydrodistillation, organic solvent extraction under ultrasonic irradiation. It was also aimed to introduce an alternative; faster and cheaper method in the pharmaceutical and food field.

MATERIALS AND METHODS

Plant materials

Nigella sativa L. seeds were harvested in June 2006 from an experimental field of Cukurova University, Adana, Turkey. Seeds were separated from the branch and dried at room temperature.

Hydrodistillation (HD)

100 g of *Nigella sativa L. seeds* were submitted for 3 h to water distillation using a Clevenger type apparatus. After HD, a liquid–liquid extraction step was performed by using 5 mL dichloromethane (DCM). Extract and DCM placed in a separation funnel and about 1 g NaCl was added to facilitate

Organic solvent extraction under reflux *(RFE)*

The 50 mL sample flask was charged with 2 g *Nigella sativa L. seeds* and 30mL of the mixture of acetone-dichloromethane(1:1; v:v) under reflux. The temperature of extracting solvent under reflux was about boiling point of solvent mixture. Extraction was held for 3 h and carried out in triplicate

Organic solvent extraction under ultrasonic irradiation *(USE)*

Ultrasound-assisted extraction was performed in an ultrasound cleaning bath (Lab-Line Instruments Inc. 9314-1 model,310 380 200mm internal dimensions) at the fixed-frequency of 35 kHz. The temperature of the sonicated bath was 25 $^{\circ}$C. A 50 mL sample flask was charged with 2 g *Nigella sativa L. seeds* and 30 mL of *tert*-butylmethylether as extracting solvent. Sonication was held for 2 h. Extraction was carried out in triplicate.

Sub-critical water extraction (SbCW)

Sub-critical water extraction was performed in a laboratory built apparatuses. The water was sonicated to remove dissolved oxygen then was used in an HPLC pump programmed for a constant flow of 1-2 mL min^{-1}. The extractor heated in a temperature programmable oven. A 2 m long pre-heated coil was used to equilibrate the water to the desired temperature. A 14 mL extraction cell equipped with 10 µm frit at the inlet and outlet, was connected to a 1 m cooling loop out side of the oven. Extraction cell contained sea sand at both ends. SbCWE was carried out using 2 g of *Nigella sativa L. seeds* at temperatures 100, 125 and 150 $^{\circ}$C, a pressure of 60 bar and 80 bar and 30 min extraction time. The optimum condition was determined at temperature of 125 $^{\circ}$C, a pressure of 80 bar a constant flow of 2 mL min^{-1}. A liquid–liquid extraction step was performed as described for HD.

Sub-critical ethanol extraction (SbEtOH)

Sub-critical ethanol extraction was performed as described for SbCW by using ethanol sonicated to remove dissolved oxygen. SbEtOH was carried out using 2 g of *Nigella sativa L. seeds* at the optimum conditions determined at temperature 150 $^{\circ}$C, a pressure of 80 bar a constant flow of 2 mLmin^{-1}.

Super critical carbondioxide extraction ($SCCO_2$)

$SCCO_2$ extraction was performed by filling 14 mL volume vessel with 2 g of *Nigella sativa L. seeds.* The plant was then extracted with supercritical CO_2 under 200 atm pressure and 40 $^{\circ}$C temperature for 20 min static followed by 30 min dynamic flow. During $SCCO_2$ extraction Perkin-Elmer Teledyne ISCO pump was used and A manual restrictor (flow rate 2 mL min^{-1}) was used in system to collect the extracted analytes. The extracted analytes were collected in a 5 mL dichloromethane in 10 ml volumetric tube. After the $SCCO_2$ extraction was performed methylation procedure (11).

GC-MS analysis

Qualification and quantification were carried out by using a Finnigan-Trace GC–MS equipped with an auto sampler. One microlitre of sample volume was injected using split method with 50 split ratio. Chromatographic separations were accomplished with a TR-MS 5 capillary colum. Analysis was carried out using helium as the carrier gas, flow rate 1.0 mL/min. The column temperature was programmed from 50 to 240 °C at 3°C/min. The injection port temperature was 250 °C. The ionization voltage applied was 70 eV, mass range *m*/*z* 41–400 a.m.u. The separated components were identified tentatively by matching with GC–MS results of National Institute of Standards and Technology (NIST) and WILEY 7 mass spectral library data. The quantitative determination was carried out based on peak area integration.

