Analysis of six populations of Commiphora myrrha (Nees) Engl. oleo-gum resin

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Abstract

True myrrh is produced by wounding the plant *Commiphora myrrha* (Nees) Engl. A number of oleo-gum-resins are produced from various species of Commiphora. Many of these gummy substances resemble myrrh; and sometimes are used as an adulteration of Myrrh. Generally, a complicating factor in the study and use of myrrh is the fact that most samples are obtained from the market where the plant source cannot be identified and there is often adulteration in the commercial myrrh. Myrrh oleo-gum-resin is composed of about 2-8% yellow or yellowish green, rather thick essential oil. Current study analyzed the chemical composition of six myrrh samples from Tehran and Fars (Shiraz and Larestan) in Iran as well as United Arab Emirates. The essential oil samples were yielded and subjected to Gas chromatography/ Mass spectroscopy (GC/MS) to analyze the constituents. Although there was a considerable difference in yields of essential oil extracted from studied gums, all samples represented Furanoeudesma-1, 3-diene as major constituent which was in line with previous studies. In addition, Curzerene and Lindestrene were also revealed as other main ingredients.

Keywords: : Adulteration, Commiphora myrrha, Essential oil.

1. Introduction

There are several species of the genera, *Commiphora* (Burseraceae family) which produce famous commercially important resins (1). Myrrh oleo-gum-resin is one of these commercial products. It is rounded or irregular resinous substance. These substances are yellow to red in color and usually they are covered with a dusty powder which has a lighter color (2). A number of oleo-gum-resins are produced in southern Arabia and northeast Africa mainly Somalia from various species of *Commiphora*. Many of these gum-

Corresponding Author: Fatemeh Ghavidel, Department of Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Email: ghavidelf@sums.ac.ir my substances resemble myrrh; and sometimes are used as an adulteration of myrrh. However, true myrrh is produced by wounding the plant, *Commiphora myrrha* (Nees) Engl. (3). From historical point of view, myrrh has been used for incense and embalming through the ancient times. It was one of the gifts of the Magi to the infant Christ (4). The first recorded medical use of Myrrh in Chinese medicine goes back to 600 A.D. and it is continued till today where it is used as a treatment for wound healing, to relieve painful swelling, as well as management of menstrual pain in different cultures' ethnomedicine (4). Today, these resinous exudates are commonly used in perfume industry (5). Myrrh also exhibits analgesic

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properties (6). There have been reports on cytotoxic, anti-cancer, antiparasitic and hypolipidemic activities of this oleo-gum resin (7). Pharmacological studies also have indicated that myrrh possesses anti-inflammatory, effects (8). This oleo-gum-resin is composed of about 2-8% yellow or yellowish green, rather thick essential oil which has the characteristic odor of myrrh and 23-40% resin (7). Myrrh oleo-gum-resin also contains 60% of gum (9). Generally, a complicating factor in the study and use of myrrh is the fact that most samples are obtained from the market where the plant source cannot be identified and there is often adulteration in the commercial myrrh with other species of Commiphora. In this study we analyzed the chemical composition of six myrrh samples from Tehran, Shiraz and Larestan in Iran also myrrh sample from Dubai in the United Arab Emirates. GC-MS analysis was performed on the essential oils of those samples to analyze the chemical volatile compositions which may represent the quality of oleo-gum-resin samples.

2. Experimental

2.1. Plant Materials

Gum samples were purchased from different medicinal plants markets at Fars and Tehran in Iran as well as markets in United Arab Emirates (Dubai). Myrrh samples were identified in Department of Traditional Pharmacy, Shiraz University of Medical Sciences. Voucher numbers was given to the samples and kept at the Herbarium of Shiraz Faculty of Pharmacy. Table 1 represented the samples information.

2.2. Preparing the samples and extracting the essential oil

Samples were rendered free from dust

and impurities manually. Each grinded sample (30 g) was soaked in 300 ml of distillated water. The mixture was then subjected to hydrodistillation for 4 hours. A Clevenger-type apparatus was used for the extraction of myrrh essential oil (10). The obtained essential oil was dried over anhydrous sodium sulphate and kept in the freezer at 4 °C.

