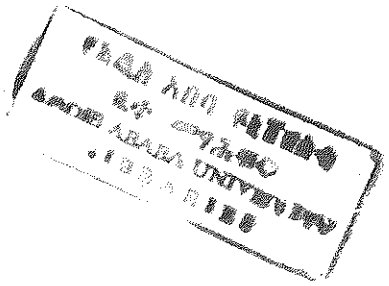
CHEMICAL INVESTIGATIONS OF THREE *OCIMUM* SPECIES OF ETHIOPIA



A THESIS SUBMITTED TO THE SCHOOL O F GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY
GETACHEW ABEBE
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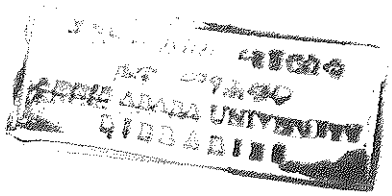

Chemical Investigations of Three *Ocimum* Species of Ethiopia: *Ocimum americanum, Ocimum basilicum* and *Ocimum lamifolium*

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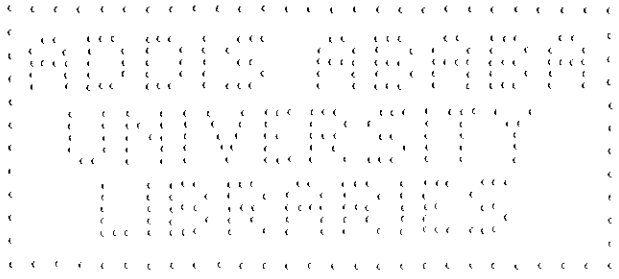

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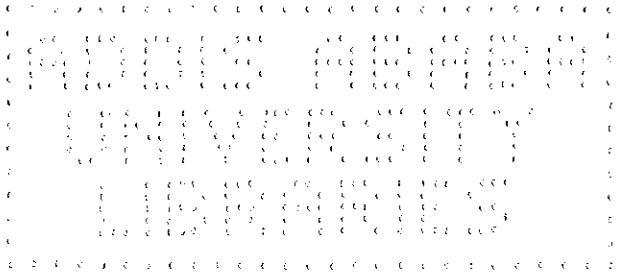

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Abstract

Chemical Investigations of three *Ocimum* Species of Ethiopia

by

Getachew Abebe

Research Advisor: Prof. Ermias Dagne

Two species of *Ocimum* locally known as 'Ajuban' and 'Besobla' are widely used in Ethiopia for fragrance and flavour purposes respectively. Until recently the two varieties were known botanically as *O. basilicum* var. *thyrsiflorum* and *O. basilicum* var. *basilicum*.

In this study the essential oils of *O. basilicum* var. *thyrsiflorum* ('Ajuban') were subjected to GC, GC/MS and ^{1}H and ^{13}C NMR analyses. Two different essential oil profiles were recoginized: linalool/estragole and 1,8-cineole/linalool/*trans*-geraniol.

Likewise, the essential oil of *O. americanum* var. *pilosum* ('Besobla'), that was collected from Wondo Genet, was analyzed by GC, GC/MS and ^{1}H and ^{13}C NMR methods. Linalool and methyl cinnamate were found to be the major components.

O. americanum var. *americanum* which is slightly different from var. *pilosum* by its less hairy fruiting calyx was collected from Meta Abo area at two different times. The essential oils obtained from this plant were analyzed by GC and GC/MS. Four different essential oil profiles were found: 1,8-cineole/linalool/methyl cinnamate,1,8-cineole/linalool, 1,8-cineole/linalool/*trans*-geraniol and 1,8-cineole/estragole.

O. lamiifolium (locally 'Dama-Kesse') , which is an important medicine in Ethiopia, has been investigated. The essential oils obtained were analyzed by GC and GC/MS.

1. Introduction

It has been known for many centuries that the flower, fruit, leaves and roots of many plants contain volatile, odoriferous substances, commonly known as essential oils [27]. Essential oils have been obtained from over 3000 plant species. The yield and composition of the oil produced depends on many factors such genetic differences, geographic locations and agricultural factors such as soil, water, nutrients, and climatic variables. Considerably, both the quantity and composition of the essential oil can also change drastically as the plant matures [8, 23].

Scented plants were mentioned in the Book of Genesis. Probably ancient Egyptians were the first people to use perfumes for religious rites and funerals as well as for making toilet water, massage preparations and fragrant materials [8]. One of the commonest essential oils, oil of turpentine, was also known to the ancient Greeks [27].

During earlier times, since methods of obtaining essential oils were not known, myrrh was the common substance which provided a powerful and lasting scent, this was where the word perfume gets its origin, *per;* means 'through' and *fumum* means 'smoke' in Latin, signifying burning of incense [8].The odour of essential oil is not due to one component but rather due to several compounds blend and are 'in tune' with each other so as to produce a perfect chord of scent. Therefore, despite efforts to make essential oil from synthetic compounds, no essential oil has yet been successfully reconstituted. It is generally believed that the therapeutic quality of an unadulterated natural oil is more effective than any synthetic or partially reconstituted equivalent.

Essential oils in plants may be stored in glands and minute amounts of the components are released to the surroundings. As pigment is bred into flowers and leaf, scent is usually

decreased. Therefore, the decreasing order of strength of perfume released by flowers with respective to their colour is as follows : white, pale pink, pale yellow and purple [8].

Beside their uses as perfumes and flavouring agents essential oils also provide a fertile source of compounds for the treatment of various types of disorders [23].

1.1 Methods of obtaining essential oils

The four most widely used methods of obtaining essential oils are:

1) **Steam or hydro-distillation:** This is by far the most common method of obtaining essential oil. Sensitive compounds may undergo rearrangement or oxidation due to the high temperature used. For instance fragile flowers whose attar cannot withstand high temperature should not be processed by this method.

2) **Solvent extraction**: The essential oils that are sensitive to heat are extracted from plant materials with light petroleum ether at room temperature. This method is usually applied to flowers since it is a more gentle method and the flowers are repeatedly washed by solvent, and the scent obtained is highly concentrated.

