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Original Article

GC-MS Chemical Profiling of Heavy Oil Derived from Commercial Variety Oman's Frankincense and their Anti Microbial Activity

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ABSTRACT

Frankincense has long ailment curing history. Local people in Oman often using frankincense resin for fragrance purpose and some varieties are edible. These edible varieties reduce the size of teratomas and some tumors but there is no scientific evidence existing for such practice. Noticeably essential oil extracted from frankincense resin by hydro-distillation (HD) method reported has enormous biological activities such as antioxidant, anti-cancer, anti-analgesic etc. Gas chromatography coupled with mass spectrometry (GC-MS) chemical profiling of this essential oil revealed that the major content is α-pinene and it is associated with other triterpenes. In this study we tried to establish soxhlet extraction procedure to isolate active molecules from commercial variety of Oman's frankincense and to reveal their chemical profiling and its anti microbial property because this variety mainly used for fragrance purpose. Inhaling frankincense fragrance has number of positive biological activities. Recent report states that frankincense fragrance enriched with α-pinene reduce the tumor growth in animal model (Ref). Our GCMS data reveal that α-pinene (48.8%) is major component in our heavy oil followed by α-amyrin (19.5%), γ-elemene (7.4%), β-amyrin (6.9%), delta-limonene (3.84%), and β-phellandrene (1.53%). It is well known that soxhlet extraction procedure is very common to obtain high yield of phytochemicals from the plant or microbial sources and it is very simple, feasible and efficient extraction method. In this study for the first time we define the soxhlet extraction based protocol to extract heavy triterpenes from commercial variety Oman's frankincense and we tested them for antimicrobial activity against different pathogens (*E.coli, klebsiella, staphylococcus and bacillus*).

1. Introduction

The genus Boswellia (family Burseraceae) consist of many species widespread thought the world. It includes approximately 23 species of small trees that grow mainly in Arabia, on eastern coast of Africa and India. Olibanum is a natural oleo-gum resin that exudes from tapping in the bark of Boswellia trees 1. Therapeutic value of Boswellia sp. resin and its essential oil is immune enhancing, antibacterial, antifungal, antiviral, antiseptic wound healing, anti-inflammatory, and anti-cancer properties 2. It has a long history of use and is considered as one of the oldest fragrant and medicinal resins known throughout the world 3. Frankincense (Boswellia sacra) trees are found in Oman, Somalia, Ethiopia, Yemen, the Southern Arabian Peninsula, and India 1, 2, 4, 5,. The discovery of pharmaceutical compounds and medicines can be traced using plants which are good sources. Plant based products; essential oils, plant extracts, natural resins and their preparations have a wide range of applications mainly in pharmaceutical industry, food technology, aroma and cosmetic industries. Extraction of medicinally active portions of the plant tissues can be done using selective solvents following standard procedures. Metabolites of relatively complex structures constitute the products so different components can be obtained in liquid, semisolid state or, after removing the solvent, in dry powder form. These products are intended for oral or external use 6. Frankincense resin has been used for variety of therapeutic purposes 7, including cancer 8, inflammation 9, arthritis 10, asthma 11, psoriasis 12, colitis 13, Crohn's diseases 14, and hyperlipidemia 15.

Essential oil extracted from frankincense resin shown to be potent active principle for many biological activities such as anti-microbial 16, food preservative 17 and anti-cancer agent 18, 19. Chemical profiling of this essential oil revealed that synergistic combination of triterpene complex responsible for observed for biological activities. Chemical profiling data of this essential oil revealed that α-pinene is major constituent and it is active principle of observed biological activities. In this study we adopted simple soxhlet extraction method to extract heavy terpenes from commercial variety of Oman's frankincense. Although hydro-distillation procedure is efficient to extract essential oil

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frankincense resin with rich amount of terpenes, still it requires sophisticated to accomplish. Achieving higher yield of medicinally important phytochemicals with cost effective manner and with least set up is interest of medicinal chemist. To the best of our knowledge there is no report available on extraction of terpenes from frankincense by soxhlet extraction method. For the first time we define the soxhlet extraction based method to extract heavy terpenes from frankincense resin and we analyzed their chemical composition by gas chromatography coupled with mass spectrometry (GCMS) and we tested for anti microbial activity.

