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Full Length Research Paper

Chemical Profiling and Anti-Microbial Activity of Frankincense (*Boswellia sacra*) Derived Heavy oil

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Frankincense considered as holy plant in Oman and its resin commonly used for fragrance purposes. In addition, frankincense resin has many ailments curing history and it is observed by local people. Essential oil extracted from frankincense resin either by hydro-distillation (HD) or microwave assisted hydro-distillation (MHD) method has numerous biological activities such as antimicrobial, antioxidant, anti-cancer, anti-analgesic etc. Gas chromatography coupled with mass spectrometry (GC-MS) chemical profiling of this essential oil revealed that the major component is α-pinene and it is associated with other triterpenes. HD and MHD widely used to extract the essential oil from frankincense but it requires sophisticated set up to achieve efficient extraction. 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 frankincense and we tested them for antimicrobial activity against different pathogens (*E.coli, klebsiella, staphylococcus and bacillus*). In addition chemical profiling of heavy oil done by GC-MS. Heavy oil extracted from frankincense exhibited considerable anti-microbial activity against the organism tested. Our data revealed that α-pinene is major content of this heavy oil about 61.5% followed by α-amyrin (20.6%), β-amyrin (8.1%), β-phellandrene (1.47%) and camphene (1.04%). Adapting simple and efficient extraction method is always warranted to obtain high yield of phytochemicals. To the best of our knowledge this is the first report on heavy oil extraction from Oman's frankincense by soxhlet extraction method.

Keywords: Frankincense; Heavy Oil; Soxhlet Extraction; Triterpene; Oman

INTRODUCTION

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 (Handa et al., 2008). Frankincense (*Boswellia sacra)* trees are found in Oman, Somalia, Ethiopia, Yemen, the Southern Arabian Peninsula, and India (Marshall, 2003; Culioli et al., 2003; Baser et al., 2003; Hamm et al., 2005).

The genus *Boswellia* (family Burseraceae) consist of many species widespread thought the world. It includes approximately 23 species of small trees that grow mainly

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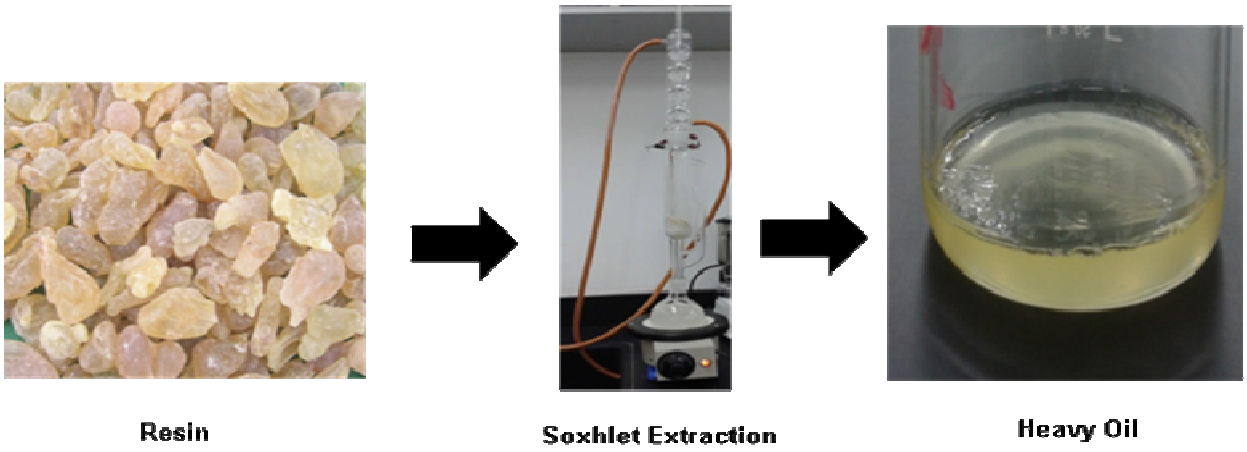

Figure 1. Extraction of heavy oil from frankincense resin

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 (Hamm et al., 2005). 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 (Crow, 2006). It has a long history of use and is considered as one of the oldest fragrant and medicinal resins known throughout the world (Culioli et al., 2003). This resin has been used for variety of therapeutic purposes (Marinetz et al., 1988), including cancer (Shao et al., 1998), inflammation (Singh and Atal, 1986), arthritis (Sharma et al., 1989), asthma (Gupta et al., 1998), psoriasis (Chopra et al., 1956), colitis (Gupta et al., 2001), Crohn's diseases (Gerhardt et al., 2001), and hyperlipidemia (Pandey et al., 2005).

Essential oil extracted from frankincense resin shown to be potent active principle for many biological activities such as anti-microbial (Hasson et al., 2011), food preservative (Saifeldin et al., 2013) and anti-cancer agent (Suhail et al., 2011; Xiao Ni et al., 2012). Chemical profiling of this essential oil revealed that synergistic combination of triterpene complex responsible for observed biological activities. However HD and MHD extraction procedure consumes more time and it needs sophisticated set up. To overcome this hurdle in this study we tried to extract heavy triterpenes by soxhlet extraction method. Data reveals that composition of heavy oil similar to essential oil extracted by other methods. Overall this study provides the evidence that potential heavy triterpenes can be extracted from frankincense by simple soxhlet extraction method.

MATERIALS AND METHODS

Extraction of Heavy Oil from Frankincense (*Boswellia sacra*) Resin

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

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

GC-MS analysis was performed on a Perkin Elmer Clarus 600 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 ^{o}C and accelerated at a rate of 3 ^{o}C/min–240 ^{o}C. The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition).

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.

