EFFECT OF DIFFERENT PARAMETER ON YIELD OF FRANKINCENSE OIL USING STEAM DISTILLATION AND GC-MS ANALYSIS

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ABSTRACT-Frankincense (also known as *Olibanum*) belonging to family Burseraceae, is an aromatic oleo-gum resin traditionally used as a medicine, a fumigant, in various cosmetic formulations and in aromatherapy in several countries. It appears to be colorless to pale yellow liquid with a balsamic, citrusy and spicy odor. Frankincense oil shows different pharmacological properties like anti-bacterial, anti-inflammatory, anti-microbial, hepatoprotective and therapeutic properties. In this study, effect of sample preparation on yield and quality, namely the chemical composition of essential oil of Indian Frankincense (*Boswellia serrata*) was identified. Two different size of sample was prepared. *Boswellia serrata* gum resin was used in the form of whole resin and ground resin (100 mesh/0.22 mm) as sample raw material. The production of frankincense oil can be obtained by using steam distillation. The yield extracted from frankincense oil was collected every 2 hour until 8 hours of extraction time. This study examined the chemical composition of frankincense oil as a function of extraction time during steam distillation. The chemical composition of the extracted essential oil was analyzed by GC-MS. α-Thujene, α-Pinene, Sabinene, α-Phellandrene, Limonene and Methyl Chavicol are the major components to determine Indian frankincense oil. The oil percentage increased gradually with time up till 8 hours, in which exceeding this duration no increase in the yield was observed. The extracted oil from whole resin resulted in 3.1% yield, whereas the ground resin resulted in 2.8%.

***Keywords*-Frankincense; Olibanum; *Boswellia spp.*; Steam Distillation; Extraction; GC-MS.**

1INTRODUCTION

Frankincense is derived from the ancient French name "franc encens" (i.e., high quality incense) [1]. As per Arabic language, frankincense is known as al-luban, which means white/cream and is a basis for its other name, olibanum [2-6]. In Chinese, it is known by the name of Ru Xiang [7]. Traditionally, *Boswellia* tree have been used worldwide for so long for treatment of various diseases [8]. Some of the important species of *Boswellia* are serrata, sacra, carterii, papyrifera, neglecta, rivae, frereana, and ovalifoliolata, etc [9-12]. The tree is commonly produced in gulf countries viz. Oman, Yemen and Sothern Saudi Arabia, in East Africa (Somalia and Ethiopia), South Asia are the gulf countries where *Boswellia* tree is commonly produced and in India, it grows in dry hilly area [13-16]. In India, it is produced on a large scale in states such as Madhya Pradesh, Bihar, Orissa and some parts of Western Himalayas [17].These trees with a thick brown inner resiniferous layer have a pale papery brown bark [18]. This gum appears to be a hardened orange-brown gum resin known as olibanum (commonly known as frankincense).

In Ayurveda, an Indian traditional system of medicine, this gum resin is used to treat a number of inflammatory diseases that affect skin, eye, gums, gastrointestinal tract (GIT) and respiratory inflammatory disorders [19]. Though, having woody, spicy and haunting smell frankincense oil is usually extracted through steam distillation of its gum resin. Currently, it is one of the most important commercial essential oils available on the international market. Indian Frankincense, Olibanum and Incense or Salai guggal is basically the dried exudate from the bark of *Boswellia serrata* tree. Its appearance is in the form of lumps or tears which are whitish and yellow in color.

Salai guggal or oleo gum resin is basically a mixture of essential oil, gum and resin. Frankincense constitutes of 60–85% resins (mixture of terpenes), 6–30% gums (mixture of polysaccharides), and 5–15% essential oils [20]. The essential oil mainly constitutes of monoterpenes, diterpenes, sesquiterpenes, phenolic compounds and a diterpene alcohol (serratol). Gum constitutes of pentose and hexose sugars along with some oxidizing and digestive enzymes. The two most stiff anti-inflammatory boswellic acids of *Boswellia* are acetyl-11-keto-beta-boswellic acid (AKBA) and 11-keto-beta-boswellic acid (KBA) [21]. On the international market, frankincense oils are obtained from several sources and distributed by various companies. Oil extracted from several *Boswellia* species are sold under the same name as “frankincense oil”.