Materials and Methods

Extraction of Heavy Oil from Frankincense (Boswellia sacra) Resin

Fresh resins of frankincense collected from Salalah, Sultanate of Oman. Collected resin was powdered by mechanical grinding and heavy oil extracted by soxhl etextraction using hexane for 4 hrs (Fig. 1). After extraction the residual solvent was removed completely from the oil by evaporation.

Drug Preparation

Stock solution of heavy oil prepared in dimethylsulfoxide (DMSO). Different concentrations such as 10, 25 and 50 mg/ml of heavy oil prepared from stock using DMSO and store in refrigerator until use for experiments.

Chemical Profiling of Heavy oil by Gas Chromatography coupled with Mass spectrometry (GCMS)

GC-MS analysis was performed on a Perkin Elmer Clarus 60^0 GC System, fitted with a Rtx®-5MScapillary column (30m×0.25mm i.d. × 0.25µm film thickness; maximum temperature, 350℃), coupled to a Perkin Elmer Clarus 600C MS. Ultra-high purity helium (99.9999%) was used as carrier gas at a constant flow of 1.0 ml/min. The injection, transfer line and ion source temperatures were 270, 240 and 240 ℃, respectively. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The injected sample volume was 1 µl with a split ratio of 50:1. The oven temperature program was 60 ° C and accelerated at a rate of 3 ° C/min–240 °C. The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition).

Preparation of test organisms

Both gram positive and gram negative bacterial strains: namely E.coli, klebsiella, staphylococcus and bacillus were used. The test microorganisms were grown on nutrient agar by following standard procedure as described elsewhere.

Antibacterial Activity Assay

The antibacterial activity of frankincense heavy oil was determined using agar well diffusion method 20. The inoculums were prepared by taking overnight bacterial culture. For sensitivity assay test 38 g of Muller Hinton agar was dissolved in 1000 ml distilled water and autoclaved at 121 °C for 15 min. The media was then poured into sterilized petri-dishes with uniform thickness and the agar was allowed to set at ambient temperature under laminar hood until solidification. These inoculums were spread evenly on the surface of solidified Muller Hinton agar with the help of sterilized spreader. On each plate equidistant wells were made with a 6mm diameter sterilized cork borer. Then 60 µl of different concentration of heavy oil was aseptically added to the respective well. This was followed by allowing the agar plate to stay for 30 min under laminar hood and then incubated at 37 °C for 24 hrs. The formations of clear inhibition zone around the wells were taken as susceptibility measurement.

