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VOLATILE CONSTITUENTS OF GINGER OIL PREPARED ACCORDING TO IRANIAN TRADITIONAL MEDICINE AND CONVENTIONAL METHOD: A COMPARATIVE STUDY

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Abstract

Background: Herbal medicines formulated as oils were believed to possess more powerful effects than their original plants in Iranian Traditional Medicine (ITM). One of the popular oils suggested for treatment of various indications was ginger oil. In the present study, to suggest a more convenient method of oil preparation (compared to the traditional method), ginger oil has been prepared according to both the traditional and conventional maceration methods and the volatile oil constituents have been compared.
Material and Methods: Ginger oil was obtained in sesame oil according to both the traditional way and the conventional (maceration) methods. The volatile oil of dried ginger and both oils were obtained by hydro-distillation and analyzed by gas chromatography/mass spectroscopy.
Results: Fifty five, fifty nine and fifty one components consisting 94 %, 94 % and 98 % of the total compounds were identified in the volatile oil of ginger, traditional and conventional oils, respectively.
Conclusion: The most dominant compounds of the traditional and conventional oils were almost similar; however they were different from ginger essential oil which has also been to possess limited amounts of anti-inflammatory components. It was concluded that ginger oil could be prepared through maceration method and used for indications mentioned in ITM.

Keywords: traditional ginger oil, conventional ginger oil, ginger essential oil, GC/MS, Iranian Traditional Medicine.

Introduction

Oils are old forms of medication used in Iranian Traditional Medicine (ITM) and they are believed to have presented more powerful effects than their original plants (Aghili Khorasani, 1999). Oils have been suggested as the most useful and suitable formulations for treatment of brain, nerve, uterus and stomach diseases; while topical application of medicines has been suggested to be more beneficial in the nervous organs compared to oral administration due to the changes in drugs during digestion. Oils have been proved to be effective as pain relievers and temperament (called '*Mizaj*" in ITM) modulators (Gharshi, 2008).

Ginger, *Zingiber officinale* (Zingiberaceae), is distributed worldwide (Mangprayool et al., 2013). It has an aromatic and pungent odor and is extensively used as spice in foods and drinks (Mangprayool et al., 2013; Razi, 1987). It has been employed as a traditional medicine around the world (Mangprayool et al., 2013).

Ginger has been used as carminative, laxative (Aghili Khorasani, 2008; Moamen Tonekaboni, 2007; Ibn sina, 2005), pain-killing agents (Aghili Khorasani, 2008; Moamen Tonekaboni, 2007) and uterine pain reliever after labor in ITM (Chashti, 2004). It is also a liver and stomach tonic and is believed to clean phlegm ("*Balgham*") and black bile ("*Sauda"*) gently (Aghili Khorasani 2008; Ansari shirazi, 1992; Ghasani, 2008).

In regard to recent studies, ginger has been used for the treatment of gastrointestinal disorders including indigestion, diarrhea (Mangprayool et al., 2013), dyspepsia Heidari et al., 2000; Heidari et al., 1997; Podlogar and Verspohl, 2012), and nausea related to pregnancy, surgery or chemotherapy (Fleming, 2002; Mangprayool et al., 2013). It has also been used as carminative (Fleming, 2002), antioxidant (Fleming, 2002; Liu et al., 2013), anti-inflammatory (Fleming, 2002; Grzanna et al., 2005; Heidari et al.; Heidari et al., 1997; Podlogar and Verspohl, 2012a), anti-prostaglandin (Grzanna et al., 2005), anti-lipidemic (Fleming, 2002) agent. Ginger has been applied for pain-killing (Mangprayool et al., 2013; Rizk, 2013), uterine smooth muscle relaxation (Buddhakala et al., 2008; Mangprayool et al., 2013) and dysmenorrhea (Kashefi et al., 2014; Ozgoli et al., 2009; Rahnama et al., 2010; Rahnama et al., 2012). It has reduced hypertension, nocturnal cough and dyspnea in asthmatic patients (Mangprayool et al., 2013) and has exhibited hepatoprotective activity (Liu et al., 2013).