Therefore, researches have been conducted to determine the best method for extraction of essential oil. The chemical composition of various frankincense oils [22-27] and their constituents changes according to the climate, harvest conditions and geographical distribution [28]. In this study, the focus is on the yield, extraction time and sample preparation. As per past research, longer extraction time gave higher yield. Some studies suggested the sample mass factor as high sample mass is proportional to higher oil yield. However, no research has been conducted for effect of these parameters on the chemical composition of the extracted frankincense oil. Hence, this research specifically focuses on the findings of the recent study conducted by the authors on this issue.

MATERIALS AND METHODS

Raw Material:

Boswellia serrata oleo-gum resin was purchased from local market of Madhya Pradesh, India. The oleo-gum resin was used in the form of two different samples. The first sample was used as whole resin i.e., lumps of gum resin as obtained from the supplier. The second sample was used as ground resin i.e., powdered using a mixer grinder to a particle size of 100 meshes (0.218 mm).

Extraction Procedure:

100g of *Boswellia serrata* oleo-gum resin was loaded into 1-Litre round bottom flask with 500ml of distilled water, which was placed in a bench scale steam distillation unit and the system was run at 100ºC and atmospheric pressure. On heating, the sample generated vapor which was condensed with a Clevenger apparatus. The condensate (steam & oil) was collected in a separating funnel where 5 ml of diethyl ether was added to the condensate and diethyl ether containing oil was separated into a conical flask. The duration of extraction was in the range of 2 to 8 hours. Then, the traces of water were removed by adding 1 gram of magnesium sulphate. The essential oil was then obtained by giving a water bath.

Fig 1: Experimental setup of Steam Distillation

Chemical Analysis:

The extracted essential oil was analyzed using Gas Chromatography Mass Spectrometer (GC-MS) to determine the chemical composition. GC was performed by Nucon 5700 Model, fitted with a fused silica capillary column with a non polar stationary phase. The condition followed was: injector temperature of 390ºC in 10ºC steps, oven temperature of 399ºC in 1ºC steps. Temperature programming was set by a Microprocessor based programmer with rates from 0.1ºC to 29.9ºC per min. with initial time holds up to 45 min. 0.10 µL of sample was injected, also FID detector was used.

MS was performed using Agilent 5977 E Model using a fused silica capillary column with a non polar stationary phase. 0.05 µL of each sample was injected in a splitting ratio of 1:10 at temperature 250ºC; helium was used as a carrier gas. Mass spectra were operated in electron impact mode with ionization energy of 70eV. The compounds of oil were identified by recorded mass spectra in the MS library.

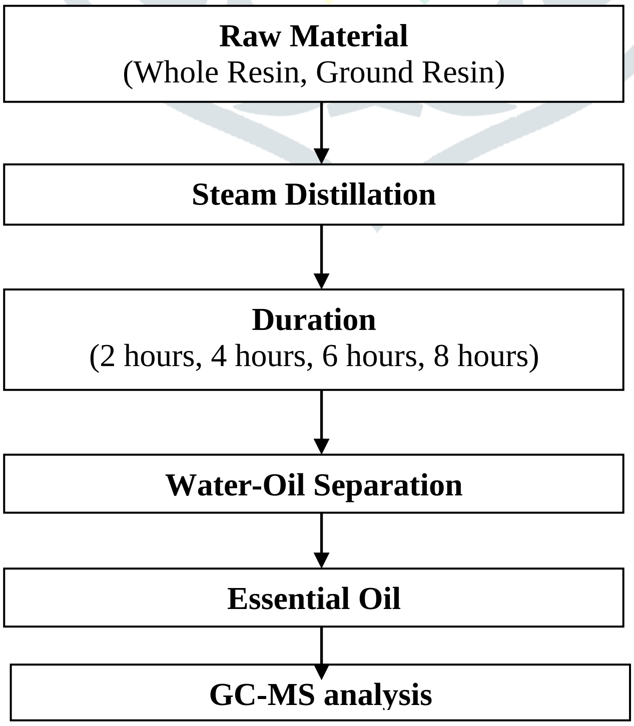

Fig 2: Flow Chart of the experimental Work

RESULTS & DISCUSSION

Effect of extraction time on yield

Extraction time required to obtain oil was taken 8 hours maximum, after which no increase in yield was observed. The first droplet was obtained at 1 hour as shown in fig. 3 & fig. 4 for both whole resin and ground resin. The ultimate essential oil yield obtained from *Boswellia serrata* was 3.1% in whole resin and 2.8% in ground resin. Both yields are conveyed as in grams of essential oil per 100 g of frankincense gum resin. These results mean whole resin yields more oil in comparison to ground resin.

$$\text{Oil Yield (\%)} = \frac{\text{quantity of essential oil extracted (g)}}{\text{quantity of raw material used (g)}} \times 100$$