3) **Enfleurage:** The flowers are enclosed for about 24 h between sheets of glass, coated with animal fat and then the flowers are replaced everyday. This operation is repeated up to a month when the fat will be saturated with the oil which then is scraped off the glass and the oil extracted with alcohol. Since the oil is obtained directly from living flowers the scent obtained is most delicate and delicious. Such perfume is usually expensive because of the time and labour involved while making it.

4) **Expression:** This method is used to extract the essential oil from the rind of citrus fruits by application of pressure. As fruits are being crushed between rollers, a heavy jet of water is

sprayed on them so as to separate the juice from the solid materials. The oil phase is then separated from the aqueous juice.

The quality of essential oil is seriously affected by the methods of isolation and subsequent processing steps.

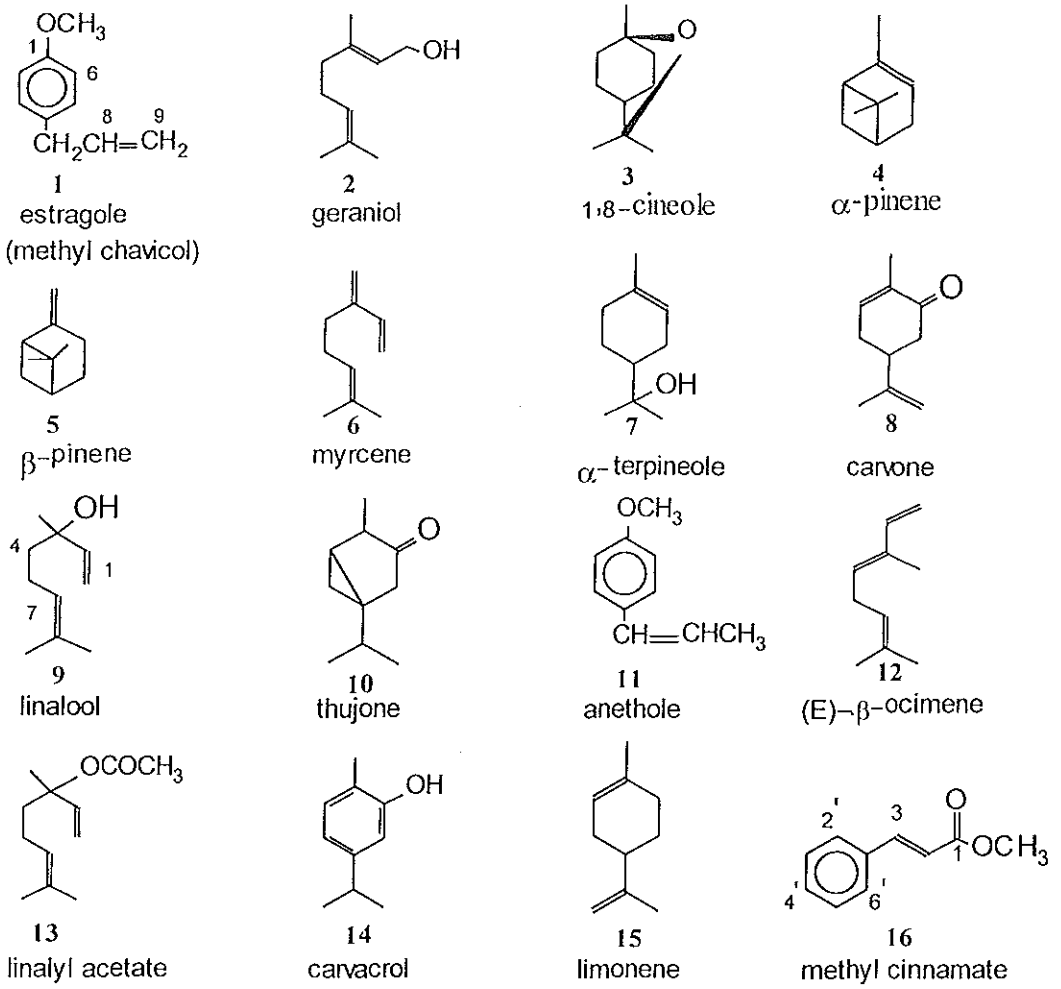


Figure 1. Structures of some of the common constituents of essential oils

1.2 Characterization of essential oil components

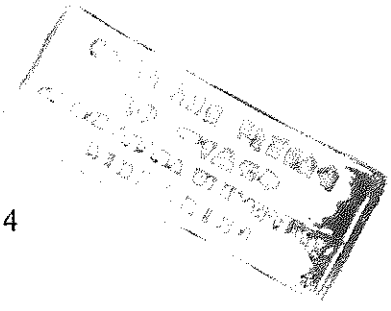
Essential oils consist of a complex mixture of compounds which may be classified as: 1) **terpenes**; 2) **nitrogen and sulfur containing compounds**; 3) **aromatic compounds** and

4) **miscellaneous compounds**. Among the hundreds of compounds that have been separated from essential oils many are monoterpenes while a small number are sesquiterpenes, containing almost all types of functional groups: alcohols, aldehydes, ketones, lactones, epoxides etc. [27].

An essential oil derived from a single plant is usually an extremely complex mixture of as many as 200-300 compounds, with a few constituents being major and many other minor ones [23]. The total mixture composing the oil is characterized by physical properties such as colour, boiling point range, specific gravity, refractive index and optical rotation. Oils for flavour or scent are usually evaluated by organoleptic tests.

The development of gas chromatography (GC) in the 1960s and the use of very long capillary columns of up to 100 m with film of adsorbent on the wall revolutionized the analysis of essential oils [23]. In GC the retention time is characteristic of the compound and depends on the solubility of the compound in the mobile and stationary phases. Therefore, it is possible to identify the components of the essential oil under a given set of conditions by comparing the retention time of an unknown with that of standard samples or published data. However, it is better if the identification is confirmed by co-injection to avoid the effect of subtle changes in GC conditions. GC/MS (gas chromatography coupled to mass spectrometric detector) is an even more powerful and a reliable method of identifying volatile compounds. The most important feature of the mass spectrometric detector is that even if the compounds co-elute positive identification is possible.

