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Biophysical and Chemical Investigations of Frankincense of *Boswellia papyrifera* from North and Northwestern Ethiopia

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ABSTRACT

This study was conducted to contribute to filling the existing knowledge gap on biophysical and chemical properties of frankincense towards revising the national grades being used in the export market. A biophysical and chemical study on resins (frankincense, also known as gum olibanum) of Boswellia species from five regions (Amhara, Benshangol, Oromia, Somali, and Tigray) with special emphasis on the Boswellia papyrifera (Tigray type frankincense) has been conducted using GC-MS, TLC, Pyrolysis methods and odor tests. 111 samples were subjected to different analytical treatments most of which were biophysical and some chemical investigations. GC-MS analysis, odor tests by women of three cities (Harar, Nazareth and Addis Ababa), TLC runs and observations of Pyrolysis experiment at 400, below 1000 and over 1200°C were used to see if age of tree, origin (Provenance) and frequency of tapping have had any influence on the quality of frankincense, a major export product of Ethiopia. The data obtained, helped to see origin, age of tree and color of product to have influences on quality of frankincense. Headspace GC-MS analysis of frankincense of B. papyrifera at different collection sites and ages of the trees indicated the presence of n-octylacetate as a major component at varying relative percentage composition. The harvests resins of Boswellia species from Borena and Ogaden, while exhibiting same even better quality frankincense for purpose of similar use, were not processed for international market. This paper also presented the findings of the investigations in such a way that further biophysical and chemical works remain to be performed so as to see conclusively how the three resin types (Tigray, Borena and Ogaden) are similar and/or different for the purpose (international trade) sought.

Key words: *Boswellia papyrifera;* frankincense; Burseraceae; headspace analysis; GC-MS analysis.

INTRODUCTION

Boswellia papyrifera (Del) Hochst, is a decidious, gum-producing, multipurpose perennial tree, which is tapped on the stem for a kind of oleo-gum called “olibanum” (true frankincense) [1-4]. This gum resin is used in medicinal preparations for the treatment of amenorrhoea. It is also used in treatment of diarrhea, asthma, and bronchitis [5,6]. The *Boswellia* plants are known to contain several acidic triterpenes, some of which show analgesic, immunosuppressant, antileukemic, hepatoprotective, and anti-inflammatory activities. Most of these activities are based on the inhibition of the enzyme 5-lipoxygenase [7,8]. Incensole acetate, a novel anti-inflammatory compound

isolated from *Boswellia* resin, inhibits nuclear factor-kappa B activation. Being a natural product, frankincense varies greatly in its ingredients. These variations can also be found in different varieties of the *Boswellia* tree; whose botanical classification is:

Division: Spermatophyta
Sub-division: Angiospermae
Tribe: Rosopsida
Sub-tribe: Rosidae s. lat.
Super-class: Rutanae
Class: Anacardiales
Family: Burseraceae
Genus *Boswellia*

The species are [9]: *B. sacra*, also known as *Boswellia* Roxo or B. carter Birdw; *B frereana*, also known as "Elenni frankincense"; *B. serrata*, also known as "Indian frankincense"; *B. papyrifera*, also known as: "Ethiopian frankincense"(syn: *Amyris papyrifera*).; *B. rivae*; *B. neglecta*.; and about 10 more species.

It is interesting to note that some literature reports mention *B. sacra* and *B. carteri* to be different species [2,10]. This may be explained by the fact that frankincense is cultivated in different countries by various means. Furthermore, the quality of frankincense is defined by geographical trade names and not by the botanical classification [9,11]. Frankincense or olibanum is a plant product. It is an oleo-gum-resin produced by several species of tree belonging to the *Boswellia*, which is characterized by resin bearing ducts. To obtain frankincense, the bark of the tree is cut several times to allow a white milky resin to seep from the wounds. The resin is left on the tree to dry in the sun for a few days, after which the so called resin tears are scraped off. The color of the resin varies from light yellow to dark brown. The resin tears consist of [11] 60% resin (of which 50% are boswellic acids), 29% rubber, 6-8% bassorine, 5-15% essential oils, 0.5% bitter and nuciliage compounds *B. papyrifera* is known to occur in Ethiopia, Sudan and in some parts of West Africa [12] (Vollesen, 1989). Its resin, commonly referred to as "Tigray" or "Keren-type" olibanum, is widely used in Ethiopia as incense at home and during religious ceremonies. It is also an important item of export for formulation of perfumes [13]. Resin from this species is normally obtained by making an incision on the bark and allowing a white emulsion to exude out, which slowly dries into different shaped tears.

Frankincense is a natural product whose ingredients may depend on many factors such as region of origin, climate, time of harvest and other environmental conditions [11,14]. An indication of this variance might be obtained when comparing the different results of the samples of the same species. As frankincense is a highly appreciated material in cosmetic products [15] and is gaining more and more importance in other fields such as medicine [16] it is necessary to develop specific criteria to assure constant quality of the traded frankincense resin and the respective frankincense products.

Comparison of samples of *B. papyrifera*, *B. pirottae*, and *B. frereana* were generally found to contain four common volatiles: camphene, limonene, oepinene, and oeterpineol. In summary, limonene was found to be the only common compound present in all samples of all investigated *Boswellia* varieties, as analysed by means of hydrodistillation. Investigations of the resin and essences or extracts of *Boswellia* with regard to the specific volatile constituents have been reported in a series of studies, with chemical characterization procedures enabling a total of 311 volatiles to be identified. The diverse volatile constituents listed in Table 1 [11] were detected by GC-MS analysis in the respective species.

In *B. papyrifera* a total of 56 compounds were identified; 42 by hydrodistillation; and 27 by SPME. In general, quantitative determinations have been based on relative peak area comparisons, as obtained by means of GC-FID analysis. Thereby, all values have been expressed as a percentage of the total overall peak area.

n-Octyl acetate was found in the greatest abundance (64.6%) in *B. papyrifera* according to [2], followed by *n*-octanol (13.9%). The relative percentage of *n*-octyl acetate in the hydrodistilled oil of *B. papyrifera* was also reported as 63.6% [17] and 56% [18] (Table 1). An indication of this variation might be obtained when comparing the different results of the samples of the same species [11]. The frankincense of *B. papyrifera* has different prices based on its grade and its quality. There were no previous studies which are based on analysis of constituents of the

frankincense of *B. papyrifera* from different collection sites, ages of the plant and grade. In this study we report the influence of regions of origin, age of plant and frequency of tapping on grade/quality using data from GC/MS, TLC, and Pyrolysis experiments.

