ESSENTIAL OILS OF SOME *EUCALYPTUS* SPECIES OF ETHIOPIA

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BY
ABERA ASEFA
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ABSTRACT

ESSENTIAL OILS OF SOME *EUCALYPTUS* SPECIES OF ETHIOPIA

By
Abera Asefa

Research advisor : Dr. Ermias Dagne

The essential oils of the leaves of six *Eucalyptus* species (*E. globulus* Labill., *E. citriodora* Hooker, *E. tereticornis* Sm., *E. viminalis* Labill, *E. robusta* Sm. and *E. camaldulensis* Dehn) acclimatized in Ethiopia were investigated for the first time. The results indicated *E. globulus* and *E. viminalis* to possess oil rich in 1,8-cineole (>70%). The principal components in oils of leaves of *E. citriodora* , *E. robusta* and *E. camaldulensis* were citronellal (>70%), α-pinene (>50%) and *p*-cymene (>30%), respectively. Essential oils of fruits of two *Eucalyptus* species (*E. globulus* and *E. citriodora*) were studied and found to be different from those obtained from their respective leaves. Essential oils from leaves of young and old plants of *E. globulus* did not show significant differences in yield and composition.

1. INTRODUCTION

Essential oils or ethereal oils are complex mixtures of odoriferous and volatile compounds occurring in, or isolated from plant materials [1]. They are the aroma giving constituents of aromatic plants and are not to be confused with other oils such as "fixed oils" which are chemically different [2,3]. Essential oils consist of terpenoids and aromatic hydrocarbons and their oxygenated derivatives and are necessarily of plant origin. Fixed oils may have plant or animal origin and are essentially triglycerides.

The function of the essential oils in plants is still not satisfactorily explained. Whether they serve as attractants, repellents , or protectants or are simply waste products is not clear [1,4].

Essential oils are mostly obtained from plant materials by steam distillation. In some cases these oils may also be obtained by cold-pressing, solvent extraction , maceration, etc. [5]. The term essential oil is derived from the consideration that the oil has the same characteristic "essence" as the plant it is obtained from. Essential oils may develop through the entire plant or in certain parts of the plant only. Some essential oils occur solely in the bark, leaves, flowers or fruits. In some cases different parts of a single plant may contain essential oils of different chemical composition.

Essential oils are employed widely for imparting odour and flavour to an almost unlimited variety of consumer goods, such as pharmaceutical and dental preparations, food products, alcoholic and nonalcoholic beverages, confectionery, chewing gums, soaps, detergents, room sprays and insecticides, cosmetics and perfumes; and for masking odours in synthetic products, such as plastic, artificial leather and rubber goods [1].

There is considerable interest in the study of essential oils since 1920. Much useful information has been published by Guenther in his six volumes "The Essential Oils" [2]. The oils are thought to be intimately connected with vital processes in the plant, and as a result they have been used in the determination of evolutionary status of the plant families. They are considered as secondary metabolites of plant products and were utilized as adjunct to classical morphological taxonomy. Attempts have been made to establish relationships between chemical oil composition and the systematic position of the plant [6]. In some cases essential oils are also used as chemotaxonomic markers [44].

1.1 Composition of essential oils

The essential oils vary widely in their chemical composition. A few are composed of almost exclusively one component. Most essential oils, however, contain a large number of constituents, 50 and more not being unusual [1]. The principal constituents of essential oils are terpenoid hydrocarbons and their oxygenated derivatives [4]. Phenyl propanoids are the other class of compounds found in essential oils. Of the terpenoids, monoterpenoids and sesquiterpenoids are the most common components of essential oils.

1.2. Isolation of Essential oils

Essential oils are most often separated by steam distillation at atmospheric pressure. The oils are insoluble in water and form an oil phase on the aqueous distillate. The oil can easily be separated and freed from water with anhydrous sodium sulphate. When small amounts of essential oils are distilled, they can be separated from the water layer by extraction with petroleum ether or diethylether. Some essential oil constituents are heat labile, and structural rearrangements can take place during isolation by steam distillation at atmospheric pressure. Therefore, steam distillation at reduced pressure is some times used or other methods of isolation are employed which are less likely to cause changes. These are cold-pressing, extraction with volatile organic solvents, expression, maceration, etc. Cold-press method avoids heat but is suitable only for materials containing large quantities of essential oils [5].

1.3. Characterization of Essential oils

Gas liquid chromatography (GLC) is an excellent tool for the separation, characterization, and quantitative analysis of essential oils. Separations of components of essential oils that formerly took days by tedious chemical and physical means, or were impossible by these older methods, can now be accomplished in minutes.

Before gas liquid chromatography (GLC) analysis terpenoid hydrocarbons can be separated from the oxygenated components of essential oils by liquid solid chromatography on silica gel. Hydrocarbon fraction could be eluted quantitatively from a column packed with silica gel with hexane as eluting agent. Later, the oxygenated fractions are eluted with ethyl acetate [5].

Sometimes preliminary GLC can be used to separate essential oil constituents on non polar packing. The compounds are eluted in the order of their boiling points, and since the monoterpenoid hydrocarbons generally have higher vapour pressure than their oxygenated derivatives, the hydrocarbons are eluted first. However, several low boiling oxygenated components, important in some essential oils, are also removed with monoterpenoid hydrocarbons. The sesquiterpenoid hydrocarbons are eluted after the oxygenated monoterpenoids, since their boiling points in general are higher than those of the oxygenated derivatives. The eluates can be collected and further separation of components made under different chromatographic conditions.

In GC analysis, under a definite set of operational conditions, the retention time is characteristic of a certain component. It would, therefore, be reasonable to identify the components of a mixture by direct comparison of their retention time with those obtained for standard compounds. This identification can be further verified by adding a supposed standard compound into the mixture and observing the enhancement of a suspected peak on the new chromatogram. If the two compounds are not identical, a new peak or break in the old peak will usually be found in the new chromatogram [67]

The best method of analysing essential oils is by using a gas chromatograph interfaced with mass spectrometer (GC-MS). Here compounds are identified on the basis of molecular weights, fragmentation pattern and retention times. The GC-MS fitted with a computer is capable of giving a more accurate information regarding the identity of a compound [68].

1.4 Objective of the project

This study deals with the essential oils of leaves of six *Eucalyptus* species (*E. globulus*, *E. citriodora*, *E. tereticornis*, *E. camaldulensis*, *E. viminalis* and *E. robusta*) and fruits of two *Eucalyptus* species (*E. globulus* and *E. citriodora*). The main objective of this research work is to study the chemical composition of the essential oils derived from the above species and to determine their potential use in industry, medicine and for other purposes.

2. ESSENTIAL OILS OF THE GENUS *EUCALYPTUS*

The word *Eucalyptus* is derived from two Greek words "eu" meaning "well" and "kalyptus" meaning "I cover" based on the nature of the lid covering the stamens until they are fully developed [8].

The genus *Eucalyptus*, family Myrtaceae, contains nearly 700 species [9]. Some of these species provide timber for construction and fire wood. *Eucalyptus* trees are indigenous with a few exceptions to Australia and Tasmania, however they are now cultivated all over the world in temperate and semitropical regions [9].

A French philologist, Mondon-Vidaillet, introduced a series of *Eucalyptus* species from Australia to Ethiopia in 1895 [10,11]. Among the introduced species *E. camaldulensis* Dehn (*Key Bahirzaf* in Amharic) and *E. globulus* Labill (*Nech Bahirzaf*) are widely cultivated and abundant. Today there are about 55 *Eucalyptus* species in Ethiopia [12].

The genus *Eucalyptus* was known to produce essential oils for a long time. One of the first natural products exported from Australia in 1788 was *Eucalyptus* oil [8]. There are a great number of *Eucalyptus* species yielding essential oils, the foliage of some being more odorous than that of others and the oils from the various species differ widely in character [13].

The *Eucalyptus* essential oils are generally classified into three types [14]:

Cineole essential oils- These are the most common essential oils in *Eucalyptus* and have medicinal importance. The world market of this 1,8-cineole (**4**) rich oil was estimated in 1984 to be 1400 metric tons. The European pharmacopoeia requires that for an oil to meet specifications it must have a minimum of 70% of 1,8-cineole (**4**), with an α-phellandrene (**21**) content of less than 1% [8]. Presently the main source of this type of essential oil is *E. globulus* [14]. Other *Eucalyptus* species which can yield this type of essential oil are *E. australiana*, *E. smithi*, *E. viridis* and *E. dives* var "C" [8].

Perfumery essential oils- The major oils in this category are oil of *E. citriodora*, oil of *E. staigeriana* and oil of *E. macarthuri*. The first one contains 65-85% of citronellal (**5**), an important product in perfumery [14]. The world production of *E. citriodora* essential oil was estimated in 1984 to be 320 metric tons. *E. staigeriana* is of value for its citral content. It is only produced in Brazil. Although only produced in small quantities, *E. macarthuri* oil is interesting for its geranyl acetate content (60-65%).

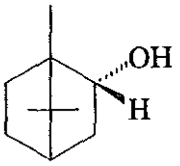
Industrial essential oils- Three species constitute the main sources of these oils: *E. radiata* var "B" yielding an essential oil rich in α-phellandrene (**21**) that is used in the manufacture of sanitary products; *E.dives* "type" and *E. radiata* var "C" which give piperitone (**26**) used for the synthesis of thymol and menthol.