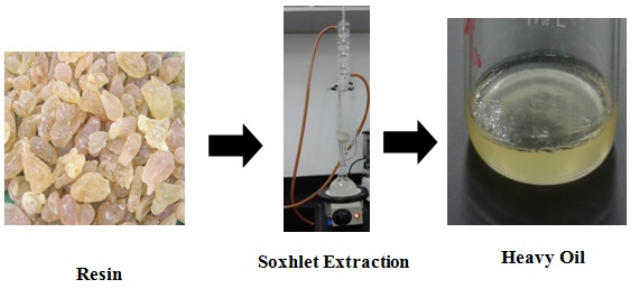
Results and Discussion

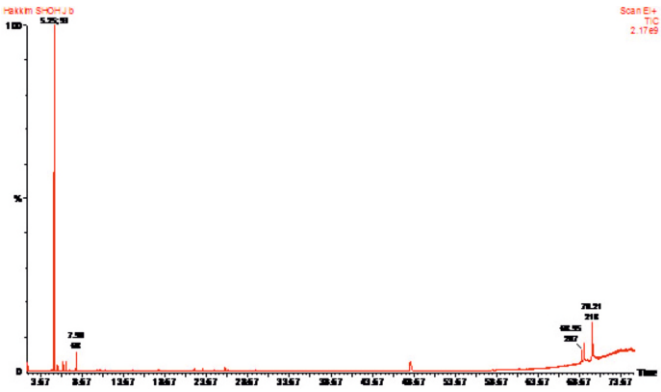
There are many different varieties of frankincense available in Oman. Some of the varieties are available commercially and it is mainly used for fragrance purpose and it has lots positive biological activities. Chemical profiling of frankincense resin fragrance containing rich amount of α-pinene and it reduce tumor burden in mice 21. Omani people ingest some commercial varieties directly for number of ailments. This traditional practice does not have scientific evidence. Our literature survey revealed that not extensive reports available on Omani frankincense but there are some reports on anticancer property of essential oil derived from Omani frankincense by hydro-distillation method against breast cancer, pancreatic cancer and bladder cancer cells 18, 19. In addition, essential oil also shown to be potent antimicrobial agent 16 .

For the first time we design the protocol to extract heavy terpenes from Omani frankincense by simple soxhlet extraction method. Our chemical profiling data reveals that α-pinene (48.8%) is major component in our heavy oil followed by α-amyrin (19.5%), γ-elemene (7.4%), β-amyrin (6.9%), delta-limonene (3.84%), and β-phellandrene (1.53%) (Table 1; Fig. 2). This data is in agreement with previous reports on frankincense essential oil extracted by HD method where α-pinene is the principal component 22. In order to ensure the proportion of major components in frankincense resin oil we compared chemical profiling of the different oils extracted by HD, MHD and soxhlet method (unpublished data). Data revealed that major component α-pinene is almost similar in all three different procedures. Establishing the stringency to extract more desired components with higher physiological activities compound by simple soxhlet extraction method is imperative to reduce the labor cost and time consumption.

Further in this study, we tested this heavy oil for anti-microbial activity against four different pathogens such as klebseilla. E.coli, staphylococcus and bacillus. We found that heavy oil derived from Omani frankincense restricted the growth of these organisms considerably (Table 2) and it is in agreement with previous reports as well (Hasson et al., 2011). Limitation of current study that we did not fractionate the heavy oil to find exact active principle responsible for observed activity. But inter and intra molecular interaction between the heavy oil constituents highly favorable to restrict the growth of the studied organisms. To address this issue we intend to fractionate the Oman's frankincense heavy oil and to study each fraction for various biological activities. This strategy would pave the way to isolate the active component from heavy oil in near future.