Summarizing the data from the analyses of hydrodistillation extract, α-pinene, limonene, *n*-octyl acetate, α-thujene, and (*E*)-β-ocinene can be regarded as those compounds that have been most frequently reported to be the most dominant volatile constituents of the frankincense distillate [19,20]. It is not clear whether, for example, oxidation or polymerization processes occurred to a different extent between samples, so that data might have been biased with regard to identification and quantification. In particular, a general well-known problem is the relative ability of diverse terpenoids that are prone to oxidation, rearrangement or cyclization processes [19].

Table 1: Relative concentration of volatiles (%) of the essential oil obtained by hydrodistillation *Boswellia papyrifera* (Martens *et al*., 2009)

No	Volatile	*B. papyrifera*	No	Volatile	*B. papyrifera*	
		Hamm *et al.* (2005)			**Camarda (2007)**	**Dekebo *et al.* (1999)**
179	*o*-Campholene aldehyde	Traces	176	Benzene, 1 methoxy-2-methyl	0.3	nd
90	Trans-Carveol	0.3	177	Endo-Borneol	nd	1.5
91	Carvone	0.3	5	Bornyl acetate	nd	0.7
15	Cembrene A	1.7	9	Camphene	0.1	0.3
21	p-Cymene	0.2	91	Canvone	0.1	nd
256	n-Decanoic acid	0.1	17	1,8 Cineole	nd	2.2
106	n-Decylacetate	0.9	21	p-Cymene	0.4	nd
189	Eucalyptol	0.1	106	Decylacetate	0.3	nd
259	n-Hexanoic acid	0.2	113	β-Elemene	nd	1.1
260	Hexylcaprylate	1.2	189	Eucalyptol	0.5	nd
121	n-Hexylhexanoate	0.9	120	Hexylacetat	nd	1.3
123	Incensol	1.0	115	Geranylacetate	0.1	nd
124	Incensolacetate	10.8	123	Incensol	0.7	nd
262	Incensol oxide	0.4	124	Incensolacetate	1.7	nd
263	Incensol oxide acetate	0.1	36	Limonene	4.7	6.5
37	Linalool	0.2	37	Linalool	o.7	3.2
197	Cis-Linalcol oxide	0.2	208	p-Mentha-6,8-dien-2-0ne	nd	0.7
138	Nerylacetate	0.1	44	Mycene	0.3	0.7
268	n-Nonanoic acid	0.5	143	Octanol	nd	8.0
143	Octanol	13.9	145	n-Octylacetate	63.5	56.0
269	n-Octanoic acid	0.8	63	*o*-Terpinene	0.4	nd
145	n-Octylacetate	64.6	64	γ-Terpinene	0.2	nd
53	*o*-Pinene	0.5	59	Terpinen-4-ol	0.1	nd
55	Trans-Pinocarveol	0.1	61	*o*-Terpineol	0.2	0.5
179	*o*-Campholene aldehyde	Traces	165	Terpinolene	nd	0.4
			68	*o*-Thujene	0.1	nd
			231	Thuribergene	0.1	nd
			171	Verticilia-4(20),7,11-triene	2.3	nd
			234	Verticellol	nd	0.5

Apart from a sensory evaluation of odor quality and intensity and a visual appraisal of color, an analytical assessment of frankincense quality is currently predominantly made by means of thin-layer chromatography (TLC) and preparative layer chromatography/gas chromatography-mass spectrometry (PLC/GC-MS), whereby a fingerprint of specific markers or quality parameters is obtained [7]. The fastest such method is the Rapid Test by TLC which was developed by Harfield *et al.*, (1984) [21].

In comparison to the data provided for the volatile composition of frankincense, investigations of volatiles from pyrolysis of frankincense are relatively rare [20]. The frankincense material was brought into contact with red-hot charcoal and the released pyrolysates were then purged into a cartridge and adsorbed in glass cartridge filled with Super Q* (Alltech). In *B. carteri*, the volatiles cemberene A, cemberene C, verticillia-4 (20), 7, 11-triene, incensole and incensyl acetate were found at relatively high concentrations without any alteration in their structure. Therefore, these structures can probably be taken as diagnostic markers for *B. carteri*. Furthermore, 1-octanol and *n*-octyl acetate were identified and said to cause an acid smell during pyrolysis. Neither [3] nor [20] took into consideration the heat dynamics during pyrolysation. However, it can be assumed that the evaporating fumes of frankincense

during pyrolysis exhibit a dynamic pattern according to the temperature curve (increase during incense process, red heat stage or glowing) and that with regard to the sensory quality of the pyrolyzed frankincense optimum temperature ranges need to be considered. Accordingly, a temperature-resolved analysis of pyrolysates formation and comparison would be of high interest.

None of the volatile components identified in the different studies have, according to our knowledge ever been attributed to the specific smell of frankincense. It is interesting to note, however, that an olibanum-like odour has been reported elsewhere for a substance found in orange oil residue [21] which was identified as *cis*-iso-cascarilla acid.

The studies summarized here are generally focused on substances with high abundances and it is not clear whether all odour contributing compounds of frankincense or of specific frankincense volatiles have thus so far been identified. Furthermore, it remains unanswered whether there are substances with frankincense specific odour qualities or whether the characteristic smell of frankincense is due to a specific blend of odorants as often observed in other food or plant aromas. Further research is therefore necessary to elucidate the specific contribution to the aroma-profile of frankincense and frankincense-pyrolysate.

The main constituents common in frankincense (depending on the different *Boswellia* species) have been reported to be α-thujene, α-pinene, myrcene, incensole acetate, *E*-β-ocimene, duva-3,9,13-triene-1,5a-diol-1-acetate, phyllociadene, limonene, *n*-octanol and *n*-octyl acetate. Moreover, special attention has been paid to verticilla-4(20),7,11-triene [3] since this compound has hitherto been identified only in frankincense and not in any other biological or plant material. Probably this substance can be suggested as a marker substance for frankincense products, although further research is needed. However, the specific smell of this compound, if any, has not been described.