2.3. Analysis of the essential oil (GC-MS)

Gas chromatography/Flame ionization detector (GC/FID) was employed to firstly carry out a proper method of isolation for those essential oil samples. GC/FID apparatus was a gas chromatograph Agilent technologies (7890A) apparatus equipped with a HP-5 column (25 m length \times 0.32 mm i.d.; film thickness 0.52 µm) connected to a FID. Nitrogen was the carrier gas (flow rate: 1 ml/min, split ratio: 1:30). Injector and detector temperatures were adjusted at 250 and 280 °C, respectively. Column temperature was linearly programmed from 60-250 °C (at rate of 5°/min) and then was held at 250 °C for 10 min.

The method of analysis (derived from GC/FID) were employed for GC/MS (GC/mass spectroscopy) analysis. GC/MS apparatus was an Agilent technologies (7890A) gas chromatograph with a HP-5MS capillary column (phenyl methyl siloxane coated, 30 m \times 0.25 mm i.d.) connected to a mass detector (Agilent technologies model 5975C). The flow rate of Helium, as the carrier gas, was 1 ml/min. The mass spectrometer was acquired in EI mode (70 eV; mass range: 30-600 m/z). The interface temperature was at 280 °C.

A homologous series of n-alkanes C_8 - C_{30} was also injected to GC/MS for identification and determination of the component via calculating the Kovats indices (KI). The identification of components was based on comparison of respective

Sample No.	Voucher No.	Store	Oil content (% V/W)		
S1	PM-506	Shiraz	2.3		
S2	PM-507	Tehran	2.2		
S3	PM-508	Dubai	7.8		
S4	PM-509	Dubai	6.5		
S5	PM-511	Dubai	7.6		
S6	PM-510	Larestan	7.2		

Table 1. Average values of essential oil content in myrrh oleo-gum-resin samples.

mass spectra with Willey (nl7), libraries of spectra (Adams') and with those reported in previous literatures.

3. Results

For all six samples of the oleo-gum-resin, yield of extracted essential oil was obtained from 2.2 to 7.8% (v/w). The oil content of each sample is presented as the mean of three times of extraction. Table 1 shows places of purchase of the samples as well as the yield of the essential oil extraction for each sample. There were considerable differences in the yield of extraction among those samples.

Chemical constituents of each sample essential oil are reported in Table 2. For all six samples, main components of total essential oil were determined as Furanoeudesma-1, 3-diene, Curzerene and Lindestrene. These components were different in their ranking amounts. According to table 2, the amount of Furanoeudesma-1, 3-diene in all samples were ranked from 29.46-51.53%. Figure 1 represented the chemical structure of those main components in all samples.

4. Discussion

Myrrh samples in this current study were purchased from local distributors of myrrh in Dubai, Larestan, Shiraz and Tehran. Medicinal plants marketers in Shiraz claim that main sources of myrrh distribution is located in Dubai. They also indicate that these samples are being purchased by Iranian medicinal plants distributers in UAE and subsequently transferred to Iran. Some intermediaries' marketers of this medicament in Fars, as reported by salesmen in Shiraz, are from Larestan. In this regard, this study focused on samples from Shiraz, Larestan and Dubai to draw a comparative profile of chemical constituents of those samples.

As shown in Table 2, Furanoeudesma-1, 3-diene, Curzerene and Lindestrene are main ingredients in selected samples. Researchers in a related study analyzed samples of Commiphora myrrha and Commiphora holtziana collected from Kenya. They reported a wide range of sesquiterpenes, mainly furanosesquiterpenes based on eudesmane, elemane and germacrene. In their work, they reported that Commiphora *myrrha* sample did not have δ -elemene, while it was present in Commiphora holtziana (11). On the contrary, all of our samples had δ -elemene as volatile ingredient. In another study, a true myrrh (a standard Commiphora myrrha) has been compared with other myrrh samples which are often the adulterated with C. sphaerocarpa Chiov, C. holtziana Engl. and C. kataf (Forssk.) Engl. In that study, it was reported that the standard myrrh have δ -elemene, but all other samples lacked this component (12).