In ITM, topical use of ginger oil has been reported to be useful for joint pain, joint stiffness (Chashti, 2007), lumbar and pelvic pain (Noorbakhsh; 2004) and spasms (Noorani, 2008). It has also been said to possess antinociceptive properties and ginger poultice was suggested as a brain, stomach and uterus tonic and a painkiller (Moamen Tonekaboni, 2007)..

Researches have shown that topical use of ginger essential oil is useful for joint pain (Sritoomma et al., 2014; Yip and Tam, 2008); also the anti-inflammatory, antioxidant and antinociceptive properties of ginger oil have been demonstrated (Jeena et al., 2013). Regarding the long time usage of ginger oil in ITM as antinociceptive and antispasmodic agents as well as the recent reports about the anti-inflammatory, antioxidant, antinociceptive and antispasmodic activities of ginger oil, we decided to prepare the oil both through the

ITM manuscripts and the conventional maceration methods. The volatile oils derived from both oils were further compared for possible differences/similarities.

Materials and Methods
Plant *m*aterial

Dried *Zingiber officinale* Roscoe rhizomes were provided from the local market (Aug 2014) and the scientific identity was confirmed by botanists at Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. A sample was kept for future reference (No 336 (HMS)).

Preparation of ginger oil

Traditional method (Ibn Abi Nasr, 1940)

Ginger rhizomes (100 g) were crushed and macerated in water (1000 mL) overnight. The mixture was heated until the volume reached half the initial. Then the mixture was filtered and sesame oil (500 mL) was added to the filtrate and the whole mixture was placed in a tin plated copper pot and heated gently until all water was evaporated. The oil was further filtered and centrifuged. The final transparent yellowish oil was kept in a refrigerator till the time of essential oil extraction.

Conventional method

Crushed ginger rhizomes (100 g) were macerated in sesame oil (1:5) with continuous shaking for 7 days. The mixture was filtered and centrifuged afterwards. The final transparent yellowish oil was kept in a refrigerator.

Essential oil extraction

The volatile oil of the dried ginger (50 g) (yield 1.9 %) and the oils obtained by the traditional and conventional methods (200 mL each) were obtained by hydrodistillation using a Clevenger type apparatus.

GC/MS analysis

The constituents of the above volatile oils were identified by gas chromatography followed by mass spectrometry that is a solvent-free, efficient and rapid method to determine the volatile compounds (Yu et al., 2007). The analysis was performed on an Agilent 5973 mass selective detector coupled with an Agilent 6890 gas chromatograph, equipped with a BPX5 capillary column SGE Analytical Science, Australia (30 m × 0.25 mm; film thickness 0.25 µm). The oven temperature was programmed 50-300 °C. The initial increasing rate was 3 °C per min.

After reaching 240 °C, the rate increased to 15 °C per min up to 300 °C. Helium was used as the carrier gas at a flow rate of 0.5 mL/min. Injector temperatures was 290 °C. The MS operating parameters were: ionization voltage, 70 eV; ion source temperature, 220 °C; mass range, 40-500. The MSD ChemStation was used as the operating software. Retention indices were calculated by using retention times of n-alkanes (C_8- C_{24}) that were injected after the oil at the same conditions. Components of the oil were identified by comparison of their retention indices (RI) with those reported in the literature and also by computer matching with Wiley library. The fragmentation patterns of the mass spectra were also compared with those reported in the literature (Adams, 2001; McLafferty and Stauffer, 1989).

Results

The composition of the volatile oils of ginger, traditional ginger oil and the conventional ginger oil with the retention times, retention indices and the percentage shares are presented in tables 1, respectively. More than 94 % of the components of ginger essential oil (55 compounds) have been identified. The most abundant compounds included α-zingiberene (15.20 %), β-phellandrene (13.51 %), camphene (7.69 %), E-E-α-farnesen (7.04 %), β-sesquiphellandrene (6.96 %) and *ar*-curcumene (5.60 %), which presented about 56 % of the essential oil compounds. α-Zingiberene (30.06 %), β-sesquiphellandrene (10.71 %), E-E-α-farnesene (9.75), β-bisabolene (6.53 %), ɣ-curcumene (5.90 %) and *ar*-curcumene (5.18 %) were the most dominant compounds in the essential oil of traditional ginger oil which included about 68 % of the oil. Around 94 % of the essential oil constituents were identified (59 compounds).