No information regarding the chemosensory contribution of individual constituents to the specific odor of frankincense resin can be drawn. Neither quantitative nor qualitative data allow for specification of the main odor constituents of these materials. Furthermore, to our knowledge, no attempts have been made to reconstitute the characteristic aroma of frankincense or its specific varieties.

Table 2. Headspace and GC operating conditions

Headspace Parameters	
Transfer Temp.	160 °C
Oven Temp.	150 °C
GC Cycle Time	45 min
Thermostat Time	20 min
Pressurization Time	3 min
Withdrawal Time	0.2 min
Carrier Gas Pressure	14 psi
GC Parameters	
Initial Temp.	60 °C
Final Temp.	200 °C
Rate	4 °C / min
Thermostat Time	20 min
Total Time	45 min
Inj. Port Temp.	170 °C
Pressurization Time	3 min
Withdrawal Time	0.2 min
Carrier Gas Pressure	14 psi
Column Size	30 m × 0.32 mm × 0.25 μm
MS Parameter	
Mass Scan	45 – 450 *m/z*
Max. prog. Temp.	350 °C
Min. Bleed at:	330 °C

EXPERIMENTAL SECTION

GC-MS Experiment

A GC-MS instrument from Agilent Technologies (Santa Clara, CA, USA) was equipped with a 6890N network GC system, 5975 inert mass selective detector, 7683B series autosampler injector (10 µL in size), G1701DA GC/MSD ChemStation and HP_5MS column (30 m length x 0.25 mm internal diameter x 0.25 µm film thickness) coated with 5% phenyl 95% methyl poly siloxane. All headspace and GC operating conditions are listed in Table 2.

TLC Experiment

Thin Layer Chromatography, TLC, experiment has been conducted on 111 samples of olibanum from north and northwestern Ethiopia. The chromatograms were developed using *n*-hexane.

Pyrolysis Experiment

For pyrolysis experiment, samples collected from three major producing sites (Humera; Metema and Metekel) and also sample from Nazareth export store were first graded into three by color (White {Light yellow}; Brown; and Dark) and each grade-sample in three replicates were subjected to heat treatment at three temperature levels. In priority, melting temperatures of the resins were determined. For all samples melting of a resin began after temperature of 300°C. Then each replica was heated at 400 ^{o}C; below 1000 ^{o}C and 1200°C (directly on red hot charcoal) until vaporization was completed. Time-data were collected at these three events: Beginning of melting/vaporization; End of melting; and finally End of vaporization. In addition, odor, color change and physical appearances of residues were checked. The pyrolysis experiment was conducted by taking equal volumes of each sample, that is using a spoon 1.5 ml of water and each measured volume of resin weighed on a top load balance of Switzerland made, SNR 1121323934 and Max. wt.; 610g, d: 0.01; Item No. Eo 6120. Each weighed frankincense in three replicates heated up first on a hotplate of UK; CAT No: CB 162; Serial: R000100044; Volts 230; Hz: 50; Power: 550; thermostated at 400°C. Similar heat treatments conducted on the samples in triplicates at temperature less than 1000°C and 1200°C. To maintain temperatures with in specified ranges a red-hot charcoal was the heat source in both instances and to keep the temperature below 1000°C samples were put in a can container and then the can with the sample in it was subjected to vaporize on a red-hot charcoal. Lastly, resins were directly transferred on a red-hot charcoal and a red-hot charcoal's temperature is assumed to be well over 1000°C and some report it to reach 1200°C.

Odor tests

Oder tests were conducted at Harar, Nazareth and Addis Ababa. Samples separated by production area (Humera, Metema, Metekel, and Nazareth Export Store) and color (White or yellow; Dark Brown and Bark) were smoked over red hot charcoal under typical domestic conditions (household coffee ceremony by women about five to seven people gathering) at all three cities. Individual reaction to odor test of each smoke was recorded.

RESULTS

GC-MS

All GC (headspace analysis) data obtained from Ambo University, Chemistry laboratory indicated that frankincense from Humera, Metema, Metekel, Nazareth store, Ogaden and Borena have more or less similar features. Chromatography peaks were significant around three major retention time ranges; 5 to 10; 15 to 23 and 35 to 43 minutes.

Ogaden and Borena samples showed no features beyond 25 minutes. Resins of these two regions seem to constitute very good amounts of volatile constituents which appeared before the retention time of 15 minutes. Borena samples maintaining being distinct from those of the Ogaden materials.

Samples from Metekel were pronouncedly varying from those of Humera and Metema in the first region of the chromatogram, which is in the 5 to 10 minutes ranges. Samples from Metekel featured proximity to those of the Borena and Ogaden types. Humera and Metema gave similar profiles.

Similar patterns were observed between Humera, Metema, and samples collected from the Nazareth store (Tigray type). Surprisingly, these features were also in congruent with samples from old and young trees of Humera. However, medium aged trees of Humera had a very different pattern from the old and young trees of same site

especially in the first retention time ranges, 5 to 10 minutes of the chromatograms and these medium aged trees of Humera gave resemblances to that of Metekel trees.

Resin samples collected from 7th, 8th, 10th and 11th cycles of tapping of all the three sites' stores of Ethiopia (Humera, Metema and Metekel) were analyzed. At this stage of the experiment and from this level of study, it was not possible to see any effect of frequency of tapping on the quality of frankincense as no difference in patterns among chromatograms have been observed.

Table 3. TLC of crude extracts of different incense samples (Solvent system: *n*-hexane)