The composition of the *Eucalyptus* leaf oils varies widely between groups of species and, to a lesser extent, within these groups and also within species. The main constituents found in crude *Eucalyptus* oils are α-pinene (**23**), β-pinene (**24**), 1,8-cineole (**4**), *p*-cymene (**10**), α-phellandrene (**21**), piperitone (**26**) and geraniol (**12**) [15].

Monoterpenoids are the major constituents of the lower boiling and less polar fraction of the essential oils. Examples of these C_{10} compounds are α-pinene (**23**), β-pinene (**24**), myrcene (**17**), limonene (**15**), 1,8-cineole (**4**) and *p*-cymene (**10**). Other groups of compounds found in the oils are sesquiterpenoids (both hydrocarbon and oxygenated compounds) such as aromadendrene (**35**), globulol (**43**), α-eudesmol (**40**), β-eudesmol (**41**), γ-eudesmol (**42**) and alloaromadendrene (**34**) [6, 15].

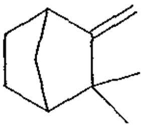
Many alcohols, esters, acids, aldehydes and ketones have been separated from the essential oils of *Eucalyptus* [15, 16]. Examples include geraniol (**12**), citronellal (**5**) and citronellol (**7**), the most valued essential oil isolates in perfume formulation, and piperitone (**26**) [17]. Other components separated and identified from *Eucalyptus* oils include globulol (**43**), methyl cinnamate, phellandral (**20**), verbenone (**33**), and myrtenal (**18**) [15,17].

Fig. 2.1 : Some monotetpenoids that were reported from essential oils of *Eucalyptus* species



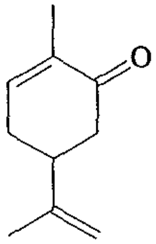
1

Borneol



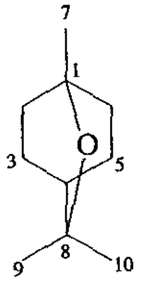
2

Camphene



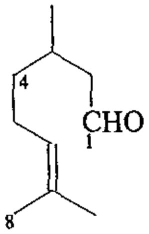
3

Carvone



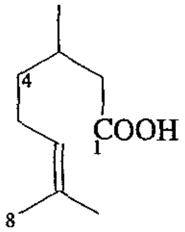
4

1,8-cineole



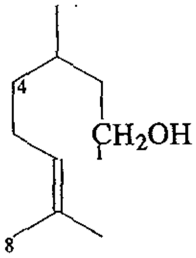
5

Citronellal



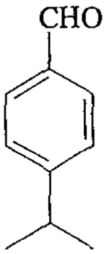
6

Citronellic acid



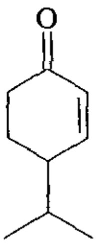
7

Citronellol



8

Cuminaldehyde



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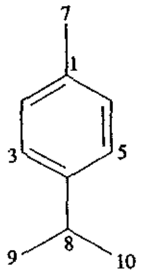
Cryptone



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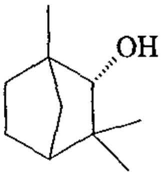
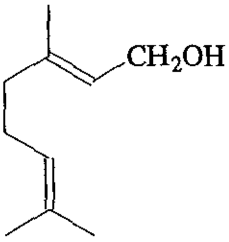
p-Cymene



11

α-Fenchyl alcohol



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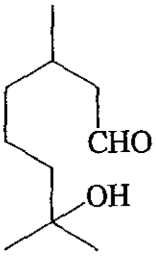
Geraniol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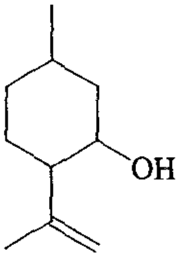
Hydroxycitronellal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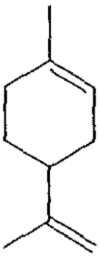
Isopulegol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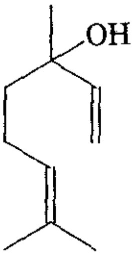
Limonene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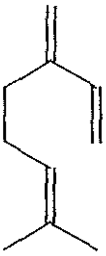
Linalool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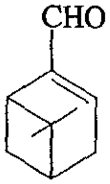
Myrcene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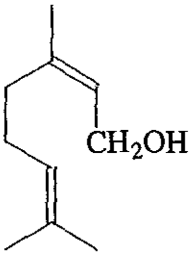
Myrtanal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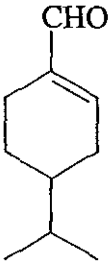
Nerol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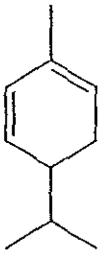
α -Phellandral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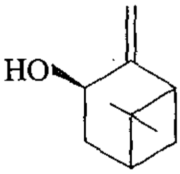
α -Phellandrene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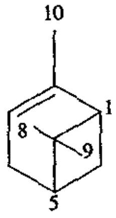
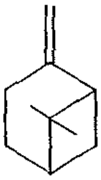
Pinocarveol



23

α -Pinene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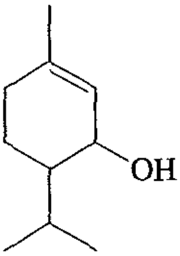
β-Pinene



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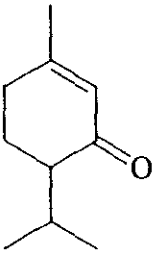
Piperitol



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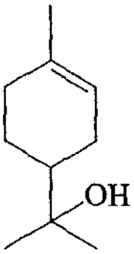
Piperitone



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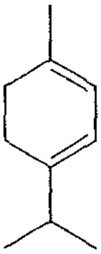
α-Terpineol



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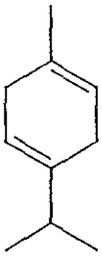
α -Terpinene



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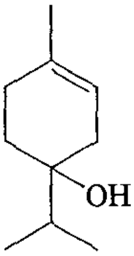
γ -Terpinene



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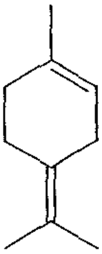
Terpinene-4-ol



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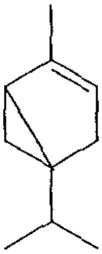
Terpinolene



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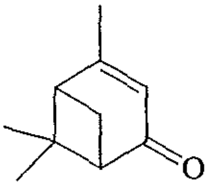
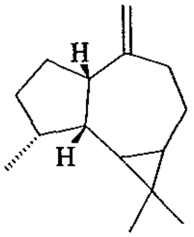
α -Thujene



33

Verbenone

Fig. 2.2 : Some sesquiterpenoids that were reported from essential oils of *Eucalyptus* species



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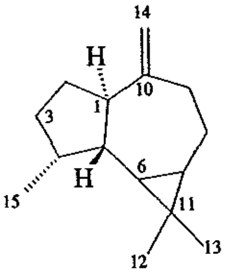
Alloaromadendrene



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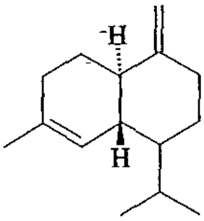
Aromadendrene



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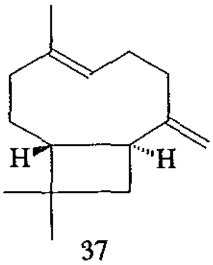
γ-Cadinene



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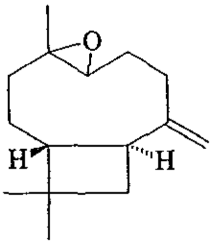
β-Caryophyllene



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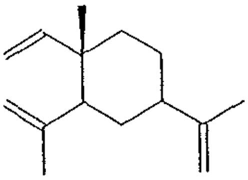
Caryophyllene oxide



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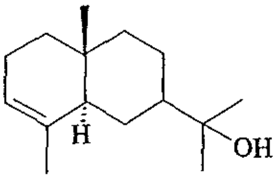
β-Elemene



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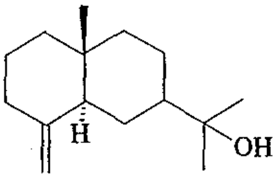
α-Eudesmol



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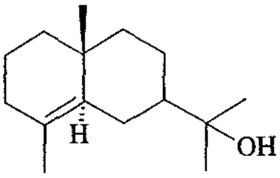
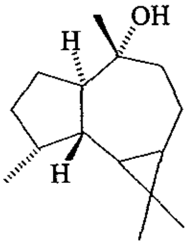
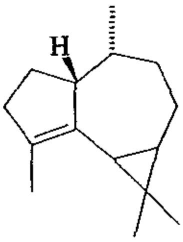


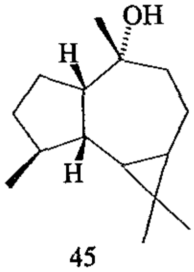
42
γ-Eudesmol



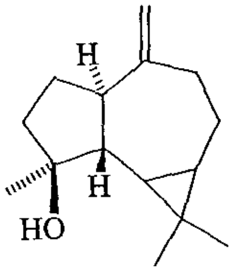
43
Globulol



44
α-Gurjunene



45
Ledol



46

Spathulenol

Generally many chemical investigations on the essential oils of different *Eucalyptus* species acclimated to different countries are documented in the chemical abstracts (1907-1993). A summary of the constituents of oils of some common *Eucalyptus* species from literature is given in Table 2.1. These investigations showed that the chemical composition of the essential oil can be affected by the geographical location in which the plant grows. However, the chemical composition of the essential oils derived from *Eucalyptus* species grown in Ethiopia has not been studied to date. For large scale production of essential oils from *Eucalyptus* species, the chemical composition of the essential oils must be studied first and the quality and the commercial value of the oils must be determined. Therefore, these considerations led us to study the chemical composition of the essential oils of six *Eucalyptus* species acclimatized in Ethiopia. A brief summary of the reported studies on these species is summarized here under.