The importance of spectroscopic methods such as UV, IR, NMR and also chemical methods cannot be overemphasized [23]. These spectroscopic methods coupled with isolation of pure fractions containing only single components has enabled the identification of numerous compounds from essential oils.



1.3 The importance of essential oils for the plants bearing them

The evolutionary *raison d' être* of essential oils, was unknown until a couple of decades ago. Now it is recognized that secondary metabolites in general and essential oils in particular have vital roles as mediators of ecological interactions. They have a function in ensuring the continued survival of particular organisms in an often hostile environment where many organisms are competing with each other [5, 15].

Plants that are under attack by herbivores insects and pathogens release a number of volatile compounds especially monoterpenes and sesquiterpenes. These chemicals attract natural enemies of herbivore insects that attack the plant. Furthermore, plants may also use these volatile molecules as semiochemicals to warn their neighbours to switch to plant defense genes or make the plant unpalatable to insects or serve as oviposition deterrent. Semiochemicals are volatile signaling compounds released by a plant under attack by herbivore insect or their pathogenic symbionites and stimulate plant defense mechanisms in the neighbouring plants. The volatile compound, methyl jasmonate, is used as a signaling molecule between tomato plants to warn their neighbours to switch to plant defense genes. This is also described for many other plant species. These assertions are substantiated by a number of studies conducted on plant herbivore and predator relationship [5, 33, 36, 37, 39].

Stress induced terpenes are produced several hours after the attack and persist for several hours, perhaps days [36], while the constituted terpenes are synthesized earlier and are stored in the plant for immediate use [41]. The delay in forming induced terpenes indicates that the physiology and biochemistry of plants changes in response to herbivore damage [36].

It is therefore worth emphasizing that the classical analytical determination of a chemotype provides only a historical record of secondary metabolites that may or may not reflect the full biosynthetic capability of a plant. The constitutive monoterpenes may

accumulate for over two years [13] and also upon external attack by pathogens or herbivores. A plant may adapt any of the metabolic pathways that produces structurally diverse compounds [15].

Not only do plants use the essential oils for protection against herbivores and pathogens but may also use them for dominance over each other. For instance, the plant *Artemsia californica* releases monoterpenes, primarily camphor and 1,8-cineole, when adsorbed by the soil in the canopy lead to inhibition of germination of seeds. It is only when fire occurs, which destroys the plant and the monoterpenes in the soil, will other species of plants appear in any number. Similar phenomenon is also exhibited by *Eucalyptus* plants where they release terpenes and phenols that hinder the growth of other plants [15].

1.4 Botanical classification of *Ocimum* L. (Labiatae)

The family Labiatae consists of annual or perennial herbs classified into more than 180 genera and 3500 species. More recently the subdivision of the Labiatae according to the existence of tricolpate or hexacolpate pollen grains has become more fashionable.

Ocimum L. is placed in the Tribe *Ocimeae* Benth. and subtribe *Ociminae*. The modern classification of *Ocimum* by Alan Paton [24] is similar to that of the classification by Bentham. Paton divided *Ocimum* into 3 sections: sect. *Ocimum*, sect. *Hierocymum* and sect. *Gymnocymum*. Sect. *Ocimum* in turn is divided into two subsections: subsect. *Ocimum* and subsect. *Gratissma*. This classification is based on morphological features.

Ocimum contains about 30 species in the tropic and subtropic regions of the world. Some species are widely cultivated in temperate areas for many have culinary herb and medicinal use. According to Alan Paton [24] there are sixteen *Ocimum* species on mainland Africa, namely: 1) *O. gratissimum*, 2) *O. natalense*, 3) *O. spicatum*,4) *O. jamesii*,5) *O.*

cufodontii, 6) *O. nummularia*, 7) *O. kilimandscharicum*, 8) *O. kenyense*, 9) *O. basilicum*, 10) *O. americanum*, 11) *O. forskolei*, 12) *O. fischeri*, 13) *O. circinatum* 14) *O. lamiifolium*, 15) *O. masaiense* and 16) *O. tenuiflorum*..

Artificial selection and breeding of *Ocimum* species have been taking place for several years resulting in hybrid formation and species changes. These have necessitated revision of the genus by Paton *et al.* [24] which meant lumping two or more species together and splitting a species into two or more other species.

Thus *Ocimum canum* Sims, which is native to Africa, is now considered as a synonym of *O. americanum*. The two main varieties that occur in Ethiopia *O. basilicum* var. *basilicum* ('Besobla') and *O. basilicum* var. *thyrsiflorum* ('Ajuban') [10] have now been delineated into two species namely *O. americanum* var. *pilosum* and *O. americanum* var. *americanum* (both 'Besobla') and *O. basilicum* var. *thyrsiflorum* ('Ajuban').

The distinction of these formerly varieties ('Besobla' and 'Ajuban') to two different species is a significant step in shading light to the taxonomy of these important culinary ('Besobla') and fragrant ('Ajuban') plants.

1.5 Importance of *Ocimum* species

The *Ocimum* species owe their importance to the essential oils they contain. Numerous studies have been conducted on the essential oils components of these economically important species. Many species for example *O. americanum* (= *O. canum* Sims), *O. gratissimum*, and *O. basilicum* etc. have been grown in tropical and subtropical regions as medicinal plants, culinary herb and insect controlling agents. *O. basilicum* known as sweet basil is a major essential oil crop [9].

The medicinal importance, bioactivity and fragrance of particular oil from plant depends on its constituents especially major ones. Basil oil may contain as many as 50 components. Therefore, characterisation of oil both by physical and chemical methods is indispensable to relate the importance and composition of the oils.