As for the essential oil of the conventional oil, about 98 % of the compounds have been authenticated (51 compounds) with α-zingiberene (29.35 %), β-sesquiphellanderen (9.60 %), E-E-α-farnesen (9.26 %), β-phellandrene (8.87 %), β-bisabolene (5.83 %), ɣ-curcumene (5.62 %) and *ar*-curcumene (5.33 %) as the most abundant compounds which comprised about 74 % of the essential oil. The most abundant compounds of each of the three essential oils are presented in table 2. The compounds which consisted more than 5 % of total volatile oil were considered as the most dominant constituents.

Table 1: Constituents of the essential oil of ginger rhizome, traditional and conventional ginger oils.

NO	Compound	GR KI	TO KI	CO KI	Standard KI*	GR RT	TO RT	CO RT	Type
1	n- Octane	-	800	-	800	-	5.82	-	Other
2	Hexanal	-	807	-	802	-	6.13	-	Other
3	n- Nonane	-	900	-	900	-	9.94	-	Other
4	α- Thyujene	-	927	-	930	-	11.33	-	MH
5	Tricyclene	924	-	924	927	11.17	-	11.18	MH
6	α- Pinene	935	935	934	939	11.74	11.71	11.72	MH
7	**Camphene**	**954**	**952**	**952**	**954**	**12.69**	**12.6**	**12.61**	MH
8	β- Pinene	981	-	981	979	14.06	-	14.06	MH
9	Myrcene	992	992	992	991	14.65	14.63	14.63	MH
10	n- Decana	-	1000	-	1000	-	15.02	-	MH
11	α- Phellandrene	1011	1011	1010	1004	15.61	15.58	15.58	MH
12	α- Terpinene	1021	1021	1021	1017	16.14	16.1	16.12	MH
13	p- Cymene	1031	1030	1030	1025	16.64	16.6	16.61	MH
14	Limonene	-	1034	1034	1029	-	16.76	16.79	MH
15	**β- Phellandrene**	**1038**	**1036**	**1036**	**1030**	**17.03**	**16.89**	**16.91**	**MH**
16	Z-β- Ocimene	-	1050	-	1037	-	17.63	-	MH
17	Benzen acetaldehyde	-	1058	-	1042	-	18.04	-	Other
18	ɣ- Terpinene	1063	1058	1063	1060	18.31	18.3	18.31	MH
19	Terpinolene	1090	-	1089	1089	19.7	-	19.69	MH
20	p- Cymenene	1098	1068	1098	1085	20.14	20.12	20.14	MH
21	Linalool	1106	1098	1106	1097	20.53	20.5	20.51	MO
22	n- Nonanal	-	1106	-	1101	-	20.85	-	Other
23	endo- Fenchol	1128	-	1128	1117	21.64	-	21.63	MO
24	cis-p Menth-2-en-1-ol	1132	-	-	1122	21.85	-	-	MO
25	Camphor	1159	-	-	1146	23.15	-	-	MO
26	Camphene hydrate	1166	-	1166	1150	23.51	-	23.5	MO
27	Iso Borneol	1174	-	1174	1162	23.92	-	23.91	MO
28	Borneol	1184	1183	1183	1169	24.4	24.35	24.36	MO
29	Terpinen-4-ol	1190	1190	1190	1177	24.71	24.69	24.7	MO
30	1- Dodecane	-	1200	-	1190	-	25.19	-	Other
31	α- Terpineol	1206	1206	1205	1189	25.51	25.47	25.47	MO
32	Citronellol	1234	1234	1234	1226	26.83	26.8	26.8	MO
33	Neral	1249	-	1248	1238	27.5	-	27.49	MO
34	Geraniol	1259	1258	1258	1230	27.98	27.94	27.94	MO
35	Geranial	1278	-	1278	1267	28.91	-	28.90	MO
36	Thymoquinone	-	1264	-	1252	-	28.22	-	MH
37	2E- Decanal	-	1273	-	1264	-	28.61	-	MH
38	Isobornyl acetate	1291	1292	1291	1286	29.52	29.52	29.51	MO
39	2- Undecanone	1299	1300	1299	1294	29.89	29.88	29.88	Other
40	Thymol	-	1311	-	1299	-	30.39	-	MO
41	2E,4E- Decadienal	-	1331	1331	1317	-	31.29	31.29	MO
42	neo iso- Carvomenthyl acetate	-	1355	-	1350	-	32.33	-	MO
43	Citronellyl acetate	1354	1354	-	1353	32.34	-	32.33	
44	Cyclosativene	1374	1375	1374	1371	33.22	33.21	33.22	SH
45	α- Copaene	1381	1381	1381	1377	33.50	33.50	33.50	SH
46	Geranyl acetate	1385	1384	1384	1381	33.70	33.64	33.64	MO
47	β- Elemene	1394	1400	1401	1391	34.11	34.11	34.10	MH
48	7-epi- Sesquithujene	1405	1405	1404	1391	34.55	34.54	34.54	SH
49	Methyl eugenol	-	1414	-	1404	-	34.91	-	PHENYL
50	E- Caryophyllene	-	1418	-	1419	-	35.10	-	SH
51	α- Funebrene	1425	-	1424	1403	35.40	-	35.38	SH
52	trans- Caryophyllene	-	-	1427	1419	-	-	35.47	SH
53	ɣ- Elemene	1435	-	1435	1437	35.81	-	35.81	SH
54	α-trans- Bergamotene	1437	1437	-	1435	35.91	35.90	-	SH
55	α- Himachalene	1457	1459	1456	1441	36.73	36.70	36.71	SH