2.1. Oil of *Eucalyptus globulus*

This species was described by Labillardiere in 1799. It is a tall tree with a smooth whitish blue bark. The tree is known in Australia by the common name of "Blue Gum" [8]. *E. globulus* is commonly called in Ethiopia as "Nech Bahrzaf". It is wide spread in villages, towns and capitals on the cold high lands.

The oil of *E. globulus* was first investigated by Cloez who isolated a substance, which he called eucalyptol. The molecular formula of eucalyptol, $C_{10}H_{18}O$, and its identity with 1,8-ceneole (**4**) was recognized by Jahns [8]. Since then oil of *E. globulus* has been the subject of many chemical investigations.

In 1972 analytical study of the essential oil of *E. globulus* leaves from Portugal by J. Cardoso do Vale [18] revealed the presence of butyraldehyde, isovaleric and valeric aldehyde, α-pinene (**23**), β-pinene (**24**), camphene (**2**), limonene (**15**), 1,8-cineole (**4**), *p*-cymene (**10**), α-terpineol (**27**), terpenyl acetate, verbenone (**33**) and aromadendrene (**35**).

In 1972 also Pradash *et al.* [19] studied the chemical composition of essential oil extracted from the fruits of *E. globulus*. This study revealed the presence of α-pinene (**23**), 1,8-cineole (**4**), α-pinocarvone (**22**), citronellal (**5**), d-myrenal, carvone (**3**), cuminaldehyde (**8**), citral, aromadendrene (**35**), globulol (**43**), cinnamic acid and eudesmyl acetate.

In 1979 Nishimura and Melvin Calvin [20] studied the essential oil of *E. globulus* fruits and reported the presence of 1,8-cineole (**4**), α-terpinene (**28**), γ-terpinene (**29**), β-pinene (**24**), terpinen-4-ol (**30**), linalool oxide, α-gurjunene (**44**), aromadendrene (**35**), alloaromadendrene (**34**), globulol (**43**), piperitone (**26**), eremophilene and γ-cadinene (**36**).

In 1980 Chennoufi *et al.* [21] studied the essential oil obtained from young and old *E. globulus* grown in Morocco. Oils extracted from the young and old leaves did not show much differences.

In 1985 the study of the chemical composition of the essential oil of *E. globulus* Labill revealed the presence of 1,8-cineole (**4**) (75%), α-pinene (**23**) and limonene (**15**) as major components [22]. And analysis of medicinal oil from *E. globulus* ssp bicostata leaves revealed the presence of 1,8-cineole (**4**) (73.09%), bicostol (15.7%), α-pinene (**23**) (4%), α-terpineol (**27**) (0.7%) and linalool (**16**) (0.009%) [23].

In 1992 Zrira and Benjilali [14] reported 1,8-cineole (**4**) (72.8%), α-pinene (**23**) (11.9%), α-terpinyl acetate (2.7%), aromadendrene (**35**) (2.0), globulol (**43**) (1.3%) and other minor components from essential oil of leaves of *E. globulus* Labill grown in Morocco.

Compounds that were reported from essential oil of *E. globulus* leaves:

alloaromadendrene, aromadendrene, borneol, butyraldehyde, camphene, 1,8-cineole,citronellal, *p*-cymene, α-*p*-dimethylstyrene, geraniol, isovaleric aldehyde, ledol, limonene, linalool, myrcene, α-phellandrene, α-pinene, β-pinene, *trans*-pinocarveol, piperitone, terpinen-4-ol, α-terpineol, α-terpinyl acetate, valeric aldehyde, verbenone.

Compounds that were reported from essential oil of *E. globulus* fruits:

alloaromadendrene, aromadendrene, γ-cadinene, carvone, 1,8-cineole, cinnamic acid, citral, citronellal, cuminaldehyde, eremophilene, eudesmol, eudesmyl acetate, globulol, α-gurjiunene, linalool oxide, myrtenal, α-pinene, β-pinene, α-pinocarvone, piperitone, α-terpinene, β-terpinene, γ-terpinene, terpinen-4-ol.

2.2 Oil of *Eucalyptus citriodora*

Eucalyptus citriodora, also known as "Spotted Gum", "Citron Scented Gum" or "Lemon Scented Gum", is a fast growing forest tree [8]. It is indigenous to Australia and has been introduced in many parts of the world including Ethiopia. The leaves of this tree yield an oil which is used in perfumery industry. The oil serves as a source of citronellal for the production of citronellol, hydroxycitronellal and menthol [39].

In 1888, for the first time Schimmel and his coworkers [8] recognized that essential oil of *E. citriodora* consists chiefly of citronellal (**5**) and the other constituents of the oil were investigated years later by Chiris in 1925. Since then oil of *E. citriodora* has been the subject of many chemical investigations.

In 1972 the study of chemical composition of *E. citriodora* oil by Barua *et al.* [25] revealed the presence of α-pinene (**23**), β-pinene (**23**), 1,8-cineole (**4**), *p*-cymene (**10**), citronellal (**5**) and geraniol (**12**). In 1973, Antonia *et al.* [26] reported citronellal (**5**) (54%), α-pinene (**23**), β-pinene (**24**), limonene (**15**), 1,8-cineole (**4**), isopulegol (**14**),α-eudesmol

(**42**), carvone (**3**), hydroxycitronellal (**13**), eugenol, fatty acids and phenols from the essential oil of *E. citriodora*.

In 1974 Concepcion [27] studied the essential oil of *E. citriodora* acclimated to Spain and reported that isopulegol (**14**), citronellal (**5**), citronellol (**7**) and 1,8-cineole (**4**) are the principal components of the oil.

In 1978 Shien Jui-Chung *et al.* [28] studied the essential oil of *E. citriodora* leaves collected from different localities of Taiwan. This study revealed citronellal (**5**), isopulegol (**14**) and citronellol (**7**) as major components and menthol, α-pinene (**23**), β-pinenes (**24**), p-cymene (**10**), 1,8-cineol (**4**), linalool (**16**), myrcene (**17**), terpinolene (**31**), camphene (**2**) and benzyl acetate as minor components of the oil.

In 1983, the study on *E. citriodora* leaves oil by Youdi *et al.* [29] confirmed that the major components are citronellal (**5**) (73%) and citronellol (**7**) (14%).

Miranda [30] in 1983 reported that oil of *E. citriodora* grown in Cuba contained approximately 10% hydrocarbons and 90% oxygenated compounds. This study also revealed the presence of four new compounds sabinene, α-terpinene (**28**), *trans*-ocimene and 3-hexen-1-ol. In 1981 Mwangi *et al.* [31] reported citronellal (**5**) (65-88%), citronellol (**7**) (2-25%) and isopulegol (**14**) (2-19%) as the major components of essential oil obtained from *E. citriodora* grown in Kenya.

In 1987 Sood *et al.* [24] observed a significant differences in the chemical composition of *E. citriodora* oils obtained from young and old leaves. The greatest difference obtained was in the content of citronellal (**5**) (32% and 67%) and citronellol (**7**) (52% and 23%) in the oils from young and old leaves respectively.

In 1992 Zrira and Benjilali [14] reported citronellal (**5**) (56%), citronellyl acetate (11%), citronellol (**7**, 8%), citronellic acid (**6**) (5%) and other minor components from essential oil of *E. citriodora* grown in Morocco.

Compounds that were reported from essential oil of *E. citriodora* leaves:

benzyl acetate, camphene, carvone, 1,8-cineole, citronellal, citronellic acid, citronellol, citronellyl acetate, *p*-cymene, α-eudesmol, eugenol, geraniol, 3-hexen-1-ol, hydroxycitronellal, isopulegol, limonene, linalool, menthol, myrcene, *trans*-ocimene, α-pinene, β-pinene, γ-terpinene, α-terpineol, terpinolene, α-terpinyl acetate

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2.3 Oil of *Eucalyptus camaldulensis*

This species is known in Australia by the name of "Murray red Gum" or "River red Gum" in order to distinguish it from the allied species [32]. In Ethiopia it is commonly called as "*Key Bahirzaf*" and cultivated in the hot low lands [10].

In 1974 Guniz [33] studied the essential oil obtained from leaves of *E. camaldulensis* grown in the Mediteranean region of Turkey and reported 1,8-cineole (**4**) (75%), isovaleraldehyde, geraniol (**12**), α-phellandrene (**21**), cuminal (**8**), phellandral (**20**). In 1977, Acosta de Iglesias *et al.* [34] reported α-pinene (**23**), β-pinene (**24**), α-phellandrene (**21**), eucalyptol (**4**) (19%), *p*-cymene (**10**) (31%), linalyl acetate, neryl acetate, α-phellandral (**20**), cuminal (**8**), piperitone (**26**), α-terpineol (**27**), nerol (**19**), fatty acid and other minor components from essential oil of *E. camaldulensis*.

In 1981 Singh *et al.* [35] studied the composition of essential oil of *E. camaldulensis* grown in India and reported α-pinene (**23**) (16%), β-pinene (**24**) (16%), α-terpinene (**28**) (7%), 1,8-cineole (**4**) (21%), *p*-cymene (**10**) (25%) and other minor constituents.