Figure 1





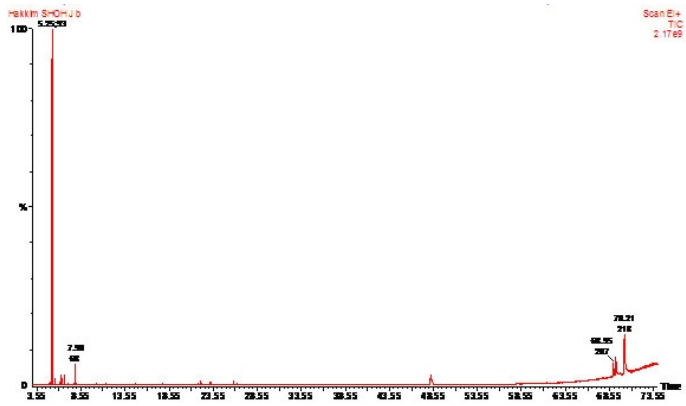


Table 1

No.	Name	RT (min.)	Area	KI	%
1	a - Pinene	5.255	67650448	934.3	48.8
2	Camophene	5.625	1304237.75	949.1	0.94
3	ß- Phellandrene	6.255	2127943.75	974.4	1.53
4	ß-Pinene	6.36	1318985.375	978.7	0.95
5	ß-Myrcene	6.7	2255086.25	992.3	1.62
6	a -Phellandrene	7.13	329438.594	1007.1	0.23
7	O-Cymene	7.311	70131.891	1012.4	0.05
8	(+)-4-Carene	7.521	50533.508	1018.7	0.03
9	O-Cymene	7.756	457253.938	1025.6	0.33
10	D-Limonene	7.896	5319989.5	1029.8	3.84
11	Eucalyptol	8.001	180688.063	1032.9	0.13
12	Trans-ß-Ocimene	8.166	154773.25	1037.8	0.11
13	ß-Ocimene	8.516	91826.438	1048.2	0.06
14	?-Terpinene	8.911	90073.844	1059.9	0.06
15	Myrtenyl Acetate	10.737	1231889.5	1112.1	0.88
16	a -Campholenal	11.372	273337.438	1128.2	0.19
17	U.I*	11.557	97879.719	1133	0.07
18	U.I*	12.733	20910.871	1162.9	0.01
19	Pinocarvone	12.818	47667.355	1165	0.03
20	Myrtenal	14.163	158455.813	1199.3	0.11
21	l-Verbenone	14.699	384966.844	1212.3	0.27
22	Bornyl Acetate	17.825	563728.813	1288.2	0.4
23	a-Terpineol acetate	20.426	314730.844	1351.8	0.22
24	COPAENE or alpha-Cubebene	21.481	150755.141	1377.7	0.1
25	(-)-ß-Bourbonene	21.842	343663.781	1386.5	0.24
26	ß-Elemene	22.142	1383993.25	1393.9	0.99
27	Caryophyllene	23.217	1012055.125	1420.9	0.73
28	Humulene	24.563	423126.594	1455	0.3
29	Alloaromadendrene	24.853	85319.492	1462.3	0.06
30	?-Muurolene	25.488	186699.641	1478.4	0.13
31	ß-Cubebene	25.648	65540.211	1482.4	0.04
32	ß-Eudesmene	25.848	1374512.625	1487.5	0.99
33	a-Selinene	26.208	688444.125	1496.6	0.49
34	?-Cadinene	26.944	92620.625	1515.9	0.06
35	delta.-Cadinene	27.314	184155.719	1525.6	0.13
36	U.I*	29.075	127092.211	1572.1	0.09
37	trans-Caryophyllene	29.535	367402.281	1584.3	0.26
38	(2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide $$ (2S,4R)-5-Isopropenyl-2-methylenecyclohexyl hydroperoxide	30.515	112233.227	1610.6	0.08
39	1-Phellandrene	36.983	236702.25	1748	0.17
40	ß-elemene	42.385	187937.172	1960.6	0.13
41	?-ELEMENE	48.268	10267871	2157.2	7.41
42	ß-Amyrin	69.266	9611855	3016.6	6.9
43	a-Amyrin	70.212	27123686	3062.1	19.5

Table 2. Anti-bacterial activity of heavy oil derived from Oman's frankincense resin

	Organism			
	Zone of inhibition (mm)			
Drg concentration (mg/ml)	***klebseilla***	***E.coli***	***Staphylococcus***	***Bacillus***
10	5.7 ± 1.7	5.5 ± 1.7	3.75 ± 0.8	4 ± 1.6
25	4.1 ± 1.1	5 ± 1.06	5.5 ± 0.5	6.2 ± 2.5
50	5.7 ± 2.6	8.7 ± 2.05	3.7 ± 0.4	6.2 ± 1.7

Data are presented as mean ± SD

Figure 2 Effect of missing RR interval of healthy subjects on Poincaré plot based HRV parameters SD1 (a) and SD2 (b)

The effects of the missing RR interval data on the Poincaré plot and DFA based HRV measures were evaluated based on the relative errors (RE), compared with the parameters calculated from the original, complete RR interval data. When X1, X2, . . . Xn (n = 2 in this study) is obtained for a HRV parameter of the data set with a missing duration, and Xorigin is the corresponding parameter value of that without any missing data, the relative errors REk are computed as |Xorigin − Xk|/Xorigin × 100 (%), where k=25. For each HRV parameter and missing duration, 1000 error values were derived and used for the statistical calculations.

Table 1 Significance level (p value) to reject the alternative hypothesis that the nonlinear HRV parameters of 25 subjects in presence of missing RR intervals is less than mean HRV indices without missing RR intervals

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