NO	Compound	GR KI	TO KI	CO KI	Standard KI*	GR RT	TO RT	CO RT	Type
56	allo- Aromadendrene	1469	1468	1468	1460	36.83	36.79	36.81	SH
57	**α- Acrodiene**	**1465**	**1465**	**1464**	**1466**	**37.09**	**37.04**	**37.06**	**SH**
58	**ɣ- Curcumene**	**1484**	**1483**	**1483**	**1483**	**37.87**	**37.80**	**37.83**	**SH**
59	**ar- Curcumene**	**1490**	**1488**	**1488**	**1481**	**38.12**	**38.02**	**38.04**	**SH**
60	β- Selinene	1494	1494	1493	1493	38.28	38.27	38.25	SH
61	**α- Zingiberene**	**1505**	**1501**	**1503**	**1494**	**38.74**	**38.57**	**38.65**	**SH**
62	Epizonarene	-	1504	-	1502	-	38.69	-	SH
63	**E-E-α- Farnesene**	**1511**	**1509**	**1511**	**1506**	**38.98**	**38.86**	**38.90**	**SH**
64	**β- Bisabolene**	**1516**	**1514**	**1514**	**1506**	**39.18**	**39.07**	**39.11**	**SH**
65	ɣ- Cadinene	1524	1523	1523	1514	39.50	39.42	39.44	SH
66	δ- Cadinene	1527	1526	1526	1523	39.62	39.56	39.58	SH
67	**β- Sesquiphellandrene**	**1534**	**1532**	**1532**	**1523**	**39.89**	**39.77**	**39.80**	**SH**
68	Selina-3,7(11)-diene	1548	1552	1547	1547	40.46	40.58	40.42	SH
69	trans- Cadinene ether	1557	-	-	1559	40.80	-	-	SO
70	E- Nerolidol	1567	1567	1567	1563	41.20	41.19	41.19	SO
71	Hexadecane	-	1600	-	1600	-	42.47	-	Other
72	Dodecanoic acid	1572	-	-	1567	41.39	-	-	Other
73	epi-α- Cadinol	1639	-	-	1640	43.97	-	-	SO
74	α- Muurolol	1656	-	-	1646	44.59	-	-	SO
75	β- Eudesmol	1671	1671	1670	1651	45.17	45.15	45.14	SO
76	n- Hexanoic acid	-	1970	-	-	-	55.47	-	Other
77	Hexadecanoic acid,ethyl ester	-	1998	-	-	-	56.38	-	Other