1.6 Essential oils of the genus *Ocimum*

The economic importance of the Labiatae family can be related to the essential oil content of important members such as *Ocimum basilicum* [14] which is used in flavour and fragrance industries as whole oil or as useful sources of important compounds like methyl chavicol, linalool etc. [10]. Some developing countries namely Egypt, Comoros Island, Malagasy Republic are known producers of basil oils [1]. Nearly 50 tonnes of essential oil is produced yearly from *Ocimum basilicum*. Israel alone earns 4 million dollars annually by exporting basil oil [25].

Oils of basil obtained from different countries have been studied so far clearly showing need to do similar analysis for species occurring in Ethiopia. Asfaw *et al.* [1] investigated the oil of *O. canum* (now *O. americanum*) collected from Ethiopia and found that it was rich in linalool, camphor and 1,8-cineole. These workers also examined *O. urticifolium* and *O. forskolei* and the major components were found to be eugenol and eugenol/myrcene respectively.

In 1977 Gulati *et al.* [14] analyzed the composition of *O. basilicum* from India and found that the major constituent was estragole. The oil of the same plant species found in Turkey was also examined by Perez-Alonso *et al.* [26] and their analysis showed that the oil was rich in linalool and methyl cinnamate.

Lawrence *et al.*[14] investigated in 1980 the chemical composition of oils of 26 accessions of plants grown in experimental garden in North Carolina whose seeds were obtained from different sources. The oils were found to possess a few major components which varied widely. Among the major components obtained were *cis*-ocimene, 1,8-cineole, linalool, estragole, methyl cinnamate,eugenol etc. In 1988 the same workers investigated the composition of about 200 basil oils produced from plants cultivated in North America. Based on a single major component they classified the oils into four chemotypes: estragole, linalool, methyl eugenol and methyl cinnamate. During these studies no eugenol rich plants were found [9].

Grayer *et al.* [9] had investigated oils of different varieties of *O. basilicum* namely *purpurscens*, *basilicum* and *difforme* that was grown under carefully controlled green house conditions. Their study contained oils of 16 accessions of plants and found five essential oil profiles: linalool, estragole, linalool/estragole, linalool/eugenol and estragole/methyl eugenol. Their classification of chemotype was based on percentages of all the major constituents which apart from the four major essential oils mentioned by Lawrence *et al.* also included eugenol.

In 1988, Chien investigated the composition of basil oil. Some of the constituents were found to be benzaldehyde, benzyl formate, octyl acetate, isobornyl acetate, methoxy cymene, bulnesol and γ-eudesmol. This work did not agree with previously published data and requires corroborative confirmation [14].

1.7 Bioactivities of essential oils from *Ocimum* species

Bioactivity studies conducted on oils of *Ocimum* species shows antitubercular, insecticidal, fungicidal, antiasthmatic and antimicrobial properties [1].

Bean leaf discs freshly sprayed with different concentrations of the acetone solutions of the oil from *Ocimum basilicum* caused mortality and induced repellency in adult females of the carmine spider mite, where egg-laying was also reduced. The same oil was also shown to have repellent activity against red flour beetle, *Tribolium castaneum,* [9]. *O. americanum* from Rwanda whose major component is linalool is used for protection of post harvest damage of stored products by insects. It was reported that the adult of *Zabrotes subfasciatus* (Coleoptera) when exposed to dried *O. americanum* leaves resulted in 100% mortality of males and 50% mortality of females after 48 hr.

Oil of *O. sanctum* which is known as holy basil by the Indians has been tested and found to exhibit remarkable anti-fungal activity against the following species of fungi: *Aspergillus flavus, A. fumigatus, A. parasiticus, Helminthosporum oxysporum, Trichderma viride,* and three fungi that are pathogens of rice [3, 34].

The leaves of *O. gratissimum* were tested for fungitoxicity against betel vine (*Piper betle* L.) pathogens and found to have remarkable fungitoxic activity. Fifteen compounds were identified from the oil and eugenol was found to be the major fungitoxic principle in the oil. The oil was either equally effective or superior to synthetic commercial fungicides and was non-phytotoxic to the host plants. Thus the oil can be used as a valuable indigenous and biodegradable agent against fungi that cause losses to the betel vine industry [35]. Essential oil of *O. gratissimum* was also studied for repellency and direct toxicity against the housefly. The oil showed 100% repellent activity [32].

Essential oil of *O. gratissimum* traditionally used as medicament in Vietnam was combined with modern antibiotics and the combination was tested for antibacterial activity. It was found that combination with antibiotics potentiated the therapeutical action of the essential oil [11].

Insecticidal properties of essential oils of *O. basilicum* and *O. sanctum* and their major constituents, were evaluated against vector mosquito species, *Anopheles stephens, Aedes aegpti,* and *Culex quinque fasciatus.* The bioassay study revealed that the essential oil of *O. basilicum* and its major constituent, estragole (1), were more effective than that of *O. sanctum* whose major component is eugenol [3].

1.8 Objective of this study

The main aim of this study is the analysis of essential oils of three *Ocimum* species of Ethiopia, *O. basilicum* var. *thyrsiflorum* ('Ajuban'), *O. americanum* var. *pilosum* and var. *americanum* ('Besobla') and *O. lamiifolium* (Dama-Kesse).

Due to the wide use attached to the above indigenous plants of Ethiopia for culinary, fragrance and medicinal purposes we decided to undertake chemical studies of their oils. Except for one previous report on *O. canum* (now *O. americanum*) from Ethiopia oils of the other species *O. basilicum* and *O. lamiifolium* have not been chemically investigated.

2.0 Results and discussion

Ocimum basilicum var. *thyrsiflorum* (locally 'Ajuban') were collected during flowering stage from Wondo Genet on February 2, 1998 and Meta Abo on June 5, 1998. Specimens were submitted to botanists at Kew, UK and National Herbarium, AAU. The plants from both collections were unequivocally identified and named as *Ocimum basilicum* var. *thyrsiflorum* (see Experimental for Voucher No.).

Ocimum americanum var. *pilosum* (locally 'Besobla') was collected from Wondo Genet on Feb. 2, 1998. Likewise, the specimen was submitted for identification and named at Kew, UK. Two collections of 'Besobla' from Meta Abo area were done on Mar. 22 and Oct. 6, 1998 the specimens identified by botanical authorities as *Ocimum americanum* var. *americanum* both at Kew, UK and National Herbarium, AAU.