In 1991 study on the essential oil of the leaves and fruits of *E. camaldulensis* showed that the oil yield was higher in the leaves than the fruits but the oils were found to be very similar. The major components in the leaf oil and fruit oil were 1,8-cineole (**4**) (45%, 49%), *p*-cymene (**10**) (23%, 20%), γ-terpinene (**29**) (8%, 4%) and α-pinene (**23**) (10%, 3%), respectively.

In the same year the study on *E. camaldulensis* leaf oil in Nepal showed that the major component of the oil was 1,8-cineole (**4**, 60-65%) and the other constituents were α-pinene

(**23**), β-pinene (**24**), limonene (**15**), β-caryophyllene (**37**) and 1(7)-p-methen-9-ol [37]. In 1992 Zrira and Benjilali [14] reported 1,8-cineole (**4**, 52%), α-pinene (**23**, 25%) borneol (**1**, 3%) and other minor components from *E. camaldulensis* in Morocco.

Compounds that were reported from essential oil of *E. camaldulensis* leaves:

alloaromadendrene, aromadendrene, benzaldehyde, borneol, camphene, campholenic aldehyde, β-caryophyllene, 1,8-cineole, citronellal, citronellol, cuminal, *p*-cymene, α-*p*-dimethylstyrene, eugenol, α-fenchyl alcohol, geraniol, globulol, isovaleraldehyde, limonene, linalool, linalyl acetate, 1(7)-*p*-methen-ol, myrcene, nerol, neryl acetate, phellandral, α-phellandrene, α-pinene, β-pinene, piperitone, α-terpinene, γ-terpinene, terpinen-4-ol, α-terpineol, α-terpinyl acetate, verbenone.

2.4 Oils of *E. tereticornis, E. viminals, E. robusta*

The constituents of these oils and that of 39 other species from literature are summarized and given in Table 2.1.

Table 2.1: Components of essential oils of 41 selected *Eucalyptus* species. See section 2.1, 2.2 and 2.3 for *E. globulus*, *E. citriodora* and *E. camaldulensis*, respectively.

Species	**Compounds**	**Ref.**
E. albens	aromadendrene, camphene, 1,8-cineole, α-p-dimethylstyrene, α-fenchyl alcohol, globulol, myrtenol, α-phellandrene, α-pinene, β-pinene, piperitone, α-terpineol, terpinen-4-ol,	14
E. astringens	alloaromadendrene, aromadendrene, borneol, campholenic aldehyde, 1,8-cineole, citronellol, α-p-dimethylstyrene, β-elemene, globulol, isoamylisovalerate, ledol, myrtenol, α-pinene, β-pinene, *trans*-pinocarveol, α-terpineol, α-terpinyl acetate.	14
E. blakeli	aromadendrene, borneol, camphene, 1,8-cineole, citronellic acid, citronellol, p-cymene, α-p-dimethylstyrene, eugenol, α-fenchyl aldehyde, geraniol, isoamyl isovalerate, linalool, β-myrcene, α-phelandrene, α-pinene, piperitone, *trans*-pinocarvaeol, terpinen-4-ol, α-terpinolene, thujene, verbenone.	14, 15
E. bosistoana	alloaromadendrene, aromadendrene, camphene, 1,8-cineole, citronellyl acetate, α-p-dimethylstyrene, globulol, isoamyl isovalerate, lendol, linalool, myrcene, α-phelandrene,α-pinene, β-pinene, *trans*-pinocarveol, γ-terpinene, terpinen-4-ol,	14
E. botroides	alloaromadendrene, aromadendrene, borneol, camphene, 1,8-cineole, citronellol, α-p-dimethylstyrene, α-fenchyl alcohol, globulol, α-pinene, *trans*-pinocarveol, spathulenol, γ-terpinene, α-terpineol, α-terpinyl acetate.	14
E. bridgesiana	1,8-cineole, p-cymene, globulol, limonene, α-terpineol	38
E. caladocalyx	alloaromadendrene, aromadendrene, borneol, camphene, camphonelic aldehyde, β-caryophllene, 1,8-cineole, p-cymene, α-*p*-dimethylstyrene, α-fenchyl alcohol, globulol, isobutyl valerate, limonene, linalool, myrcene, myrtenol, α-phelandrene, α-pinene, β-pinene, *trans*-pinocarveol, γ-terpinene, terpinene-4-ol, α-terpineol, thujene.	14
E. cinerea	aromadendrene, 1,8-cineole, *p*-cymene, geraniol, limonene, linalool, myrcene, α-pinene, β-pinene, piperitone, terpenolene, terpinen-4ol, α-terpineol, terpinyl acetate, thujene.	15, 39-42
E. crebra	aromadendrene, 1,8-cineole, citronellal, *p*-cymene, geraniol, linalool, myrcene,α-pinene, piperitone, terpenolene, α- terpinolene.	15
E. dealbata	alloaromadendrene, aromadendrene, camphoenic aldehyde, β-caryophyllene, caryophyllene oxide, 1,8-cineole, cryptone, cuminaldehyde, *p*-cymene, α-*p*-dimethylstyrene, globulol, limonene, linalool, myrcene, α-phelandrene, α-pinene, β-pinene, *trans*-pinocarveol, piperitone, γ-terpinene, terpinen-4-ol, α-terpineol, thujene.	14, 43
E. delegatensis	1,8-cineole, limonene, *cis*-*p*-methene-1-ol, *trans*-*p*-methene-1-ol, methylcinnamate, myrcene, α-phellandrene, 4-phenyl-2-butanone, α-pinene, β-pinene, *trans*-pipritol, α-terpinene, γ-terpinene, thujene.	44

E. diversicolor	camphene, 1,8-cineole, α-dimethylstyrene, α-phellandrene, α-pinene, γ-terpinene, α-terpinyl acetate	14
E. exerta	alloaromadendrene, aromadendrene, borneol, camphene, β-caryophyllene, 1,8-cineole, citronellic acid, citronellol, cuminaldehyde, α-*p*-dimethylstyrene, α-fenchyl alcohol, globulol, ledol, linalool, myrcene, α-phellandrene, α-pinene, β-pinene, *trans*-pinocarveol, α-terpinene, γ-terpinene, terpinen-8-ol, α-terpineol.	14, 45
E. ficifolia	3- or 4-carene, p-cymene, limonene, α-pinene, β-pinene, γ-terpinene, α-thujene,	46
E. grandis	aromadendrene, 1,8-cineole, *p*-cymene, eugenol, geraniol, linalool, α-pinene, piperitone, α-terpinene, terpenolene, thujene.	15
E. longifoli	aromadendrene, 1,8-cineole, *p*-cymene, limonene, linalool, myrcene, α-pinene, piperitone, α-terpinene, terpenolene, thujene.	15
E. macarthuri	aromadendrene, 1,8-cineole, *p*-cymene, α-eudesmol, β-eudesmol, geraniol, geranyl acetate, linalool, myrcene, α-pinene, β-pinene, piperitone, α-terpinolene,thujene.	15, 47, 48
E. macrorhyncha	α-eudesml, β-eudesmole, γ-eudesmol, 1,8-cineole.	49
E. maculata	alloaromadendrene, aromadendrene, borneol, 3-carene, (+)-carvone, β-caryophyllene, 1,8-cineole, citronellol, *p*-cymene, *p*-cymen-8-ol, α-*p*-dimethylstyrene, dipentene, geraniol, isomenthone, isopulegol, limonene, linalool, myrcene, α-phellandrene, α-pinene, β-pinene, *trans*-pinocarveol, α-terpinene, spathulenol, γ-terpinene, terpinen-4-ol, α-terpineol.	14, 50
E. maideni	alloaromadendrene, aromadendrene, borneol, 1,8-cineole, *p*-cymene, α-*p*-dimethylstyrene, dipentene, eugenol, geraniol, globulol, isoamyl isovalerate, isopulegol, linalool, myrcene, α-phellandrene, α-pinene, β-pinene, piperitone, *trans*-pinocarveol, terpinen-4-ol, α-terpineol, α-terpinolene, thujene	14, 15, 51
E. mellidora	aromadendrene, camphene, 1,8-cineole, citronellal, α-*p*-dimethylstyrene, isoamyl isovalerate, linalool, α-phellandrene, α-pinene, β-pinene, *trans*-pinocarveol, γ-terpinene, terpinen-4-ol, α-terpineol.	14
E. mitchelliana	1,8-cineole, *p*-cymene, α-eudesmol, β-eudesmol, α-phellandrene, α-pinene, piperitol, piperitone, α-terpineol.	52
E. moluccana	alloaromadendrene, borneol, β-caryophyllene, 1,8-cineole, citronellol, cryptone, cuminaldehyde, *p*-cymene, α-*p*-dimethylstyrene, α-fenchyl alcohol, globulol, *p*-isopropylphenol, limonene, linalool, myrcene, α-pinene, *trans*-pinocarveol, spathulenol, α-terpinene, γ-terpinene, terpinen-4-ol, α-terpineol, thujene.	14, 53
E. nova-anglica	aromadendrene,α-eudesmol, β-eudesmol, γ-eudesmol, globulol, nevolidol.	54
E. numerosa	aromadendrene, camphene, 1,8-cineole, cryptone, linalool, α-phellandrene, α-pinene, piperitone.	66
E. oblonga	1,8-cineole, α-eudesmol, β-eudesmol, (+)-α-pinene	52
E. occidentalis	allo-aromadendrene, campholenic aldehyde, *p*-cymene, globulol, limonene, linalool, myrtenol,α-pinene, β-pinene, spathulenol, terpinen-4-ol.	14
E. oviformis	camphene, 1,8-cineole, α-*p*-dimethylstyrene, myrcene, α-phellandrene, α-pinene, β-pinene, piperitone, α-terpinene, γ-terpinene, terpinen-4-ol, thujene.	14