Group: A	**Typ**	**Cyl**	**R_F Values**							
Humera-Bkr-KnKr	5	7th				5.0	5.5	6.5	
Humera-Bkr-KnKr	8	8th					5.5		
Metekel-Gub-Fengiso	4	10th		3			5.5	5.9	
Metekel-Awi-ZirZir	6	8th					5.8	6.5	7
Metekel-	7	8th					5.8	6.5	7
Metema-Zbbr-WowGot	1	11th					5.5	6.4	
Metema-Zbbr-WowGot	2						5.5	6.4	
Metema-Zbbr-shashge	3	10th				5.2	5.5		
Group: B	**Typ**	**Cyl**	**R_F Values**							
Humera-Bkr-KnKr	5	7th	2.0			5.0	5.5		6.5	
Humera-Bkr-KnKr	8	8th		3				5.8	6.5	7
Metekel-Gub-Fengiso	4	10th		3			5.5	5.8		
Metekel-Awi-ZirZir	6	8th						5.8	6.5	7
Metekel-	7	8th	2	3				5.8	6.5	7
Metema-Zbbr-WowGot	1	11th	2.0	3		5.0	5.5	5.8	6.5	
Metema-Zbbr-WowGot	2		2.0	3		5.0	5.5	5.8	6.5	
Metema-Zbbr-shashge	3	10th	2.0			5.0	5.5			
Group: C	**Typ**	**Cyl**	**R_F Values**							
Humera-Bkr-KnKr	5	7th		3				5.8	6.6	
Humera-Bkr-KnKr	8	8th		3				5.8	6.6	
Metekel-Gub-Fengiso	4	10th			4			5.8	6.6	7
Metekel-Awi-ZirZir	6	8th			4			5.8	6.6	7
Metekel-	7	8th			4			5.8	6.6	7
Metema-Zbbr-WowGot	1	11th								
Metema-Zbbr-WowGot	2			3				5.8	6.6	
Metema-Zbbr-shashge	3	10th		3				5.8	6.6	
Group: D	**Typ**	**Cyl**	**R_F Values**							
Humera-Bkr-KnKr	5	7th							6.6	
Humera-Bkr-KnKr	8	8th								
Metekel-Gub-Fengiso	4	10th								
Metekel-Awi-ZirZir	6	8th								
Metekel-	7	8th						5.8	6.6	
Metema-Zbbr-WowGot	1	11th						5.8	6.6	
Metema-Zbbr-WowGot	2							5.8	6.6	
Metema-Zbbr-shashge	3	10th								
Group: Trees	**Age**	**Typ**	**R_F Values**							
Humera-Bkr-KnKr	Old	1								
Humera-Bkr-KnKr	Old	2	2.4				5.5			
Humera-Bkr-KnKr	Old	3	2.4				5.5			
Humera-Bkr-KnKr	Old	10	2.4				5.5	6.5		
Humera-Bkr-KnKr	Old	11					5.5	6.5		
Humera-Bkr-KnKr	old	12						6.5		
Humera-Bkr-KnKr	Med	4	2.4				5.5			
Humera-Bkr-KnKr	Med	5	2.4				5.5			
Humera-Bkr-KnKr	Med	6	2.4				5.5			
Humera-Bkr-KnKr	Sml	7	2.4				5.5			
Humera-Bkr-KnKr	Sml	8	2.4				5.5			
Humera-Bkr-KnKr	Sml	9	2.4				5.5			

Table 3. Continued

Group: Trees	Age	Typ	R_F Values							
Metekel-Gub-Feng	Old	53		3			5.5			
Metekel-Gub-Feng	Old	54		3			5.5			
Metekel-Gub-Feng	Old	55		3			5.5			
Metekel-Gub-Feng	Old	62					5.5	6		
Metekel-Gub-Feng	Old	63								
Metekel-Gub-Feng	old	64		3			5.5			
Metekel-Gub-Feng	Med	59		3			5.5	6		
Metekel-Gub-Feng	Med	60		3			5.5			
Metekel-Gub-Feng	Med	61		3			5.5	6		
Metekel-Gub-Feng	Sml	56		3			5.5			
Metekel-Gub-Feng	Sml	57		3			5.5			
Metekel-Gub-Feng	Sml	58		3			5.5	6		
Metekel-Awi-kebtale	Med	76		3			5.5	6	6.6	
Metekel-Awi-kebtale	Sml	78		3			5.5	6	6.6	
Metekel-Awi-kebtale	Sml	79		3			5.5	6	6.6	
Metekel-Awi-kebtale	Sml	80		3			5.5	6	6.6	
Metekel-Awi-Bbs	Old	81					5.5	6	6.6	
Metekel-Awi-kebtale	Old	82		3			5.5	6	6.6	
Metekel-Awi-kebtale	Old	83		3			5.5	6	6.6	
Metekel	Sml	84			4.2				6.6	
Metekel	Sml	85			4.2				6.6	
Metekel	Sml	86			4.4				6.8	
Metekel	Med	87			4.5				6.8	
Metekel	Med	88			4.6				6.8	
Metekel	Med	89			4.7				6.8	
Metekel-Bbs		90							6.5	7
Group: Trees	**Age**	**Typ**	**R_F Values**							
Metema-Bbr	Old	27	2.4					5.6	6.5	
Metema-Bbr	Old	29	2.4							
Metema-Bbr	Old	36	2.4						6.5	
Metema-Bbr	Old	37	2.4					5.6	6.5	
Metema-Bbr	Old	38	2.4					5.6	6.5	
Metema-Bbr	Med	30					5.5	5.6	6.5	
Metema-Bbr	Med	31		3			5.5			
Metema-Bbr	Sml	33	2.4					5.6	6.5	
Metema-Bbr	Sml	34	2.4							
Metema-Bbr	Sml	35						5.6	6.5	
Group: Nazareth-Store	**Age**	**Typ**	**R_F Values**							
Nazareth-Store	White	1A		3					6.5	7
Nazareth-Store	White	2A		3					6.5	7
Nazareth-Store	White	3A		3	4.4				6.5	7
Nazareth-Store	Brwn	4A		3	4.4				6.5	7
Nazareth-Store	Brwn	4S			4.4				6.5	7
Nazareth-Store	Bark	5A		3					6.5	7
Nazareth-Store	Brwn	1B		3	4.4				6.5	7

TLC

Thin Layer Chromatography, TLC (Table 3) experiment has been conducted on 111 samples of olibanum from north and northwestern Ethiopia. The chromatograms were developed using *n*-hexane and showed that in all cases three distinct spots were identified confirming to what has been the case with GC results. Based on the TLC results alone, it was not possible to see differences between regions, age, and frequency of tapping.

Pyrolysis Experiment

Vaporization lasted longer at low temperature (400°C) heat dynamics and to almost same period when heat temperature has been raised to 1200°C (Table 4). The average time for all cases at 1200°C was 2-4 minutes.

Odor Test

The odor test conducted at three towns (Harar, Nazareth, and Addis Ababa) over charcoal heating indicated that the white resin from Humera had a different acid smell than the smokes from the other two regions (Metema and Metekel). And the frankincense from Metema had thicker smokes but without the good pleasant smell.