The third *Ocimum* species locally known as 'Dama-Kesse' was also collected from Meta Abo. It was identified as *Ocimum lamiifolium*.

2.1. Essential oil of *Ocimum basilicum* var. *thyrsiflorum* Baker

The aerial parts of the plant material were hydro-distilled using Clevenger type apparatus for about one and half hours at atmospheric pressure, to give yellowish oil (yield 0.4%) with very pleasant odour: $[\alpha]_D^{22}$ -9.5 (neat), ref. ind. 1.486 and sp. gr. 0.91.

Likewise, the aerial parts of *Ocimum basilicum* var. *thyrsiflorum* collected from Meta Abo area were hydro-distilled to give oil with yield of 0.14%. Due to paucity of material other physical data could not be generated on this oil.

The essential oils obtained from the aerial parts of the *Ocimum basilicum* var. *thyrsiflorum* from two localities that were collected at different times were analyzed by GC

and GC/MS (see **Table 1**). The major components linalool and estragole were isolated by combination of CC and PTLC and their structures confirmed by ^{1}H and ^{13}C NMR analyses. The ^{1}H NMR of estragole is given in Fig. 2.

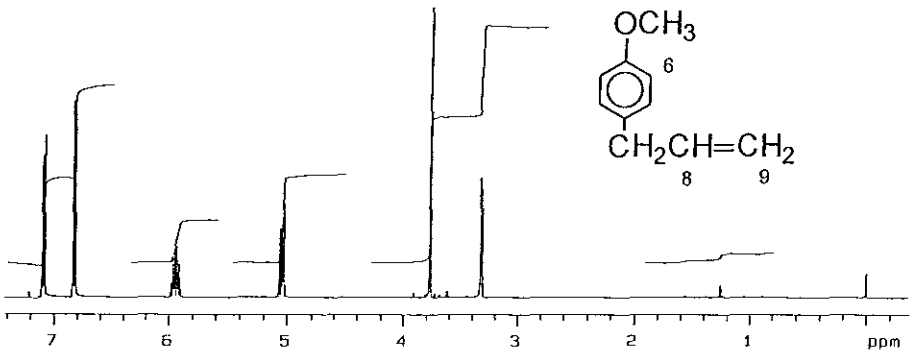


Figure 2. ^{1}H NMR spectrum of estragole

Table 1. Constituents of *O. basilicum* var. *thyrsiflorum* analysed by GC, GC/MS, listed according to increasing retention times.

components	*Ocimum basilicum* var. *thyrsiflorum*			
	str. No.	2/98 W.G	6/98 M.A	10/98 W.G
β-pinene	5		1.9	
β-myrcene	6		0.5	
1-octen-3-ol	-	0.4		
limonene	15		0.5	
1,8-cineole	3	1.7	27.7	
γ-terpinene	-		0.6	
sabinene hydrate	18		1.14	
linalool*	9	46.7	32.4	50.5
β-fenchyl alcohol	-		0.72	
p-menth-1-en-4-ol	19		4.7	
linalyl propionate	28	0.32	2.2	0.5
cis-geraniol	-		0.4	

estragole*	1	38.3	0.3	42.6
citral	22	0.6	0.6	
trans-geraniol	2		14.1	
β-elemene	29			0.4
trans-geranic acid methyl ester	23		0.3	
sabinene acetate	-		0.2	
unknown	-	1.1	0.8	1.3
neryl acetate	25		1.8	
methyl cinnamate	16	10	2.0	
germacrene D	27	0.24		
α-farnesene	-		7.8	
germacrene B	30		0.5	
γ-cadinene	31			0.5
β-cubebene	-	0.34		
unknown	-	1.2		2.4

* Confirmed by ^{1}H and ^{13}C NMR

As seen from **Table 1** two essential oil profiles are discernible for var. *thyrsiflorum*. The major components of the species growing in Wondo Genet were found to be linalool and estragole while the oil from Meta Abo area is clearly different with 1,8-cineole, linalool and *trans*-geraniol as the major components. This results clearly shows that these plants although belonging to the same species and variety are distinct chemotypes. Seasonal variation is also discernible for the oil composition, with linalool and estragole levels increasing in the October collection at the expense of other components. The change in the level of methyl cinnamate, one of the constituents of sweet basil from 10% to nil is worthy of mention.

2.2 Essential oil from *Ocimum americanum* var. *pilosum* (Willd.) Paton

Ocimum americanum var. *pilosum* locally known as 'Besobla' was collected from Wondo Genet on Feb. 2, 1998 during flowering stage. The aerial parts of the plant material

were hydro-distilled (yield was 0.3%) to give colourless oil with very pleasant odour: $[\alpha]_D^{22}$ - 7.8 (neat), ref. ind. 1.5, sp. gr. 0.96. *Ocimum americanum* 'Besobla' is most important culinary herb in Ethiopia and it is also used as medicine against malaria and headache [10].

GC and GC/MS analysis of the above crude oil was found to contain 11 components with linalool and methyl cinnamate as the major constituents. Therefore, the oil can be considered as linalool-methyl cinnamate chemotype (see **Table 2**).

Table 2. Constituents of *Ocimum americanum* var. *pilosum*

Components	str. No.	73-77A
1,8-cineole	3	7.8
sabinene hydrate	18	0.4
linalool*	9	37.9
p-menth-1-en-4-ol	19	1.1
linalyl propionate	28	0.72
estragole	1	9.1
compound A	-	1.2
methyl cinnamate*	16	29
germacrene D	27	1.2
compound B	-	1.4
compound C	-	0.4

Legend:

*Confirmed by ^{1}H and ^{13}C NMR

Compound A = (-)-endo-2,6-dimethyl-6, (4-methyl-3-pentyl)bicyclo[3.1.1]hept-2-ene

Compound B = 2-methyl-6-(4-methyl-3-cyclohexene-1-yl)- 2,5-heptadiene

Compound C = (E)-5-acetyl-2,2-dimethyl-1-(3'-methyl-1',3'-butadien-1'-yl)bicyclo [2.1.0]pentane

The crude essential oil was subjected to column chromatography using silica gel 60 and gradient elution with petrol and ethyl acetate. The fractions containing two major compounds were mixed and the two components separated by PTLC using petrol and ethyl

acetate (9:1). The purity of the two isolated compounds was monitored by GC and their identity established as methyl cinnamate and linalool by ^{1}H NMR (Fig. 3) and ^{13}C NMR (Fig. 4).