E. paniculata	alloaromadendrene, aromadendrene, borneol, camphene, campholenic aldehyde, 1,8-cineole, citronellol, *p*-cymen-8-ol, α-*p*-dimethylstyrene, β-elemene, α-fenchyl alcohol, globolol, ledol, linalool, myrtenol,α-pinene, β-pinene, *trans*-pinocarveol, spathulenol, terpinen-4-ol, α-terpineol, verbenone	14
E. polyanthemos	alloaromadendrene, aromadendrene, borneol, campholenic aldehyde, β-caryophyllene, 1,8-cineole, citronellal, cuminaldehyde, *p*-cymene, α-*p*-dimethylstyrene, eugenol, geraniol, globulol, isoamyl isovalerate, limonene, linalool, myrcene, α-phellandrene, α-pinene, β-pinene, *trans*-pinocarveol, piperitone, α-terpinene, γ-terpinene, terpinen-4-ol, α-terpineol, terpinolene, α-terpinyl acetate, thujene.	14, 15
E. pulverulenta	camphene, 1,8-cineole, limonene, myrcene, α-phellandrene, α-pinene, β-pinene, α-terpinene.	56
E. punctata	aromadendrene, benzaldehyde, borneol, camphene, carvone, 1,8-cineole, citronellol, cryptone, cuminaldehyde, *p*-cymene, α-p-dimethylstyrene, dipentane, α-fenchyl alcohol, globulol, linalool, myrcene, myrtenol, α-phellandrene, α-pinene, β-pinene, *trans*-pinocarveol, s-quaiazulene, α-terpinene, γ-terpinene, terpinen-4-ol, α-terpineol, verbenone.	14, 55, 51
E. radiata	camphene, 1,8-cineole, γ-elemene, α-phellandrene,α-pinene, β-pinene, piperitone, β-selinene, α-terpinene, terpinen-4-ol, α-terpineol.	58
E. robusta	aromadendrene, 1,8-cineole, *p*-cymene, geraniol, limonene, linalool, myrcene, α-pinene, piperitone, α-terpinene, terpinolene, thujene.	15
E. rudis	aromadendrene, 1,8-cineol, citronellal, *p*-cymene, eugenol, geraniol, limonene, linalool, myrcene, α-pinene, piperitone, terpenolene, thujene.	15
E. saligna	benzaldehyde, borneol, δ-cadinene, camphene, camphor, campholenic aldehyde, β-caryophyllene, 1,8-cineole, cuminaldehyde, *p*-cymene, *p*-cymen-8-ol, α-*p*-dimethylstyrene, α-fenchyl alcohol, geraniol, globulol, limonene, linalool, myrtenol, α-phellandrene, α-pinene, β-pinene, *trans*- pinocarveol, α-terpinene, γ-terpinene, terpinen-4-ol, α-terpineol, α-terpinyl acetate, verbenone.	14, 59-61
E. sidroxylon	alloaromadendrene, aromadendrene, β-caryophyllene, 1,8-cineole, p-cymene, α-p-dimethylstyrene, β-elemene, globulol, linalool, myrcene, α-phellandrene, α-pinene, β-pinene, piperitone, α-terpinene, γ-terpinene, terpinen-4-ol, α-terpineol, terpinolene, α-terpinyl acetate, thujene,	14, 15
E. sieberiana	Δ^3-carene, 1,8-cineol, p-cymene, α-phellandrene, α-pinene, β-pinene,	62
E. tereticornis	alloaromadendrene, aromadendrene, borneol, β-caryophyllene, 1,8-cineole, α-p-dimethylstyrene, β-elemene, globulol, ledol, myrcene, α-phelandrene, α-pinene, β-pinene, *trans*-pinocarveol, piperitone, spathulenol, γ-terpinene, terpinen-4-ol, α-terpineol, verbenone.	14, 63
E. viminalis	aromadendrene, 1,8-cineole, citronellal, citronellyl acetate, *p*-cymene, geraniol, linalool, myrcene, α-phellandrene, α-pinene, β-pinene, piperitone, sabinene, α-terpinene, terpinen-4-ol, α-terpineol, terpinolene, α-terpinyl acetate, thujene.	15, 40, 41, 64
E. viridis	camphene,1,8-cineole, cryptone, *p*-cymene, geraniol, isopulegol, α-pinene, β-pinene, α-terpineol,	65
E. youmanii	1,8-cineole, *p*-cymene, α-eudesmol, β-eudesmol, γ-eudesmol, limonene, myrcene, α-phellandrene, α-pinene, terpinen-4-ol, α-terpineol	49

3. RESULTS AND DISCUSSION

The analysis of the oils was carried out using mainly GC and NMR techniques. Identification of components were done mainly by comparison of retention times with those of standard samples. These identifications were further verified by peak enhancement techniques using standard samples.

3.1 *E. globulus* leaves oil

The essential oil of leaves of *E. globulus* from young plant was obtained by steam distillation at atmospheric pressure. The yield was determined to be 1.1% and it has yellow colour. The physicochemical properties of the oil can be seen in Table 3.2.

Table 3.1 : Essential oil yields (%v/w)

Species and parts	Yield (%fresh wt. basis)	Yield (% dry wt. basis)
E. globulus leaves (young plant)	1.1	-
E. globulus leaves (old plant)	1.2	-
E. citriodora leaves	1.4	-
E. camaldulensis leaves	0.7	1.4
E. tereticornis leaves	1.0	-
E. viminalis leaves	0.7	1.3
E. robusta leaves	0.5	1.0
E. globulus fruits	0.7	-
E. citriodora fruits	0.2	0.3

The chromatogram obtained by running the crude oil of *E. globulus* from the young plant on DB-5 30 m x 0.23 mm fused silica capillary column showed 20 components (see Fig. 3.1). There is only one major component (76%) in the oil . The ^{1}H NMR data of the crude oil was found to be similar with that reported for 1,8-cineole in the literature [69]. The ^{13}C NMR spectrum of the crude oil also displayed 10 carbon resonances corresponding to three primary, four secondary, one tertiary and two quaternary carbon atoms (see Fig.2). From these data the major component of the oil could be identified as 1,8-cineole (**4**). This is in agreement with early findings [14, 22]. The ^{13}C NMR spectral data of the crude oil is given in Table 3.3. It is in good agreement with ^{13}C NMR data for 1,8-cineole reported in the literature [9].

Table 3.2 : Physicochemical properties of the oils

no.	Species and parts	Specific gravity(d^{20})	Refractive index (n^{20})	Optical rotation
1.	*E. globulus* leaves (young plant)	0.9207	1.4624	+3.9
2.	*E. globulus* leaves (old plant)	0.9130	1.4606	+3.9
3.	*E. citriodora* leaves	0.8618	1.4525	-0.1
4.	*E. camaldulensis* leaves	0.9049	1.4904	+28.2
5.	*E. tereticornis* leaves	0.8827	1.4844	-32.1
6.	*E. viminalis* leaves	0.9122	1.4691	+0.5
7.	*E. robusta* leaves	-	1.4711	-
8.	*E. globulus* fruits	0.8930	1.4836	-33.8
9.	*E. citriodora* fruits	-	1.4725	-

The oil of leaves of *E. globulus* from old plant was also obtained by steam distillation at atmospheric pressure. The yield and physicochemical properties of the oil are given in Table 3.1 and 3.2. The oil has yellow colour. The chromatogram of the crude oil was almost similar to that of the essential oil of *E. globulus* leaves from young plant (see Fig.3.1). It has one major component (82%) and 19 minor components. The major component of the oil was identified as 1,8-cineole (**4**) from ^{1}H and ^{13}C NMR data of the crude oil.

The ^{1}H and ^{13}C NMR data of this oil was identical to that of oil of *E.globulus* leaves from young plant. The ^{13}C NMR data of the crude oil is given in Table 3.4. The 1,8-cineole content is slightly greater in the oil of *E. globulus* leaves from old plant than leaves from young plant.

Table 3.3 : ^{13}C NMR data of crude oil of *E. globulus* leaves from young plant (22.5MHz, $CDCl_3$) indicating 1,8-cineole (**4**) to be the major component. See Fig. 3.2

C	δ PPM
1	69.4
2	31.1
3	22.6
4	32.8
5	22.7
6	31.1
7	27.3
8	73.3
9	28.6
10	28.6

Table 3.4 : ^{13}C NMR data of crude oil of *E. globulus* leaves from old plant (22.5 MHz, $CDCl_3$) indicating 1,8-cineole (**4**) to be the major component.