Table 4. Pyrolysis Experiment of incense samples

	Average
9A,Naz	30.333
9B,Naz	19.817
9C,Naz	15.1
9D,Naz	29
	Average
9A,Naz	14.867
9B,Naz	10.05
9C,Naz	12.667
9D,Naz	29
	Average
9B,Naz	2.2
9C,Naz	13.12
9D,Naz	2
	Average
8A,Hum	29
5A,Hum	31.333
8B,Hum	25.667
4B,Hum	28
8C,Hum	15
5C,Hum	11.667
8D,Hum	34.333
	Average
8A,Hum	14.767
5A,Hum	16.333
8B,Hum	12
5B,Hum	12.333
8C,Hum	11.667
5C,Hum	14.667
8A,Hum	3
8B,Hum	6.38
5B,Hum	7
8C,Hum	6.48
8D,Hum	2.48
5D,Hum	1
	Average
7A,Mtk	27.333
6A,Mtk	39.667
4A,Mtk	26.333
4B,Mtk	20
4C,Mtk	13
7D,Mtk	29.333
6D,Mtk	31.667
	Average
7A,Mtk	14.667
6A,Mtk	24.667
4A,Mtk	25.667
4B,Mtk	13
4C,Mtk	12.667
7D,Mtk	23
6D,Mtk	22.667
7A,Mtk	3.22
6A,Mtk	2
4A,Mtk	4.8
7B,Mtk	13.12

Table 4. continued

	Average
4B,Mtk	4.48
5C,Mtk	4.4
7D,Mtk	1.3
6D,Mtk	2
	Average
3A,Mtm	21.667
2A,Mtm	21.557
1A,Mtm	20.763
3B,Mtm	14.667
2B,Mtm	15
1BMtm	15.667
3C,Mtm	15.333
2C,Mtm	7.3833
3D,Mtm	25.367
2D,Mtm	25
1D,Mtm	22.667
	Average
3A,Mtm	8.6667
2A,Mtm	16.667
1A,Mtm	10.667
3B,Mtm	8.1
2B,Mtm	10
1B,Mtm	15
3C,Mtm	15
2C,Mtm	13.667
3D,Mtm	6
2D,Mtm	6.15
1D,Mtm	7.05
	Average
3A,Mtm	2.45
2A,Mtm	3.5
1A,Mtm	4
3B,Mtm	4
2B,Mtm	4.1
1B,Mtm	7.7
3C,Mtm	3.48
2C,Mtm	3.1
2D,Mtm	1
1D,Mtm	1.4

Ambient Temperature

In Table 5 the minimum and maximum ambient temperatures of the three regions where samples of frankincense were collected are shown. The samples were collected in the first two weeks of October, 2009, and data on ambient temperatures of the regions were available for up to December 2006; consistent data for the three regions in October were those of the year 1999 and 2000, and hence referred.It could be said that the ambient night and day temperature around Humera is the highest and that of Metekel is the lowest of the three regions.

Table 5. Ambient Temperature of different incense samples

Station	Element	Year	Oct	Station	Element	Year	Oct
Metekel	TMPMIN	1999	17.3	Metekel	TMPMAX	1999	28.9
Metekel	TMPMIN	2000	17.1	Metekel	TMPMAX	2000	29.3
Metema	TMPMIN	1999	18.8	Metema	TMPMAX	1999	31.4
Metema	TMPMIN	2000	18.6	Metema	TMPMAX	2000	33.4
Humera	TMPMIN	1999	20.2	Humera	TMPMAX	1999	34
Humera	TMPMIN	2000	20.5	Humera	TMPMAX	2000	36.2

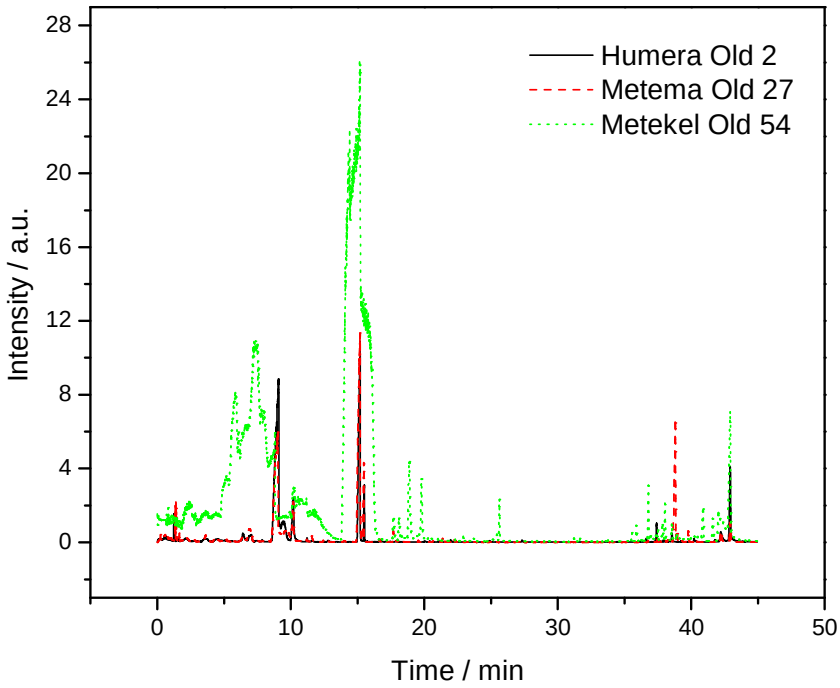
DISCUSSION

Figures 1-8 and Tables 6-8 show data from GC-MS analysis conducted at Ambo University instrumental lab of the Chemistry Department, Ethiopia. Relative percentages could not tell much about variations of volatile constituents present in samples brought to the lab from Humera, Metema, Metekel, and Nazareth export store. Tables 6, 7, and 8 in their first columns showed 28 to 30 different substances. Any one constituent has been shown to be present in almost all samples. The relative percentage of a composition is slightly varying between samples. Headspace analysis is one of the rapid methods to analyze volatile components of frankincense samples. In all samples analyzed *n*-octylacetate was the major component but its relative percentage was less than that of hydrodistilled oil of the same plant resin [2,11,17,18].