Preparation of test organisms

Both gram positive and gram negative bacterial strains: namely *E.coli, klebsiella, staphylococcus and bacillus*

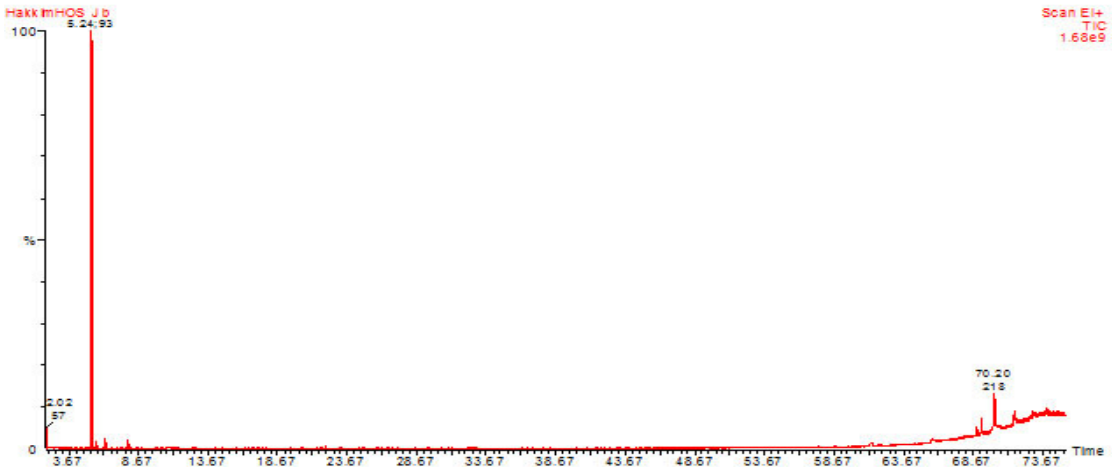

Figure 2. GC Chromatogram of heavy oil

Table 1. Chemical composition of heavy oil derived from Oman's frankincense

Name	RT	Area	KI	%
α - PINENE	5.24	52421768	933.7	61.56
CAMPHENE	5.615	891046.5	948.7	1.04
β - PHELLANDRENE	6.24	1253243.5	973.8	1.47
β - PINENE	6.345	824030.25	978.1	0.96
β - MYRCENE	6.685	189273.984	991.7	0.22
α - PHELLANDRENE	7.111	125742.477	1006.5	0.14
O-CYMENE	7.296	37991.113	1012	0.04
(+)-4-CARENE	7.491	22333.596	1017.8	0.02
O-CYMENE	7.736	142446.609	1025.1	0.16
D-LIMONENE	7.886	1351695.375	1029.5	1.58
EUCALYPTOL	7.986	99737.57	1032.5	0.11
TRANS-.BETA.-OCIMENE	8.146	105841.359	1037.2	0.12
β - OCIMENE	8.501	46717.734	1047.8	0.05
γ - TERPINENE	8.891	31123.676	1059.3	0.03
MYRTENYL ACETATE	10.712	605367.313	1111.5	0.71
α - CAMPHOLENAL	11.347	172759.047	1127.6	0.2
PINOCARVONE	12.798	50390.559	1164.5	0.05
Myrtenal	14.138	52837.758	1198.6	0.06
l-Verbenone	14.679	189862.406	1211.8	0.22
BORNYL ACETATE	17.81	242122.313	1287.8	0.28
α-Terpineol acetate	20.406	130338.734	1351.3	0.15
(-)- β -BOURBONENE	21.822	108914.273	1386	0.12
β-Elemene	22.127	401735.656	1393.5	0.47
CARYOPHYLLENE	23.202	242665.406	1420.5	0.28
HUMULENE	24.548	59947.055	1454.6	0.07
ALLOAROMADENDRENE	24.843	26928.473	1462.1	0.03
γ - MUUROLENE	25.483	22515.51	1478.3	0.02
β-Eudesmene	25.838	268771.656	1487.2	0.31
α-Selinene	26.199	114555.25	1496.4	0.13
γ-Cadinene	26.939	27214.281	1515.8	0.03
Delta - Cadinene	27.309	51775.66	1525.5	0.06
trans-Caryophyllene	29.53	77984.422	1584.1	0.09
1-PHELLANDRENE	36.978	58851.047	1747.9	0.06
β - AMYRIN	69.267	6971952	3016.6	8.1
α - AMYRIN	70.202	17608366	3061.6	20.6
α - Cubebene	21.462	56306.059	1377.2	0.06

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	3.5 ± 0.5	5.75 ± 1.3	3.5 ± 0.5	5.2 ± 0.8
25	4.5 ± 0.5	3.5 ± 0.5	4.25 ± 0.46	5.7 ± 1.5
50	8 ± 3.7	6.5 ± 1.6	3.75 ± 0.8	6.7 ± 2.1

Data are presented as mean ± SD

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 (Taye et al., 2011). The inoculums were prepared by taking overnight bacterial culture. For the sensitivity assay test, 38g 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 an 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

Frankincense considered as holy herb in Oman and it is used for curing many ailments without scientific background. It is mainly used for fragrance purpose. 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 HD method against breast cancer, pancreatic cancer and bladder cancer cells (Suhail et al., 2011; Xiao Ni et al., 2012). In addition, essential oil also shown to be potent antimicrobial agent (Hasson et al., 2011).

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 (61.56%) is the major component of this heavy oil followed by α-amyrin (20.68%) and β-amyrin (8.18%) (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 (Basar, 2005). 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 positive biological activities 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). Noticeably heavy oil does not exhibit the linearity in zone of inhibition according to drug concentration and it remains unclear. 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 might 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.

Disclosure of conflict of interest

Authors have no conflicts of interest

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