C	δ ppm
1	69.7
2	31.5
3	22.8
4	33.0
5	22.8
6	31.5
7	27.5
8	73.57
9	28.84
10	28.84

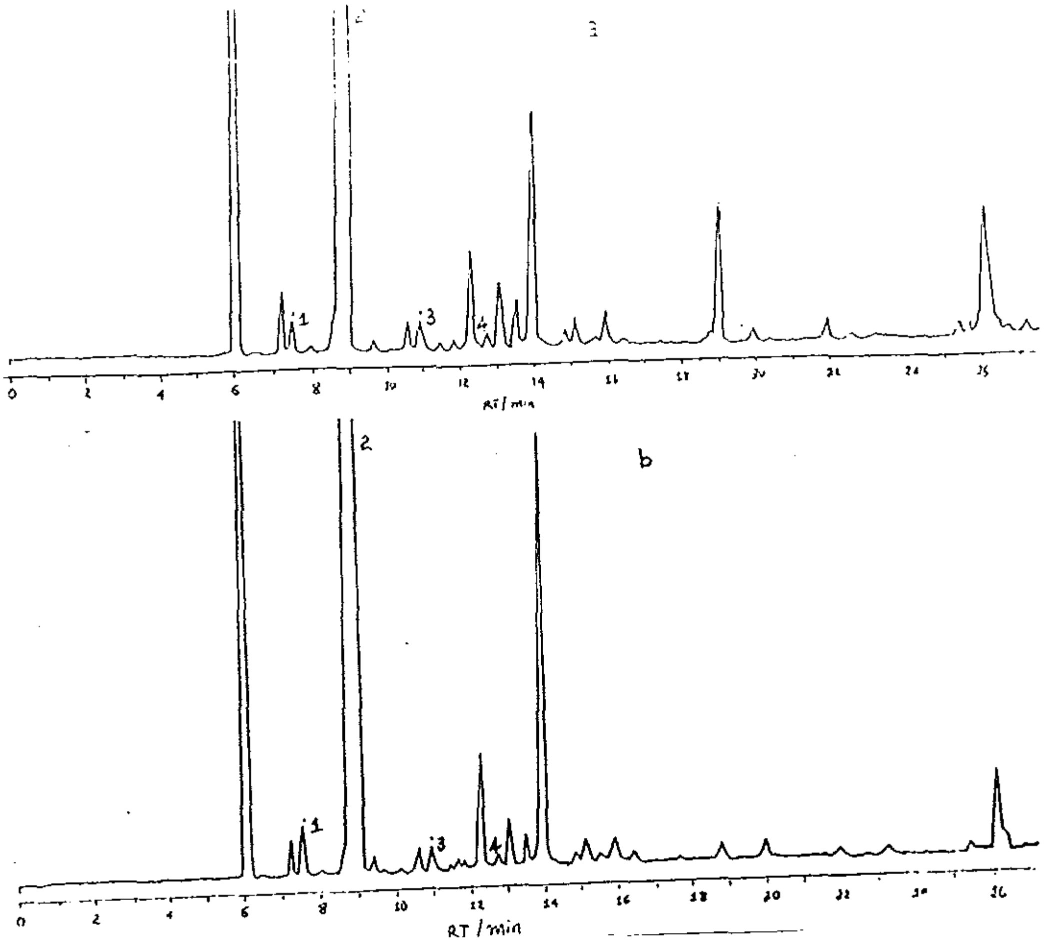

Fig. 3.1 a) Gas chromatogram of oil of *E. globulus* leaves (from young plant): 1, Myrcene; 2, 1,8-Cineole; 3, Linalool; 4, Citronellal

b) Gas chromatogram of oil of *E. globulus* leaves (from old plant): 1, Myrcene; 2, 1,8-Cineole; 3, Linalool; 4, Citronellal

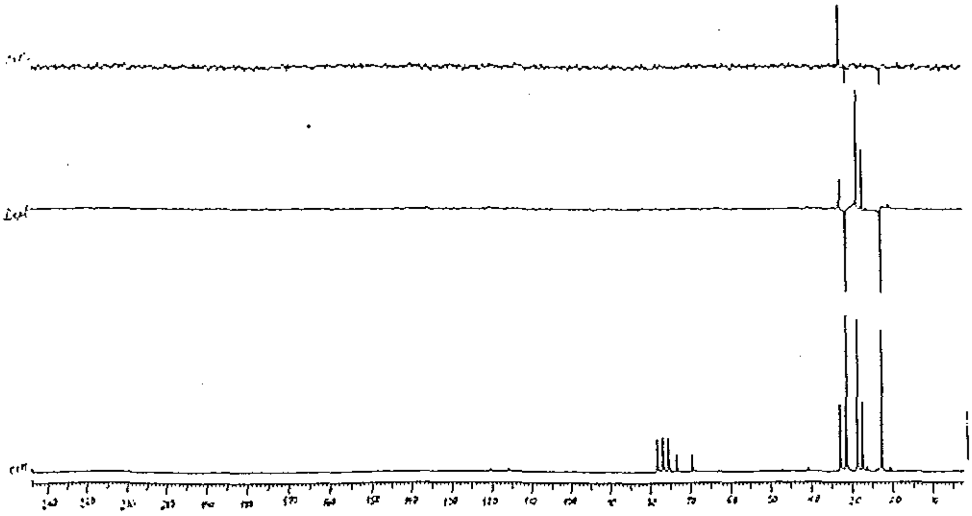

Fig. 3.2 ^{13}C NMR spectrum of crude oil of *E. globulus* leaves (from young plant) confirming identity of the major component as 1,8-cineole (4).

1,8-cineole (**4**) is the most widely used component of *Eucalyptus* oil. It is an important ingredient of many cough mixtures and expectorant preparations [15]. The 1,8-cineole content of the essential oil of *E. globulus* leaves exceeded 70%. According to the European pharmacopoeia specifications [8], this species could be used as a source of medicinal *Eucalyptus* oil. Other minor components of the oils were identified by comparison of the retention times with standard samples using DB-5 30 m x 0.23 mm fused silica capillary column. Identification of the components were further verified by adding a small amount of standards into the oil and observing the growth of suspected peaks on the new chromatograms. Table 3.5 shows the retention times of the identified components in the oil of *E. globulus* leaves under programmed oven temperature.

Table 3.5 : Identified components of oils of *E. globulus* leaves from young and old plants

Peak no.	Compounds	Retention times/min.	%of oil young	%of oil old	Method of identification
1	Myrcene (**17**)	7.6	0.2	0.3	GLC
2	1,8-Cineole (**4**)	9.1	76.0	82.0	GLC,NMR
3	Linalool (**16**)	10.9	0.2	0.2	GLC
4	Citronellal (**5**)	12.8	0.2	0.2	GlC

3.2- *E. citriodora* leaves oil

The oil of *E. citriodora* leaves was obtained by steam distillation at atmospheric pressure. The oil which is obtained in 1.4% yield has light yellow colour. The chromatogram obtained by running the crude oil on DB-5 30 m x 0.23 mm fused silica capillary column revealed the presence of 20 components and one of them was major (76%) (see Fig. 3.3). The physicochemical properties of the oil are given in Table 3.2.

The ^{1}H NMR spectrum of the crude oil revealed the presence of aldehyde group (δ 9.75, t, 1H), two methyl groups on double bond (δ 1.60, 1.70) and another methyl group (δ 0.95).This data was found to be similar with that in the literature for citronellal (**5**) [69]. The IR spectrum of the crude oil displayed absorption band for carbonyl group (1725 cm^{-1}). The ^{13}C NMR spectrum of the crude oil showed the presence of carbonyl group at δ 202.2 and olefinic carbons at δ 124.0 and 131.4. The DEPT spectrum of the oil displayed 10 carbon resonances corresponding to three primary, three secondary, three tertiary and one quaternary carbon atoms (see Fig. 3.4). The ^{13}C NMR data of the oil is given in Table 3.6. From these data the major component of this oil could be identified as citronellal (**5**). This is in agreement with early findings [14, 28, 29]. The ^{13}C NMR data of the crude oil is in good agreement with the standard ^{13}C NMR spectra for citronellal in reference [9].

Table 3.6 : ^{13}C NMR spectral data of crude oil of *E. citriodora* leaves (22.5 MHz, CDCl$_3$) indicating citronellal (5) to be the major component. See also Fig. 3.3

C	δ PPM
1	202.2
2	50.8
3	27.7
4	25.3
5	36.8
6	124.0
7	131.4
8	19.7
7-CH$_3$	25.4
3-CH$_3$	17.4

Citronellal (**5**) is very expensive compound. It is used in perfumes, for the scenting of soaps, and in the production of citronellol, hydroxycitronellal and menthol [24, 70]. To small extent it also serves in artificial citrus flavours [70]. This important compound was isolated and purified by liquid solid chromatography from essential oil of *E. citriodora* leaves.