The figures 1-8 (Chromatograms), though, clearly depicted existence of variation among samples. Those resins from Metekel gave different feature from those of Humera, Metema and Nazareth. While samples from the three sources (Humera, Metema and Nazareth) did show similarities (Figures 1, 7, & 8).

When Figures 5 and 6 are scrutinized frankincense samples from Metekel seemed to contain substances that are also predominant in samples of Borena and Ogaden. Components which appeared before fifteen minutes on the GC showed certain similar features between Metekel, Borena and Ogaden resins. Additionally, the three samples in no uncertain terms were found to be different physico-chemically, which was also clear on their chromatograms. The Borena and Ogaden resins came out with profiles of GC-MS analysis very different from each other.

Fig. 1 Comparison of headspace analysis of different incense samples from Humera, Metema and Metekel.

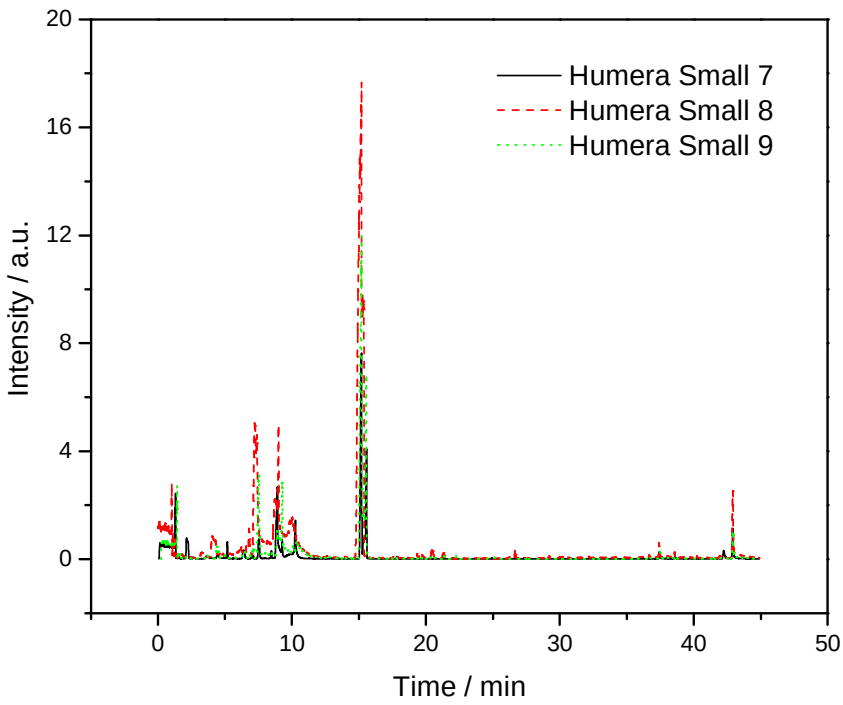


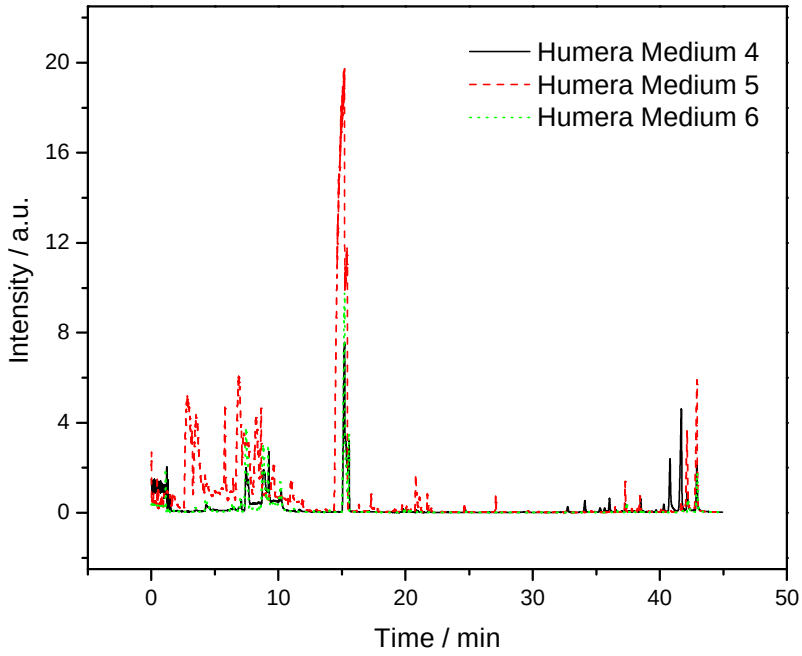
At this stage of GC-MS analysis result one could guardedly state that frankincense varies with locality as could be witnessed from the GC-MS data presented in this paper. The Tigray (Humera, Metema, and Metekel) type-frankincense itself when subjected to GC-MS, TLC, Pyrolysis, and Odor treatments did show quality variations. Variation has been observed with respect to origin and age of trees. Frequency of tapping, in this study showed no

influence on the quality of resin in all cases. Color of olibanum was found to matter in the quality of the tree product. The bark was found to give similar volatile constituents more or less as that of the white or light yellow grade of frankincense. The black or brown frankincense from all sites gave similar results. The frankincense from Humera gave acid smell during the odor test conducted in three cities. Frankincense from Metema was very smoky without the sweet odor of frankincense. Metekel, Borena and Ogaden types gave more or less similar pleasant odor during red charcoal treatment. The literature search to similar works on Ethiopian olibanum indicated the lack of related work in general and publications in particular.

Volatile components of resins of *B. papyrifera* might be used in perfume industry and as additives in preparation of antibacterial incense sticks [22]. Previous study on volatile components of the resin of *Pistacia lentiscus* using GC-MS indicates presence of sesquiterpenoids as major components [23].

Fig. 2 Comparison of headspace analysis of different incense samples from Humera.