MH: Monoterpene Hydrocarbons; MO: Oxygenated Monoterpenes; SH: Sesquiterpene Hydrocarbons; SO: Oxygenated Sesquiterpenes.
*: Adams, 2001; McLafferty and Stauffer, 1989)

Table 2: The most abundant constituents of the essential oils.

	Compounds							
Oil	**α-zingiberene**	**B-phellandrene**	**camphene**	**E-E-α-farnesen**	**β-sesquiphellandrene**	**ar-curcumene**	**β-bisabolene**	**ɣ-curcumene**
Ginger oil	15.20	13.51	7.69	7.04	6.96	5.60	-	-
Traditional oil	30.06	-	-	9.75	10.71	5.18	6.53	5.90
Conventional oil	29.35	8.87	-	9.26	9.60	5.33	5.83	5.62

-: compound comprised less than 5 % of the essential oil

Discussion

There are previous studies about the anti-inflammatory effects of *Zingiber officinale* volatile oil and some of its constituents. Jeena *et al*. have analyzed ginger essential oil to find α-zingiberene, *ar*-curcumene and α-sesquiphellandrene as the most dominant components. They have evaluated the effects of the oil in a carrageenan, dextran and formalin model for inducing chronic inflammation. The results demonstrated significant reduction in acute inflammation (Jeena et al., 2013. Ginger essential oil (rich in *ar*-curcumene) has also shown anti-inflammatory properties through affecting leukocyte migration both *in vitro* and *in vivo* (de Melo et al., 2011). The volatile oil of *Zingiber officinale* with zingiberene, *ar*-curcumene, β-bisabolene and α-sesquiphellandrene as the major components, has shown to be effective in decreasing severity and extent of inflammation in a rat model of colitis (Rashidian et al., 2014). In another study, evaluating the volatile oil of ginger (with zingiberene as the dominant composition) *in vitro* and *in vivo* has suggested the impact of the oil in cell-mediated immune response and nonspecific proliferation of T-cell which might play a role in inflammatory conditions (Zhou et al., 2006).

Conclusions

The anti-inflammatory properties of ginger volatile oil and some of its constituents like zingiberene (Jeena et al., 2013; Johji et al., 1988; Mustafa et al., 1993; Rashidian et al., 2014; Türkez et al., 2014; Zhou et al., 2006), sesquiphellandrene (Jeena et al., 2013; Rashidian et al., 2014) and curcumene (de Melo et al., 2011; Jeena et al., 2013; Podlogar and Verspohl, 2012; Rashidian et al., 2014) () somehow explain the application of ginger oil in ITM for various indications. As it is shown in table 2, except for β-phellandrene, other dominant components of the volatile oil of both traditional and conventional oils almost have similar amounts. The conventional method

of oil preparation is more convenient than the traditional method which needs special cupper made pot that need to be tin plated prior to use and the conventional maceration method seems more convenient compared to the multi-step traditional method of oil preparation. It could be concluded that instead of following the traditional method of ginger oil preparation, it is possible and more convenient to prepare ginger oil according to the conventional method so that it could be used in the numerous indications suggested in ITM.

As observed in the present study, the amount of zingiberene in both traditional and conventional ginger oil was approximately two times compared to ginger essential oil; while the amount of sesquiphellandrene in both traditional and conventional ginger oil was nearly one and a half times in comparison with ginger essential oil. On the other hand, unlike traditional and conventional oils, ginger essential oil didn't seem to possess curcumene and β-bisabolene. Regarding that zingiberene, sesquiphellandrene, curcumene and β-bisabolene are important anti-inflammatory agents, it could be suggested that the traditional and conventional ginger oils possess more anti-inflammatory effects than ginger essential oil and it could be used instead of ginger essential oil as anti-inflammation agents.

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