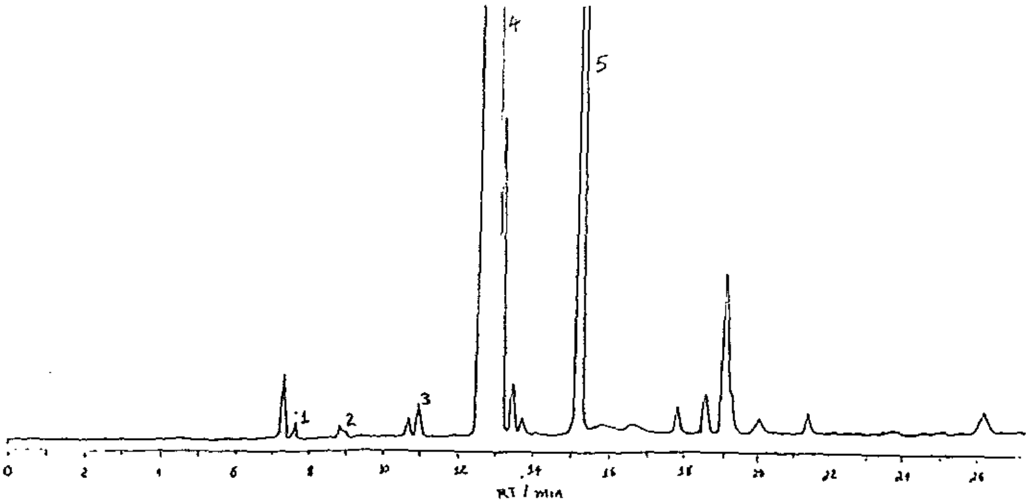

Fig. 3.3 Gas chromatogram of oil of *E. citriodora* leaves: 1, Myrcene; 2, 1,8-Cineole; 3, Linalool; 4, Citronellal; 5, Citronellol

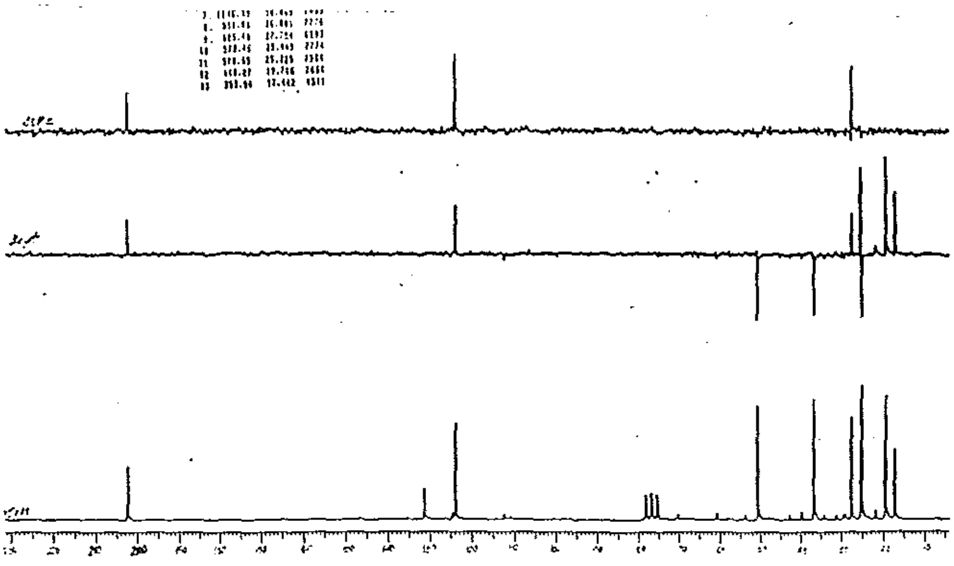

Fig. 3.4 ^{13}C NMR spectrum of crude oil of *E. citriodora* leaves confirming identity of the major component as citronellal (5).

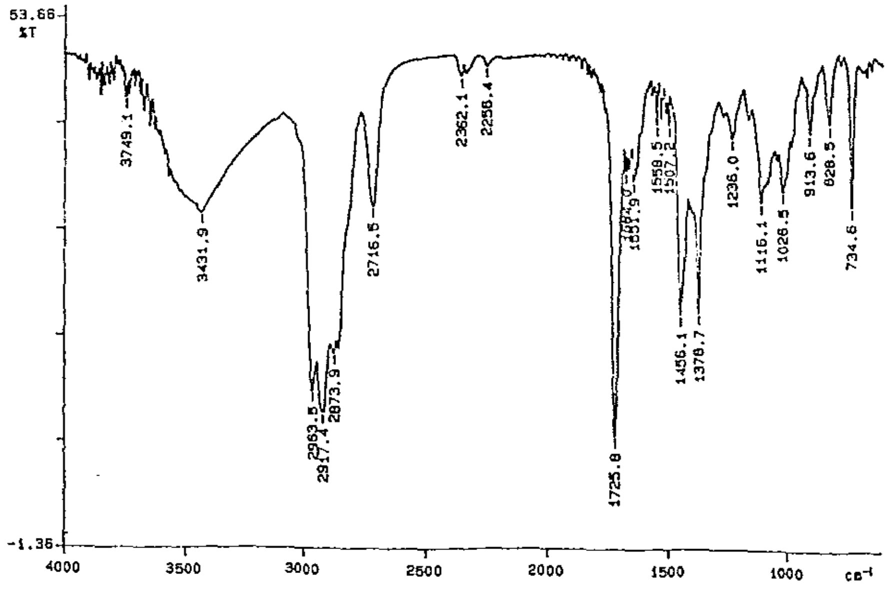

Fig. 3.5 IR spectrum of oil of *E. citriodora* leaves

3.2.1 Isolation and purification of citronellal (5)

Oil of *E. citriodora* leaves was applied on a column of silica gel and eluted with hexane and mixtures of hexane and EtOAc of increasing polarity and about 10 ml fractions were collected. The hexane fraction gave one major peak and a few minor peaks on GC. The retention time of the major peak was identical with that of citronellal (**5**). The yield obtained after evaporation of hexane was 69% from the oil and its purity was 87% on GC. To increase its purity this fraction was again applied on a column of silica gel and eluted with hexane and about 50 ml fractions were collected. Fractions 4, 5 and 6 gave citronellal in 93%, 98% and 99% purity on GC, respectively.

Other minor components of the oil were identified by their retention times and the identity of each peak was established by co-injection with reference compound. The identified components of the essential oil of leaves of *E. citriodora* are given in Table 3.7.

Table 3.7 : Identified components and percentage composition of the essential oil of *E. citriodora* leaves

Peak no.	Compounds	Retention time/min.	% of oil	Method of identification
1	Myrcene (**17**)	7.6	0.1	GLC
2	1,8-Cineole (**4**)	9.1	trace	GLC
3	Linalool (**16**)	11.1	0.2	GLC
4	Citronellal (**5**)	12.9	76.0	GLC, NMR
5	Citronellol (**7**)	15.2	7.0	GLC

Citronellal (**5**) in the oil of *E. citriodora* was found to be readily reduced to citronellol (**7**) with $NaBH_4$. This gave an oil in which citronellol (**7**) is a major component. The aroma of the oil was completely changed. The oil obtained by reduction of citronellal (**5**) to citronellol (**7**) has a some what milder and sweeter aroma than the original oil.

Citronellal (**5**) in the oil of *E. citriodora* leaves was also oxidized to citronellic acid (**6**) with silver oxide, which was prepared in situ from silver nitrate in alkaline medium [57]. The aroma of the oil obtained was compared to the original oil. It has less aroma.

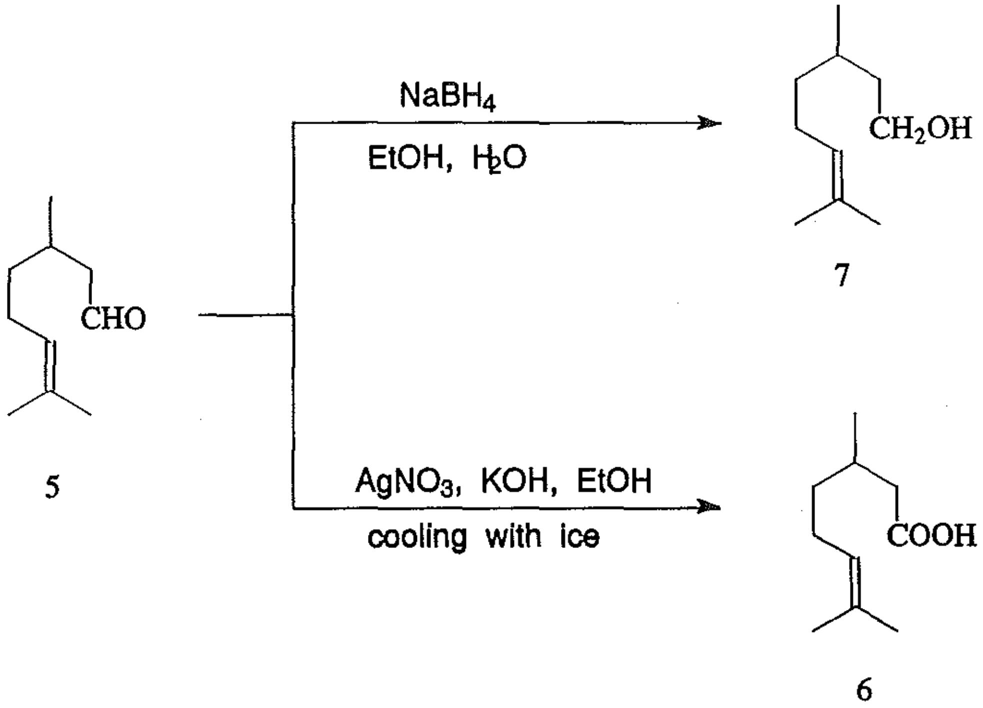

Table 3.8: The ^{13}C NMR data of citronellol (**7**) and citronellic acid (**6**).

C	7	6
1	60.7	179.3
2	39.7	41.5
3	29.3	29.7
4	25.6	25.8
5	37.1	36.7
6	124.8	124.4
7	130.8	131.4
8	19.6	19.8
7-CH_3	25.2	25.3
3-CH_3	17.1	17.3

The ^{13}C and ^{1}H NMR of the oils in which citronellal (**5**) was reduced and oxidized were found to be similar with the ^{13}C and

^{1}H NMR of citronellol (**7**) and citronellic acid (**6**) respectively [9, 69]. The ^{13}C NMR data of the oils in which citronellal (**5**) reduced and oxidized are given in Table 3.8.

The essential oil derived from leaves of *E. citriodora* is rich in citronellal (**5**). Therefore, this oil could be used in perfumery, for scenting of soaps and as source of citronellal (**5**).