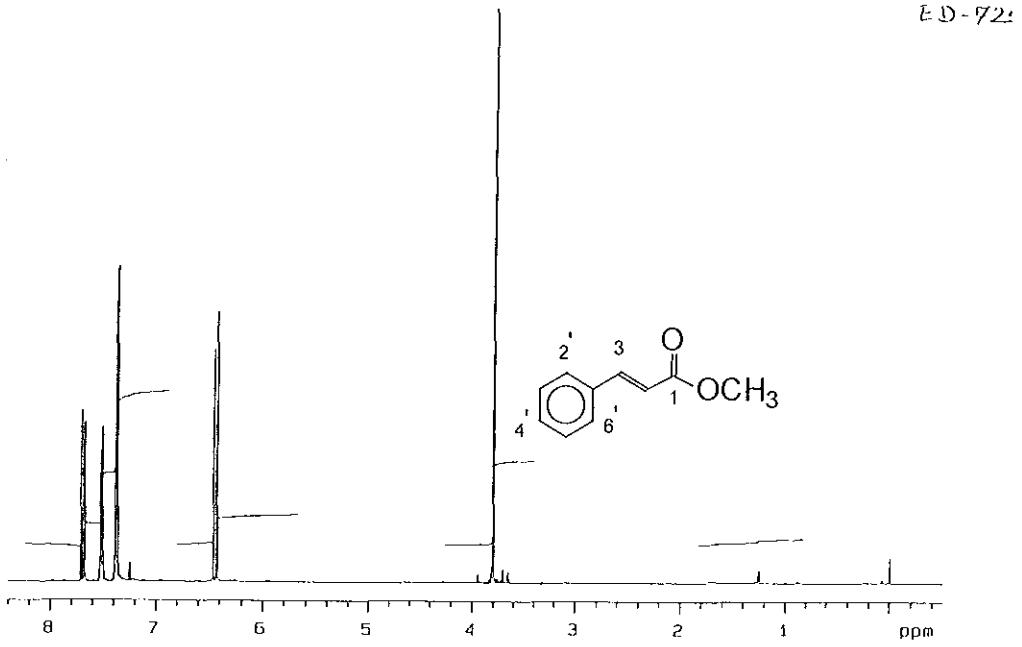


Figure 3. ^{1}H NMR spectrum of methyl cinnamate

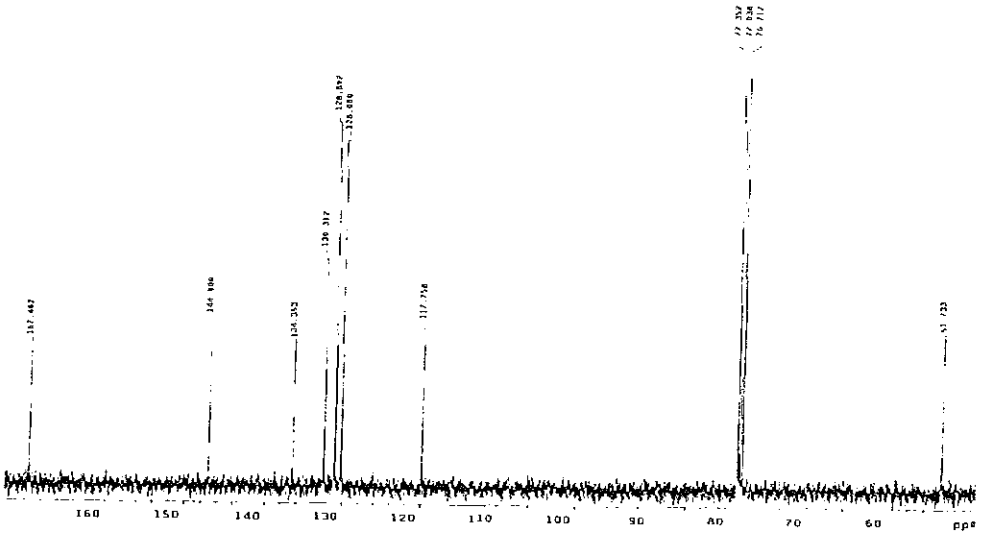


Figure 4. ^{13}C NMR spectrum of methyl cinnamate

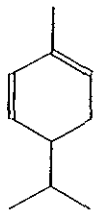
2.3 Essential oils from *Ocimum americanum* Sensu A. J. Paton var. *americanum*

Specimens from the second variety of *Ocimum americanum* var. *americanum* was collected from Meta Abo area on March 22, 98 and October 6, 98, and hydro-distilled to give oils with yield of 0.3% and 0.7% respectively.

The essential oils were analyzed by GC and GC/MS and results are shown in **Table 3**. Both oils indicate a 1,8-cineole/linalool chemotype for this variety.