RESULTS

The chemical composition of the extracts is reported in Table 1. Different extraction compositions could be obtained by different extraction methods applied to natural products (12). As shown by the results, the composition of HD, RFE, USE and sub and super critical fluids extracts were significantly different.

145, 103, and 152 signals were gained by HD, RFE, and USE extracts, respectively while 107, 120, and 53 signals were gained from SCO_2, SbCW, and SbEtOH extracts. Major products of $SCCO_2$ extraction were unsaturated fatty acids such as linoleic acid(44,09%), and oleic acid(20,28%), saturated acid such as palmitic acid(11,28%). The other components were cymol (3,69%), tymoquinone (6,84%), stearic acid (1,77%) and 11,13 eicosadienoic asid. The major components of SbEtOH extract were cymol(20,07%), 1,4-dimetoxy-2,3-dimethyl benzene (16,53%), α-thujene(11,07%), thymoquinone (10,11%), linoleic acid (8,63%) and oleic acid (4,34%) and the following components were ledol (3,38%), 5 caranol (3,15%), junipene (2,63%), α-pinene(2,58%), β-pinene (2,32%), carvacrol (2,15%), and palmitic acid (2,05%). Essential oil of SbCW extract was poor for cyclic monoterpenes, however, oxygenated compounds were found in higher amounts (thymoquinone 23,18%; carvone 8,47%; 1,4-dimehoxy-2,3-dimethyl benzene 37,64%; α-thujene 4,87%; limonene 4,20%) (10). SbEtOH technique is similar to $SCCO_2$, and to SbCW too. Furthermore SbEtOH method is among the conventional techniques.

SbCW, SbEtOH and $SCCO_2$ extraction methods appears to be cost-effective technique at laboratory scale, but an accurate economic evaluation for large scale units requires supplementary experiments. The advantages of these three methods over HD, RFE and USE are shorter extraction period, high selectivity in the extraction of compounds. In addition, the advantages of $SCCO_2$ extraction over the HD, RFE and USE include: low temperature hence, no thermal degradation of most of the labile compounds; no solvent residue(10-12,13). $SCCO_2$ extract had highest concentration of fatty acid compounds than the other extraction methods. Finally, from the extraction results different extraction techniques can be offered for different compounds determined in natural products. For example, HD for α-thujene and cymol; RFE or USE for thymoquinone; SbCW for 1,4-dimetoxy-2,3-dimethylbenzene; $SCCO_2$ for linoleic acid and oleic acid.

Table 1. Essential oil and fixed oil of *Nigella sativa L. seeds determined by GC-MS*