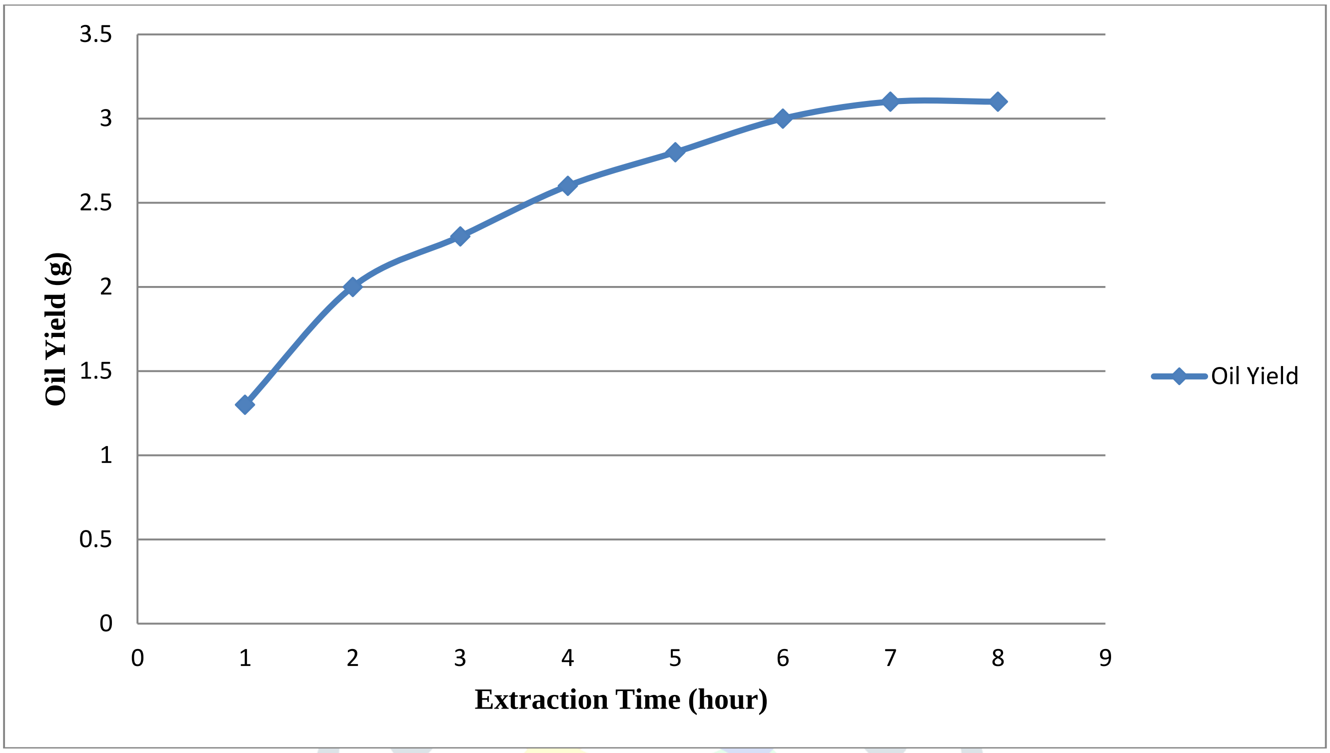

Fig 3: Effect of extraction time on oil yield of whole resin

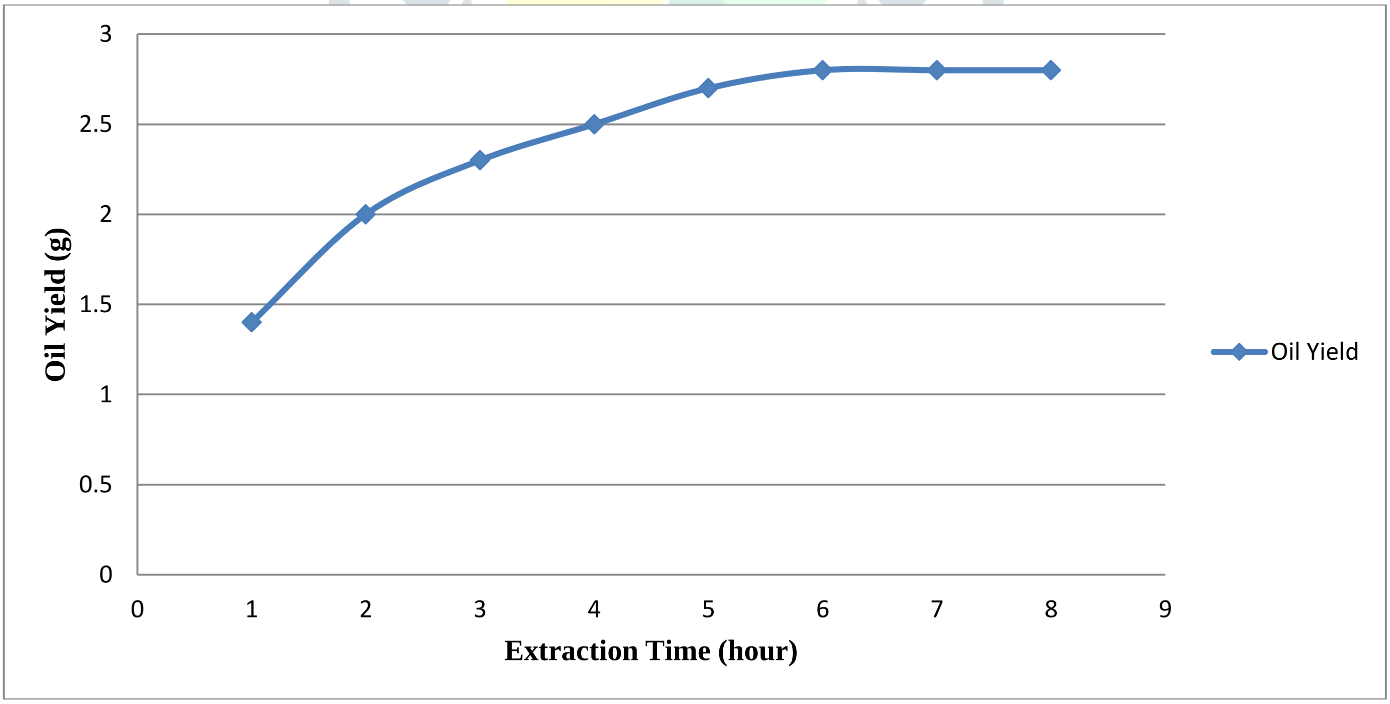

Fig 4: Effect of extraction time on oil yield of ground resin

Chemical composition analysis

GC-MS analysis identified 53 compounds of which 44 compounds were completely identified (Table 1).α-Thujene, α-Pinene, Sabinene, α-Phellandrene, Limonene and Methyl Chavicol were the main identified components, all reported in Indian olibanum (*Boswellia serrata*) obtained by steam distillation[23].

Table 1. Chemical composition analysis of extracted essential oil of *Boswellia serrata*

S. No.	Compound	Retention Time (Min)	Whole Resin (%)	Ground Resin (%)
1	Tricyclene	4.66	0.15	0.21
2	α-Thujene	4.85	44.56	39.61
3	α-Pinene	5.02	18.18	8.34
4	Thuja-2,4(10)diene	5.19	0.37	0.98
5	Camphene	5.32	0.46	0.16
6	Sabinene	5.90	8.31	8.95
7	β-Pinene	5.99	0.79	0.71
8	β-Myrcene	6.28	0.86	1.76
9	α-Phellandrene	6.71	2.09	5.16
10	δ-3-Carene	6.89	5.61	7.15