Table 3. Constituents of *Ocimum americanum* var. *americanum* from two seasons

Components	*Ocimum americanum* var. *americanum*		
	str. No.	3/98	9/98
α-pinene	4	0.62	
β-pinene	5	2.5	
β-myrcene	6	0.6	
L-phellandrene	17	0.74	
limonene	15	1.0	
1,8-cineole	3	23	12.4
γ-terpinene	-	0.64	0.3
sabinene hydrate	18	2.1	1.1
linalool	9	16.2	43.1
p-menth-1-en-4-ol	19	6.1	3.2
α-terpineol	20	1.5	
fenchyl acetate	21	0.83	0.61
estragole	1	0.18	1.4
citral	22	0.32	1.4
trans-geraniol	2		15.7
trans-geranic acid methyl ester	23	1.52	0.94
myrtenyl acetate	24	0.52	
neryl acetate	25		1.3
lavanduyl acetate	26	2.9	
methyl cinnamate	16	4.7	5.5
germacrene D	27	0.83	0.6



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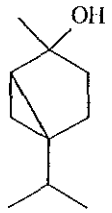

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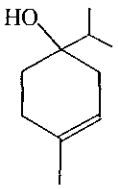

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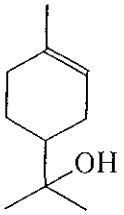

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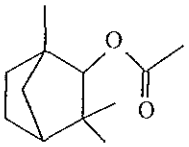

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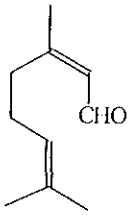

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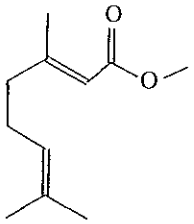

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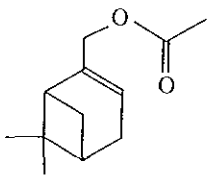

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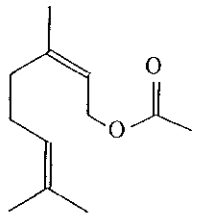

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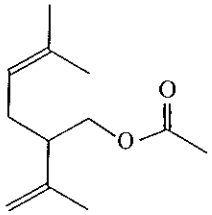

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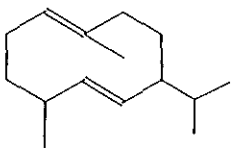

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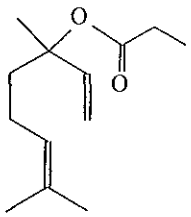

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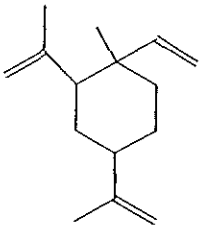

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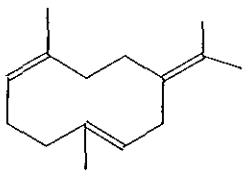

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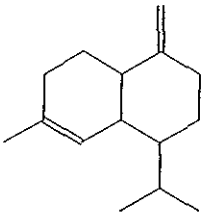

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2.4 Essential oils from *Ocimum lamiifolium* Hochst. ex Benth.

O. lamiifolium locally known as 'Dama-kesse' was collected on two occasions from Meta Abo area on Mar. 28, and Nov.19, 1998. The hydro-distillation gave small amount of oil (0.15%) that was subjected to GC/MS analysis and the result is shown in **Table 4**. An attempt was also made to extract compounds from aerial parts using solvents mixture (methanol and chloroform in 1:1). No constituents were found other than pigments up on spraying the TLC of crude with fast blue B reagent.

O. lamiifolium is perhaps the most popular traditional medicine used every where in Ethiopia. The water extract of its leaves is taken through the nasal cavity during fever. This plant is also found in Kenya, Uganda, Tanzania, Cameroon, Zaire, Rwanda, Zambia and Malawi at the altitude of (1000-2100 m) [24].

Table 4. The major and some common minor components of *O. lamiifolium*

components	3/98	10/98
α-pinene	1.3	
N-(2-phenyl ethyl) acetoamide		3.1
β-pinene	27.4	
unknown		26.9
β-myrcene	1.7	2.6
terpinene	13.3	
compound A		9.2
limonene	0.62	0.9
trans-ocimene	2.9	3.2
cis-bicyclo[5.1.0]octene	16.8	
amyl vinyl carbinol		12.9
linalool	2.2	1.6
p-menth-1-en-4-ol	1.1	1.7
α-copaene	0.2	
β-bourbonene		1.5
trans-(β)-caryophyllene	7.3	
β-elemene		8.0

compound B	9.0	
germacrene D		9.14
bicyclogermacrene	4.0	
α-cubebene	0.4	1.1
nerolidol B	0.14	0.2

Legend:

compound A = 4,4-dimethyl-1,5-cyclohexadiene carbaldehyde

compound B = 1-methyl-5-methylene-8-(1-methyl)-[5-(E,E)]-1,6-cyclodecadiene

3. Experimental

General: Optical rotations were measured using Perkin Elmer Polarimeter model 241. Refractive indices were determined on Abbe's refractometer. Specific gravity of crude oils was determined by a pycnometer. Analytical TLC were done by using 0.25 mm thick layer of silica gel GF_{254} (Merck). Identification of spots was achieved by UV light 254 nm wavelength and 1% vanillin-H_2SO_4 reagent heating with hot dry air. Column chromatography was performed using silica gel 60 (0.04-0.063 mm particle size). Gas chromatorgraphic analyses of the crude oils, and compounds obtained from PTLC were performed using HP 6890 GC series chromatograph using HP-5 5% phenyl methyl siloxane capillary 30m x 320 μm id and 0.25 μm film thickness. The oven was programmed at 50-210 °C at program rate 3°C/min using N_2 as carrier gas and air as auxiliary gas with flow rates of 2.3 ml/min and 300 ml/min respectively and injector temperature of 220 °C and detector temperature of 270°C. The GC was equipped with flame ionization detector (FID) with hydrogen flow rate of 40 ml/min.

The reference samples for identification were provided by the Natural Products Chemistry Project of AAU and Essential Oil Research Center (EORC). Neat sample with volume of 0.2 μl. was injected in each case. GC/MS was performed on a Fison GC model 8000 series coupled to a mass spectrometer, MD 800 quadrupole analyzer operating at 70 ev. The capillary column type was DB-17 (30 m x 0.25 mm id x 0.25 μm film thickness) with helium as the carrier gas (5 psi).

The components were identified by comparing their mass spectral fragmentation patterns with that of Wiley [16], NIST[17], and user generated mass spectral data libraries and also by comparing their retention times with that of authentic samples. Moreover, isolation of the major components of some of the oils was performed and NMR (^1H, ^{13}C) data were generated.