Humera Medium 4
Humera Medium 5
Humera Medium 6
Intensity / a.u.
Time / min
0
4
8
12
16
20
0
10
20
30
40
50

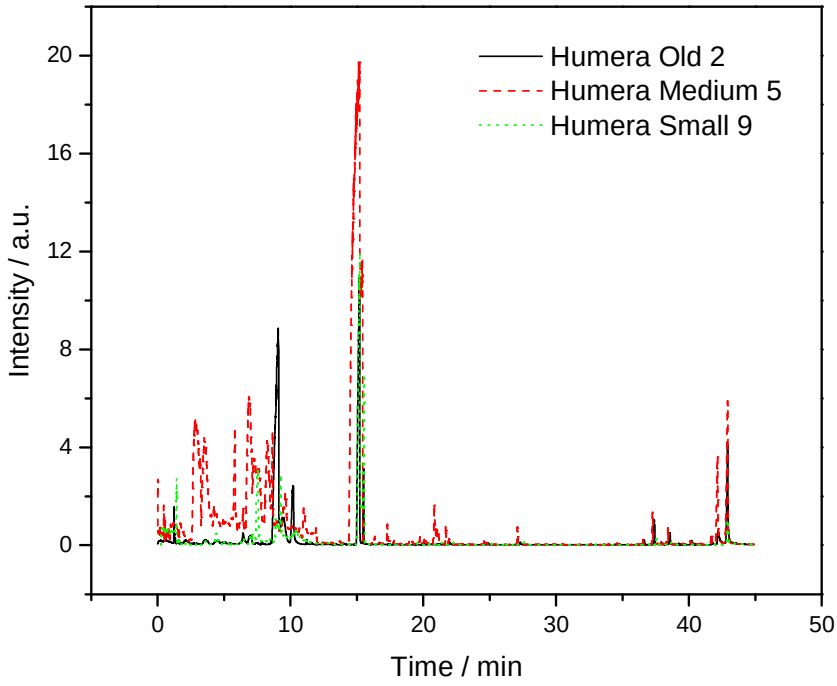

Humera Old 2
Humera Medium 5
Humera Small 9
Intensity / a.u.
Time / min
0
4
8
12
16
20
0
10
20
30
40
50

Fig. 3 Comparison of headspace analysis of different incense samples from Metema.

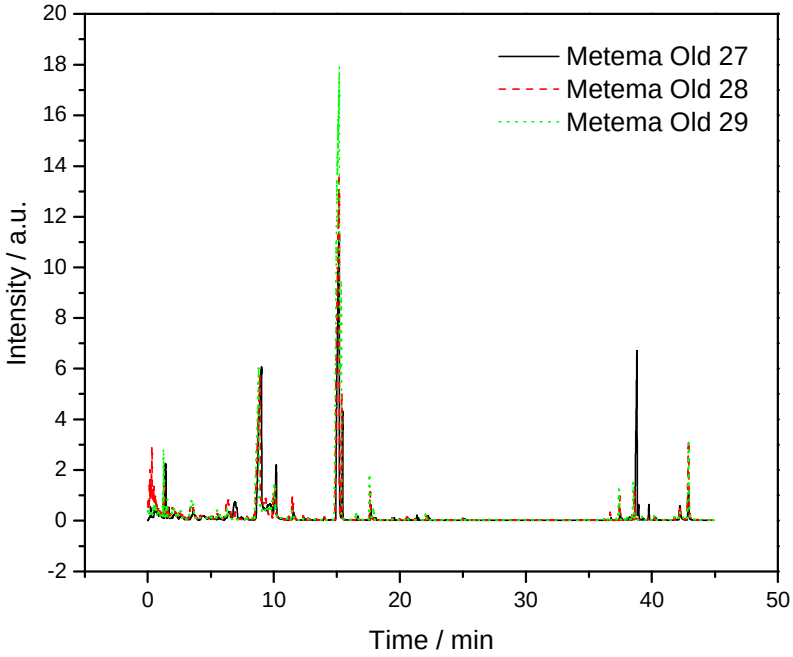


Fig. 4 Comparison of headspace analysis of different incense samples from Metekel

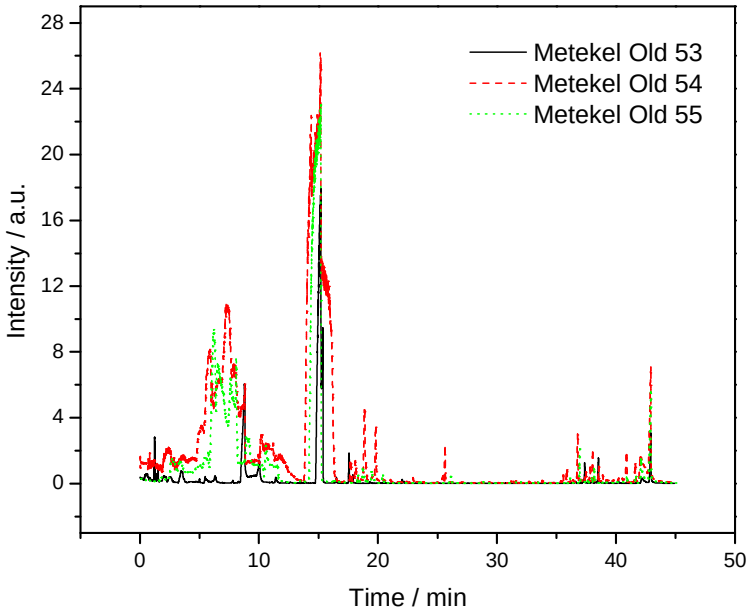


Fig. 5 Comparison of headspace analysis of different incense samples from Tigray, Borena, and Ogaden

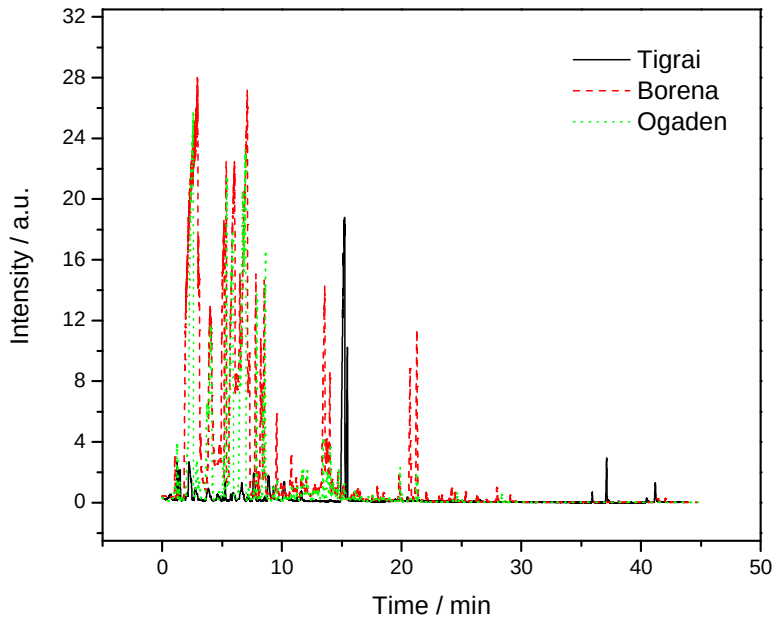


Fig.6 Comparison of headspace analysis of different incense samples from Metekel, Borena, and Ogaden.