11	α-Terpinene	7.06	0.26	1.06
12	m-Cymene	7.21	0.15	0.17
13	p-Cymene	7.30	4.21	3.57
14	Thujol	7.37	0.52	0.18
15	Limonene	7.43	3.60	5.01
16	E-β-Ocimene	7.66	0.47	1.09
17	Z-β-Ocimene	8.00	0.22	0.66
18	γ-Terpinene	8.38	0.69	2.11
19	cis-Sabinene hydrate	8.67	0.23	0.19
20	α-Terpinolene	9.39	0.28	0.71
21	Linalool	9.77	0.39	0.35
22	Thujone	10.43	0.31	0.34
23	3-Ethoxyaniline	10.55	0.17	0.07
24	trans-Sabinol	11.29	0.15	0.13
25	cis-Sabinol	12.41	0.15	0.16
26	4-Terpineol	12.77	1.06	1.62
27	α-Terpineol	13.30	0.08	0.09
28	Methylchavicol	13.63	3.08	5.10
29	Linalyl Acetate	15.96	0.10	0.06
30	α-Copaene	20.90	0.14	0.24
31	β-Bourbonene	21.27	0.58	1.30
32	allo-Aromadendrene	21.85	0.08	0.14
33	Methyleugenol	22.10	0.17	0.23
34	β-Ylangene	22.64	0.04	0.13
35	β-Cubebene	23.33	0.05	0.10
36	γ-Muurolene	23.67	0.02	0.08
37	α-Cadinene	24.97	0.03	0.08
38	Germacrene D	25.13	0.09	0.39
39	δ-Cadinene	26.81	0.01	0.06
40	5-Methylcyclopent-1-ene-1-carboxylic acid	26.96	0.16	0.24
41	β-Santalol	32.56	0.22	0.20
42	α-Santalol	34.05	0.05	0.08