^{1}H and ^{13}C NMR spectra in $CDCl_3$ were recorded on 300 & 500 MHz and 75 & 125 MHz respectively and are reported as ppm values relative to TMS as internal standard.

3.1 Plant material collection and specimens identification

Ocimum basilicum var. *thyrsiflorum* ('Ajuban') was collected from Wondo Genet on Feb. 2, and Meta Abo on Jun. 5, 1998 . This plant was originally from Ziway and it is sometimes called 'Ajuban Ziway'. It was planted at Wondo Genet by Dr. Tadele Worku.

The plant collection numbers were S1011 and S1012 respectively. The specimer were identified by Dr. Sebsebe Demissew and deposited at National Herbarium Addis Ababa University with herbarium number 072762 and 072763, respectively.

Ocimum americanum var. *pilosum* ('Besobla') was collected from Wondo Genet on Feb. 2, 1998. The plant collection number was S1010. The identification of specimen was done by Dr. Alan Paton, world authority on the subject, at Kew Botanic Garden. The specimen was deposited at National Herbarium Addis Ababa University with herbarium number 072764.

Ocimum americanum var. *americanum* ('Besobla') was collected from Meta Abo area on Mar. 22, and Oct. 6 1998. The plant collection numbers were S1004 and S1014. The specimens were identified at Kew Botanic Garden by Dr. Alan Paton and at Addis Ababa University by Dr. Sebsebe Demissew. The specimens were deposited at National Herbarium Addis Ababa University with herbarium numbers 072766 and 072767, respectively.

3.2 Hydro-distillation

Essential oil was obtained by means of hydro-distillation using Clevenger type apparatus. The yield recorded for the different collections of the above varieties varied between 0.4-0.7% for *O. basilicum* and 0.1-0.3% for *O. americanum.*

3.3 Isolation of linalool and estragole

The essential oil of *Ocimum basilicum* var. *thyrsiflorum* (2g) from specimen collected from Wondo Genet (Feb., 1998) was applied onto a column (3 cm x 30 cm) of silica gel 60 and step gradient elution with petrol-EtOAc. 24 Fractions each 50 ml were obtained and monitored by TLC. Fractions 12 and 13 were combined, concentrated and applied on PTLC using solvent system petrol-EtOAc (9:1). Two compounds linalool (**9**) and estragole (**1**) were obtained. The yields obtained were 9% and 30% respectively. Estragole has R_f value of 0.8.

Linalool: Aromatic; colourless oil soluble in alcohol and $CHCl_3$; GC R_t min: 9.196; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.2 (3H, *s,* H-3), 1.5 (2H, *dt,* H-5), 1.55 (3H, *d,* H-7), 1.63 (3H, *d,* H-8), 2 (2H, *t,* H-4), 5 (1H, *cis, dd,* H-1), 5.06 (1H, *m,* H-6), 5.16 (1H, *trans, dd,* H-1), 5.86 (1H, *dd,* H-2,); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 17.6 (C-8), 22.9 (C-5), 25.6 (C-9), 27.8 (3-CH_3), 42.0 (C-4), 73.4 (C-3), 111.6 (C-1), 124.291 (C-6), 131.8 (C-7), 145.0 (C-2)

Estragole: Aromatic; colourless oil, soluble in $CHCl_3$; GC R_t min:16.721; ^{1}H-NMR (350 MHz, $CDCl_3$): δ 3.4 (2H, *d,* H-7), 3.8 (3H, s, 1-OCH_3), 5.1 (2H, *dd,* H-9), 6 (1H, *m,* 8-H), 6.8 (2H, *dd,* 3,5-H), 7.11 (2H, *dd,* 2,6-H); ^{13}C NMR (125, MHz, $CDCl_3$): δ 39.3 (C-7), 55.8 (OCH_3), 113.2 (C-8), 115.6 (C-9), 128.8 (C-3,5), 132.0 (C-4), 137.2 (C-2,6), 157.9 (C-1).

The ^{1}H NMR of the crude oil of *Ocimum basilicum* var. *thyrsiflorum* that was collected on October 10 1998 was performed it was evident that the oil is about 1:1 mixture of linalool (**9**) and estragole (**1**).

3.4 Isolation of methyl cinnamate and linalool

Two grams of the crude oil of *Ocimum americanum var. pilosum* (Wondo Genet 2/98) was applied on column of silica gel 60 and eluted first with petrol and, petrol-ethyl acetate mixture and ethyl acetate successively. Total of 24 fractions each with 50 ml were obtained. Fractions 15, 16, 17, 18 and 19 were combined and applied on PTLC and developed using solvent system petrol and EtOAc (9:1). Two major compounds methyl cinnamate (**16**) and linalool (**9**) with R_f values of 0.4 and 0.3 respectively were isolated and characterized using NMR. The yields obtained were 10% and 8% respectively.

Methyl cinnamate: White crystalline solid m.p 35 °C; soluble in $CHCl_3$; ^{1}H-NMR (500 MHz): δ 3.8 (3H, *s*, $-OCH_3$) 6.4 (1H, *d*, H-2), 7.4 (3H, *t*, 3', 4',5'-H), 7.5 (2H, *dd*, 2', 6'-H), 7.7 (1H, *d*, 3-H); ^{13}C (75 MHz, $CDCl_3$): δ 51.7 ($-OCH_3$), 117.8 (C-4'), 128.1 (C-5'), 128.9 (C-6'), 130.3 (C-2), 134.4 (C-1'), 133.9 (C-3), 167.5 (C-1); IR ν_{max} (KBr) cm^{-1} : 3080, 2922.7, 2854.7, 1709.6, 1636.5, 1450.2, 1381.0, 1318.3, 1200.1, 1176.9, 1027.4.

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Declaration

The thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Getachew Abebe

Signature:

Place and date of submission: Chemistry Department

Addis Ababa University

March, 1999

This thesis has been submitted for examination with my approval as a university advisor.

Prof. Ermias Dagne