RT	Component	Area %					
		HD%	RFE	USE	$SCCO_2$	SbCW	SbEtOH
14,98	**α-Tujene**	**13,26**	**8,43**	**9,08**	**---**	**4,87**	**11,07**
15,45	**α-pinene**	**4,46**	**1,87**	**2,60**	**---**	**---**	**2,58**
15,78	3-ethyl-1-octen	---	---	---	---	1,57	---
17,36	**Sabinene**	**2,08**	**1,12**	**1,25**	**---**	**---**	**0,98**
17,76	**β-pinene**	**4,93**	**2,24**	**2,65**	**---**	**---**	**2,32**
19,56	α-terpinene	0,96	---	---	0,34	---	0,43
20,05	**cymol**	**39,41**	**18,59**	**18,33**	**3,69**	**2,12**	**20,07**
20,21	**dl limonene**	**2,08**	**1,58**	**1,66**	**--**	**4,20**	**1,27**
21,63	γ-terpinene	0,82	0,52	0,43	0,48	---	0,60
23,48	L-β-pinene	0,68	0,87	0,61	---	0,61	0,58
24,67	**5-caranol**	**10,49**	**---**	**---**	**---**	**---**	**3,15**
24,68	**p-menth-6en-2-yl-methylether**	---	**4,43**	**3,42**	**---**	---	---
26,63	camphor	---	---	---	---	0,42	---
27,03	trans-2-caren-4-ol	0,29	0,35	---	---	---	---
28,04	4-terpineol	2,99	0,60	0,41	---	1,36	0,48
28,34	4-hydroxy-4-methyl-2-penthanone	---	0,46	----	---	---	---
28,88	1,3,4-trimethyl-3-cyclohexenyl-1-carboxaldehyde	0,96	1,01	0,74	---	---	0,58
29,33	cis-dihidrocarvone	---	---	---	---	0,63	---
31,24	carvone	0,59	---	0,47	---	8,47	---
31,52	**p-mentha-3,6-diene-2,5-dione(Thymoquinone)**	**0,47**	**33,70**	**32,49**	**6,84**	**23,18**	**10,11**
32,83	piretrone	---	0,42	---	---	---	---
33,56	**carvacrol**	**4,26**	**2,99**	**1,77**	**0,89**	**1,24**	**2,15**
36,00	α-Longipinene	0,83	1,19	0,74	---	---	0,61
37,34	dodecane	---	---	--	--	0,85	---
38,81	5-isopropyl-3-methoxy-2-methylbenzo-1,4-quinone	---	---	---	1,66	---	---
38,97	**junipene**	**7,11**	**3,30**	**2,57**	**1,02**	**----**	**2,63**
45,33	1,4-dimethoxy-2,3-dimethylbenzene	---	1,95	1,14	---	37,64	16,53
47,72	dillapiol	---	---	---	----	2,99	---
46,63	apiol	---	---	---	---	1,50	---
51,09	ץ-gurjnenepoxide	---	---	0,49	---	---	---
54,85	nonadecane	---	---	---	---	0,56	---
56,51	cis-7-tetraadecen-1-ol	---	0,63	0,96	---	---	---
56,73	(2Z)-3,7,11-trimethyl-2,10-dodecadiene-1-ol	---	---	0,40	---	---	---
56,90	Tetradecyl 2-methylpropanoate	---	---	0,38	---	---	---
57,01	**Palmitic acid**	---	---	---	**11,28**	**---**	**2,05**
59,39	**Ledol**	**0,39**	**4,73**	**7,74**	**---**	**---**	**3,38**
60,40	C20H32	---	2,83	1,39	---	---	0,51
60,51	Kaur-16-en	---	1,61	4,80	---	---	---
63,23	Nerolidil asetat	---	---	0,37	---	---	---
64,1	linoleic acid	---	---	---	44,09	---	8,63
64,25	oleic acid	---	---	---	20,28	---	4,34
65,05	Stearic acid	---	---	---	1,77	---	0,40
71,62	11,13-eicosadienoic acid	---	---	---	1,35	---	
	Recovery	97,06	95,41	96,89	93,69	92,21	95,45

CONCLUSION

(1) Essential oil of sub and super critical fluids extracts was poor for cyclic monoterpenes, however, oxygenated compounds were found in higher amounts. The quality of the oil can be linked to the amount of oxygenated compounds present in it. According to these facts, sub and super critical fluids can be suggested as a good extractant for the extraction of essential oils from *Nigella sativa L. seeds.*

(2) SbCW, SbEtOH and $SCCO_2$ extraction methods are clearly quicker than conventional alternatives. Total extraction time of 50 min (consisting of 30 min of static extraction plus 20 min of dynamic extraction) was used during SFE fluid extractions whereas 3 h is required in hydrodistillation and 2 or 3 h for organic solvent extraction.

(3) SbCW, SbEtOH and $SCCO_2$ extraction methods are also a very clean method which avoids both the use of large amounts of organic solvent and generation of toxic residue.

(4) For fatty acids, $SCCO_2$ could be preferred while for oxygenated compounds, SbCW extraction method is suitable. SbEtOH extracts all of these compounds in equally. According to goal of using in industry could be performed by extraction method determined.

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