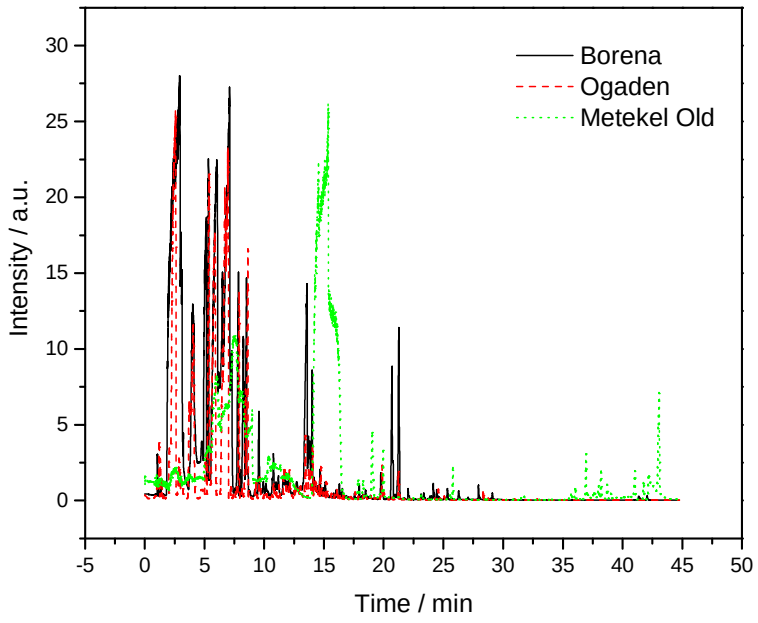


Fig. 7 Comparison of headspace analysis of different incense samples from Tigray (The seven Grades) and Humera

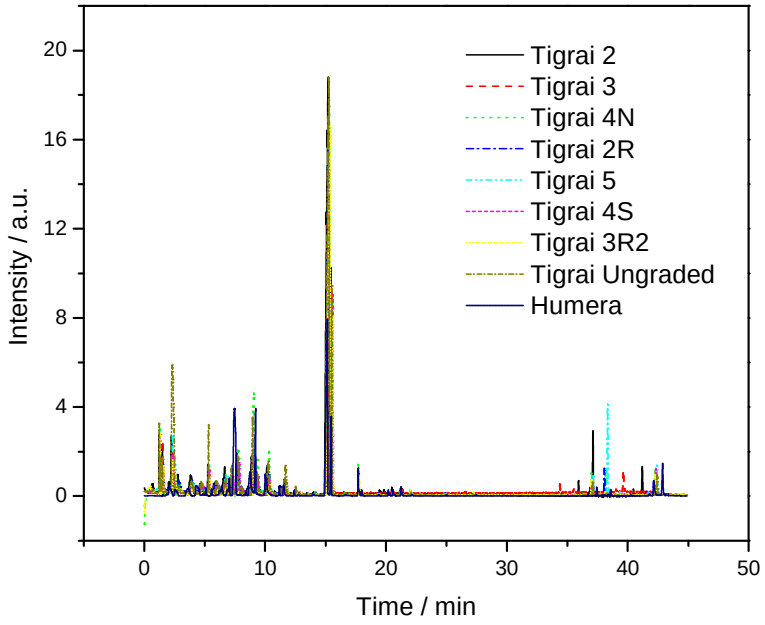
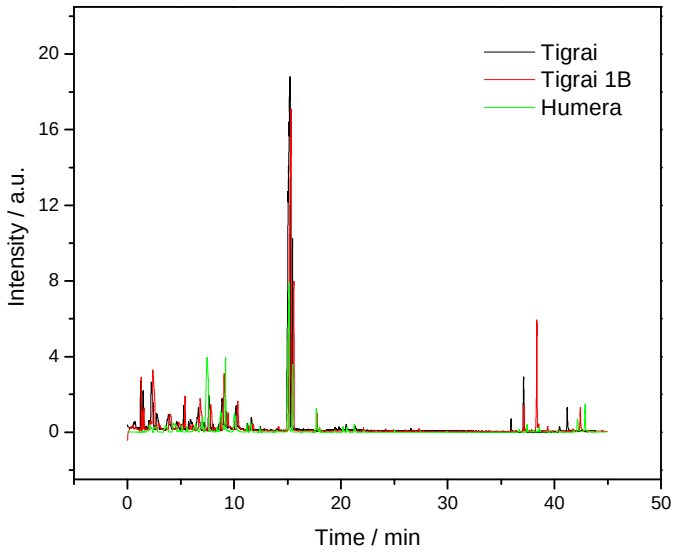


Fig. 8 Comparison of headspace analysis of different incense samples from Tigray, Tigray 1B, and Humera.



CONCLUSION

Quality of frankincense for the purpose of trade varies with color, provenance and age of tree. At this stage, it would be unwise to conclude that the frankincense from Humera, Metema and Metekel are chemically different. However, quality of tree product goes with color (white or light yellow, brown to dark brown). Dark is not to be taken as inferior as it was repeatedly determined to give similar results of volatile constituents as that of the first grade frankincense. Thus the current grading system based on color and size should take into account this. Borena and Ogaden types were found to have substances of similar nature as that of the Metekel in particular with regard to marketing frankincense for similar reason. Frankincense from Borena and Ogaden need require further study (both in terms of product quality and handling methods) so that the current system could also be revisited and these products could be marketed in the international market as or even at a better quoted price than frankincense from Tigray, currently exported frankincense. Our preliminary biochemical data obtained so far, which needs to be supplemented with further studies, shows that the potential from Borena and Ogaden trees seems very high. Using these findings in the practice of product harvesting and handling will have huge commercial implications in making products from Borean and Ogaden area ready for the export market, and in redefining the currently used conventional grading system in Ethiopia.

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