As shown in table 2 in this current study, sample 3 which was purchased from Dubai contained the highest amount of Furanoeudesma-1, 3-diene, the main reported component of myrrh oleo-gum-resin. Also it contained the least variation of chemical compositions which might be due to the least adulteration. (Tables 1 and 2). It is shown in table 1 that this sample had the highest amount of essential oil which (7.6% V/W of the oleo-gum-resin). According to the reports,



Figure 1. Chemical structures of major constituents of collected myrrha. Lindestrene (1), Furanoeudesma-1,3-diene (2), Curzerene (3).

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the amount of myrrh oleo-gum-resin essential oil is usually yielded as 2.5-8% of the oleo-gum-resin (9).

In this study (Table 2), all samples represented Furanoeudesma-1, 3-diene as major component with amount from 51.53-29.46%. Studies

Table 2. Chemical composition of essential oils obtained from Commiphora myrrha samples HP-5MS
capillary column.

NO	Component	KIC	KIR	S1%	S2%	S3%	S4%	S5%	S6%
1	Carvacrol	1298	1298	-	-	-	-	0.57	-
2	δ-Elemene	1338	1335	1.00	1.32	0.96	1.03	1.07	1.28
3	α-Copaene	1377	1374	-	1.36	-	-	-	-
4	β-Bourbonene	1388	1387	0.90	0.59	-	-	0.56	0.66
5	β-Elemene	1391	1389	5.95	3.61	3.07	3.63	4.27	4.47
5	β-Ylangene	1421	1419	1.00	0.58	-	0.44	0.55	0.69
7	γ-Elemene	1437	1434	0.66	-	0.80	0.68	0.59	0.78
3	Caryophyllene	1464	1464	0.30	-	-	-	-	-
)	β-eudesmene	1483		0.52	-	-	-	-	-
0	Germacrene-D	1485	1484	0.81	2.16	2.00	1.80	1.89	2.21
1	β-Selinene	1490	1489	1.34	0.79	0.55	0.50	-	0.67
2	Curzerene	1499	1499	19.86	11.66	17.22	24.27	20.49	22.87
3	γ-Cadinene	1514	1513	0.45	0.67	-	0.58	0.93	1.09
14	Calamenene	1521	1521	0.49	-	-	-	-	1.02
5	δ-Cadinene	1523	1522	0.42	-	-	-	-	0.39
6	α-Calacorene	1544	1544	0.37	-	-	-	-	-
7	Elemol	1548	1548	0.30	-	-	-	-	-
8	Veltonal	1555	1555	0.40	-	-	-	-	-
9	Germacrene-B	1559	1559	3.93	2.55	5.00	4.53	2.53	4.90
20	Caryophyllene oxide	1582	1582	0.83	0.71	0.67	0.65	0.56	1.03
21	Tetradecanal	1611	1611	-	1.74	-	-	-	-
22	Furanoeudesma-1,3-diene	1649	1645	29.46	49.28	51.53	40.72	45.78	37.55
23	Lindestrene	1653	1651	10.67	12.41	13.38	11.07	11.64	11.52
24	7-epiα-Eudesmol	1662	1662	-	-	-	-	-	2.41
5	Intermedeol	1665	1665	0.58	-	-	-	-	-
26	Amyl cinnamaldehyde	1667	1667	0.80	-	-	-	-	-
.7	Eudesma-4(15)-7-diene-1β-ol	1687	1687	2.06	1.02	-	-	0.60	-
28	Farnesol (2Z,6Z)	1698	1698	0.59	-	-	-	0.61	-
.9	Calamenene-10-one	1702	1702	2.39	1.59	1.05	1.23	1.69	2.57
0	Furanodiene	1706		1.71	0.76	-	0.59	-	-
2	Farnesol (2Z,6E)	1722	1722	5.87	2.58	1.57	5.90	4.27	3.90
33	Elemodiol	1746	1746	-	1.36	-	-	-	-
34	Epoxypseudoisoeugenyl	1793	1793	0.35	-	-	-	-	-
5	Farnesyl acetate	1821	1821	0.74	-	-	-	-	-
6	Eudesm-7(11)-en-4-ol	1839	1839	0.45	-	-	-	-	-
57	Oxofuranoeremophilane	1848	1848	0.45	-	-	-	-	-
8	2-Acetoxyfuranodiene	1884	1884	0.38	0.95	-	-	-	-
39	Cembrene-A	1937	1937	0.39	-	-	-	-	-
10	Hymecromone	1981	1981	1.53	-	0.89	1.40	-	-
den	tification (%)			97.95	97.69	98.69	99.02	98.07	100.00

reported that a mixture of Furanoeudesma-1,3diene and Lindestrene is responsible for the myrrh odor (3). Numerous reports also indicated that the major constituent of myrrh essential oil is Furanoeudesma-1, 3-diene (13-16). However, in a study on a similar sample from Shiraz, Curzerene was appeared as major component of essential oil (17).