43	p-Mentha-1,5-diene	36.70	0.02	0.09
44	cis-Salvene	48.09	0.09	0.16

Comparison of main components

Fig. 5 shows the comparison of main components present in the essential oil of *Boswellia serrata* among the whole resin and the ground resin. The amount of α-Thujene, α-Pinene and p-Cymene were found to be higher in the oil obtained from whole resin, whereas that of Sabinene, α-Phellandrene, δ-3-Carene, Limonene and Methyl Chavicol were higher in the ground resin.

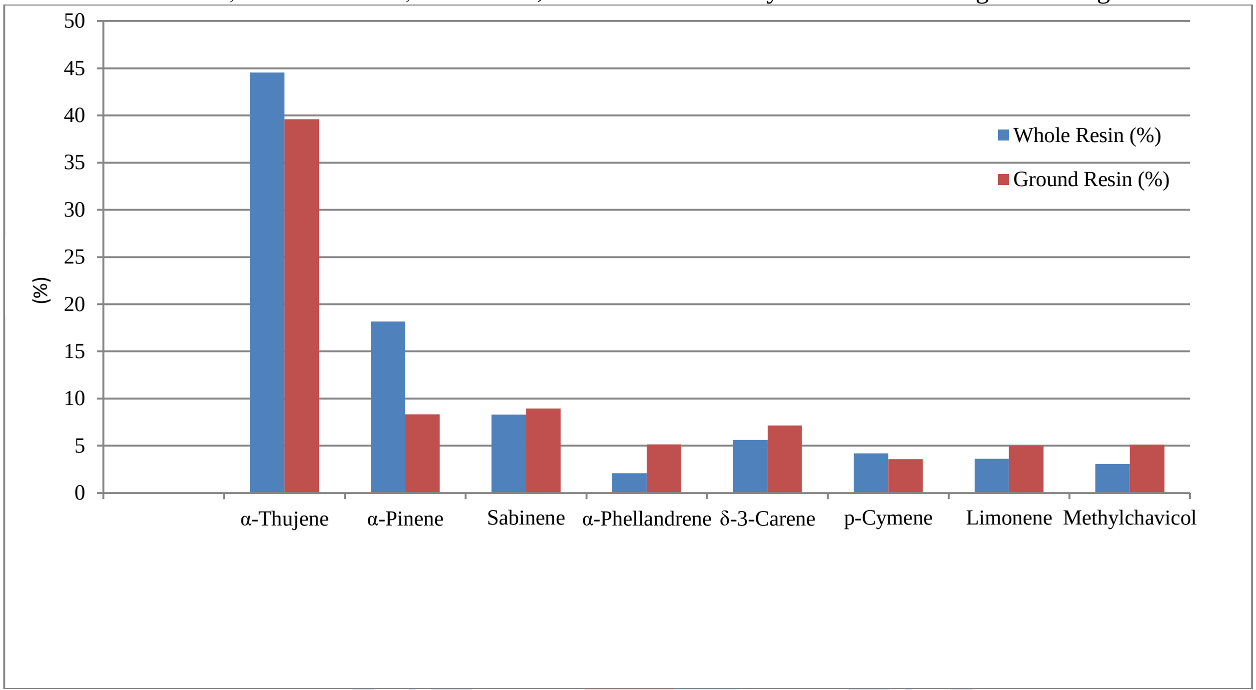

Fig 5: Comparison of main components in essential oil obtained by steam distillation

CONCLUSION

In this study we identified total 44 compounds in frankincense oil. In these compounds, α-Thujene, α-Pinene p-Cymene, Sabinene, α-Phellandrene, δ-3-Carene, Limonene and Methyl Chavicol are main compounds. The whole resin with large surface area has essential oil than ground resin with small surface area. Large particle size will yield more oil (3.1%) in comparison to small particle size (2.8%) at short distillation time.

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