Taken as a whole, all populations of myrrha in current study were in the safe side of quality or major volatile content as compared to previous

5. References

1. Baser KHC, Demirci B, Dekebo A, Dagne E. Essential oils of some Boswellia spp., myrrh and opopanax. *Flavour Frag J.* 2003;18:153-6.

2. Tucker AO. Frankincense and Myrrh. *Econ Bot.* 1986;40: 25-32.

3. Hanus LO, Rezanka T, Dembitsky VM, Moussaieff A. Myrrh--Commiphora chemistry.

Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2005;149:3-27.

4. Zhu NQ, Sheng SQ, Sang SM, Rosen RT, Ho CT. Isolation and characterization of several aromatic sesquiterpenes from *Commiphora myrrha*. *Flavour Frag J*. 2003;18:282-5.

5. Simson J, Crist J, Strehin I, Lu Q, Elisseeff JH. An orthopedic tissue adhesive for targeted delivery of intraoperative biologics. *J Orthop Res.* 2013;31:392-400.

6. John G. Voyages and travels in the years 1809, 1810, and 1811: containing statistical, commercial, and miscellaneous observations on Gibralter, Sardinia, Sicily, Malta, Serigo, and Turkey. University of St. Andrews. Library. Reserve Collection. 1812.

7. Bener M, Özyürek M, Güçlü K, Apak R. Polyphenolic contents of natural dyes produced from industrial plants assayed by HPLC and novel spectrophotometric methods. *Ind Crops Prod.* 2010;32:499-506.

8. Afzelius BA. Section staining for electron microscopy using tannic acid as a mordant: A simple method for visualization of glycogen and collagen. *Microsc Res Tech.* 1992;21:65-72.

9. Tyler VE. pharmacognosy. 9 ed. United

studies. However, the yield of extracted essential oil was different which is mainly related to the storage conditions as well as time of collection. In all, GC/MS analysis of volatile components is a rapid procedure to chemically assess and report the quality and content of various populations of an aromatic plant part in herbal medicine.

Conflict of Interest

None declared.

States of America: Lea and Febiger; 1988.

10. Barnett JR, Miller S, Pearce E. Colour and art: A brief history of pigments. *Opt Laser Technol*. 2006;38:445-53.

11. Cammack R, Joannou CL, Cui XY, Torres Martinez C, Maraj SR, Hughes MN. Nitrite and nitrosyl compounds in food preservation. *Biochim Biophys Acta*. 1999;5:2-3.

12. Nusko G, Schneider B, Ernst H, Wittekind C, Hahn EG. *Melanosis coli*--a harmless pigmentation or a precancerous condition? *Z Gastroenterol*. 1997;35:313-8.

13. Tian J, Shi S. [Constituents of essential oil of imported myrrh and gum opoponax]. *Zhongguo Zhong Yao Za Zhi*. 1996;21:235-7, 256.

14. Blay G, Cardona L, Garcia B, Pedro JR, Sanchez JJ. Stereoselective Synthesis of 8,12-Furanoeudesmanes from Santonin. Absolute Stereochemistry of Natural Furanoeudesma-1,3-diene and Tubipofurane. *J Org Chem.* 1996;61:3815-9.

15. Brieskorn CH, Noble P. Two furanoeudesmanes from the essential oil of myrrh. *Phytochemistry*. 1983;22:187-9.

16. Dolara P, Corte B, Ghelardini C, Pugliese AM, Cerbai E, Menichetti S, *et al.* Local Anaesthetic, Antibacterial and Antifungal Properties of Sesquiterpenes from Myrrh. *Planta Med.* 2000;66:356-8.

17. Morteza-Semnani K, Saeedi M. Constituents of the Essential Oil of *Commiphora myr-rha* (Nees) Engl. var. molmol. *J Essent Oil Res.* 2003;15:50-1.

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