3.3 *E. camaldulensis* leaves oil

The yellow coloured essential oil of *E. camaldulensis* leaves was obtained by steam distillation. The yield and physicochemical properties of the oil are given in Table 3.1 and 3.2. The GLC analysis of the oil showed the presence of 40 components (see fig. 3.6). There are two major components (33% and 22%). Early investigations of essential oil of *E. camaldulensis* leaves showed that the chemical composition of the oil could be affected by geographical location in which the plant grows. For example, the major component of the essential oil of *E. camldulensis* leaves from Turkey [33], Morocco [14, 36] and Nepal [37] was reported as 1,8-cineole (**4**), but the major component of the oil obtained from leaves of *E. camaldulensis* grown in India [35], Spain [34] and South Africa [15] was *p*-cymene (**10**). In this study, the major component of the essential oil derived from leaves of Ethiopian *E. camaldulensis* was identified as *p*-cymene (**10**) (33%).

3.3.1 Isolation and characterization of *p*-cymene (10)

Oil of *E. camaldulensis* leaves was applied on a silica gel column and eluted successively with hexane and EtOAc to obtain hydrocarbon and oxygenated fractions respectively. Both fractions were subjected to GLC analysis. The gas chromatogram of the hexane fraction showed the presence of one major peak (73%) and this major peak has similar retention time with the major component of the crude oil. This component was identified as *p*-cymene (**10**) from the ^{1}H and ^{13}C NMR data of the hexane fraction of the oil.

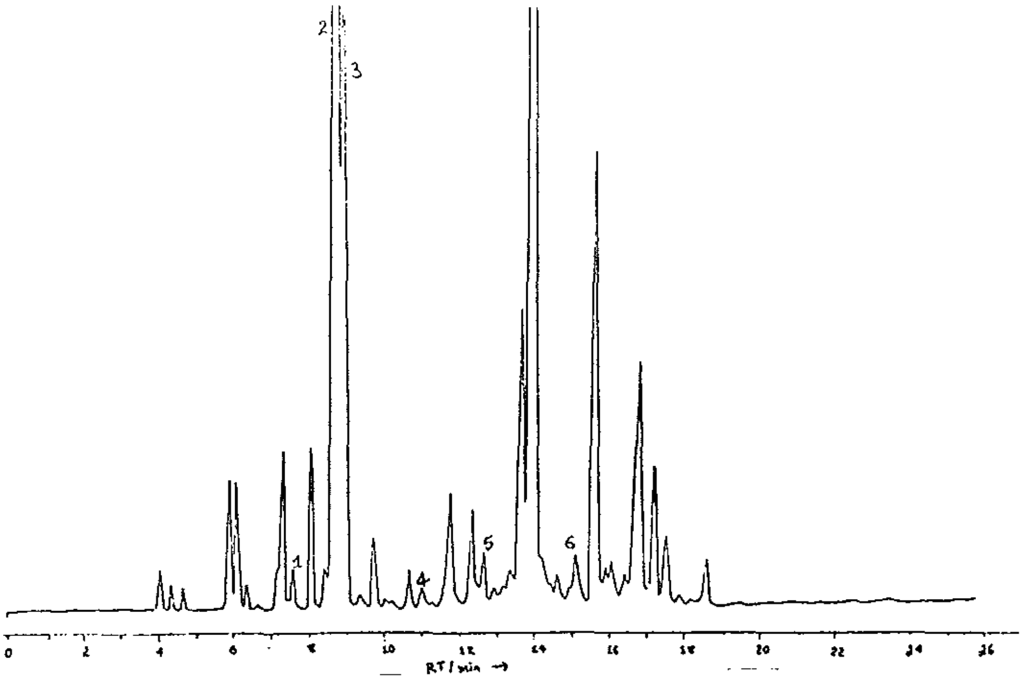

Fig. 3.6 Gas chromatogram of oil of *E. camaldulensis* leaves: 1, Myrcene; 2, *p*-Cymene; 3, 1,8-Cineole; 4, Linalool; 5, Citronellal; 6, Citronellol

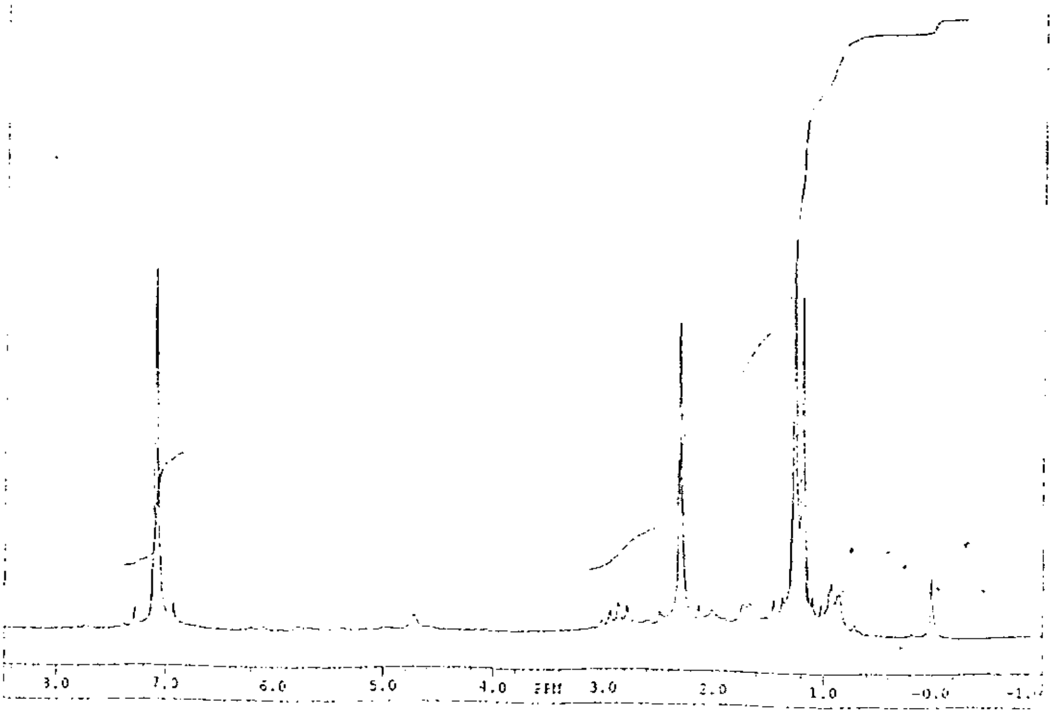

Fig. 3.7 ^{1}H NMR spectrum of p-cymene (**10**) isolated from *E. camaldulensis* leaves oil

The ^{1}H NMR of the hexane fraction of the oil revealed the presence of four aromatic protons (δ 7, s, H-2, H-3, H-5, H-6), one methyl group on aromatic ring (δ 2.3, s, H-7) and other two equivalent methyl groups due to symmetry (δ 1.25, d, H-9, H-10) (see Fig. 3.7). The ^{13}C NMR spectrum of the oil showed the presence of two equivalent methyl groups at δ 24.1, another methyl group at δ 20.9, two pairs of equivalent aromatic carbons at δ 126.0 and 128.0 and two quaternary aromatic carbons at δ 135.0 and 145.0. The ^{13}C NMR data of this fraction was found to be similar with the ^{13}C NMR data of *p*-cymene (**10**) in the literature [9]. It is given in Table 3.9.

Table 3.9: ^{13}C NMR chemical shift assignment for *p*-cymene (**10**) (22.5 MHz, $CDCl_3$)

C	δ PPM
1	135.0
2	126.0
3	128.0
4	145.0
5	128.0
6	126.0
7	20.9
8	33.7
9	24.1
10	24.1

Some other minor components of the oil were identified by adding small amount of the pure compounds and run a new chromatogram. Peak enhancement was then regarded as conformation of the identity of the compounds. The identified components of the oil are given in Table 3.10.

Table 3.10 : Identified components of *E. camaldulensis* leaves oil

Peak no.	Compounds	retention time/min.	% of oil	Method of identification
1	Myrcene (**17**)	7.7	0.3	GLC
2	*p*-cymene (**10**)	8.8	33.0	NMR
3	1,8-cineole (**4**)	9.0	7.0	GLC
4	Linalool (**16**)	11.0	0.2	GLC
5	Citronellal (**5**)	12.7	0.5	GLC
6	Citronellol (**7**)	15.1	0.6	GLC

3.4 *E. tereticornis* leaves oil

The essential oil of leaves of *E. tereticornis* from an old tree was obtained by steam distillation at atmospheric pressure. It has light yellow colour . The yield and physicochemical properties of the oil are given in Table 3.1 and 3.2. The chromatogram of the oil obtained by running on DB-5 30 m x 0.23 mm fused silica capillary column showed 30 components (see Fig. 3.8). Four of them are major components (33%, 27%, 12%, and 10%). The ^{13}C NMR and ^{1}H NMR spectra of the crude oil were a little bit complicated and it was not possible to identify the major component of this oil from these data.

The major component of the oil was identified as 1,8-cineole (**4**) by its retention times and co-injection with pure compound. The identified components of the oil are given in Table 3.11

Table 3.11: Identified components of the essential oil of *E. tereticornis* leaves

Peak. no.	Compounds	Retention time/min.	% of oil	Method of identification
1	Myrcene (**17**)	7.7	1.0	GlC
2	1,8-cineole (**4**)	9.1	33.0	GLC

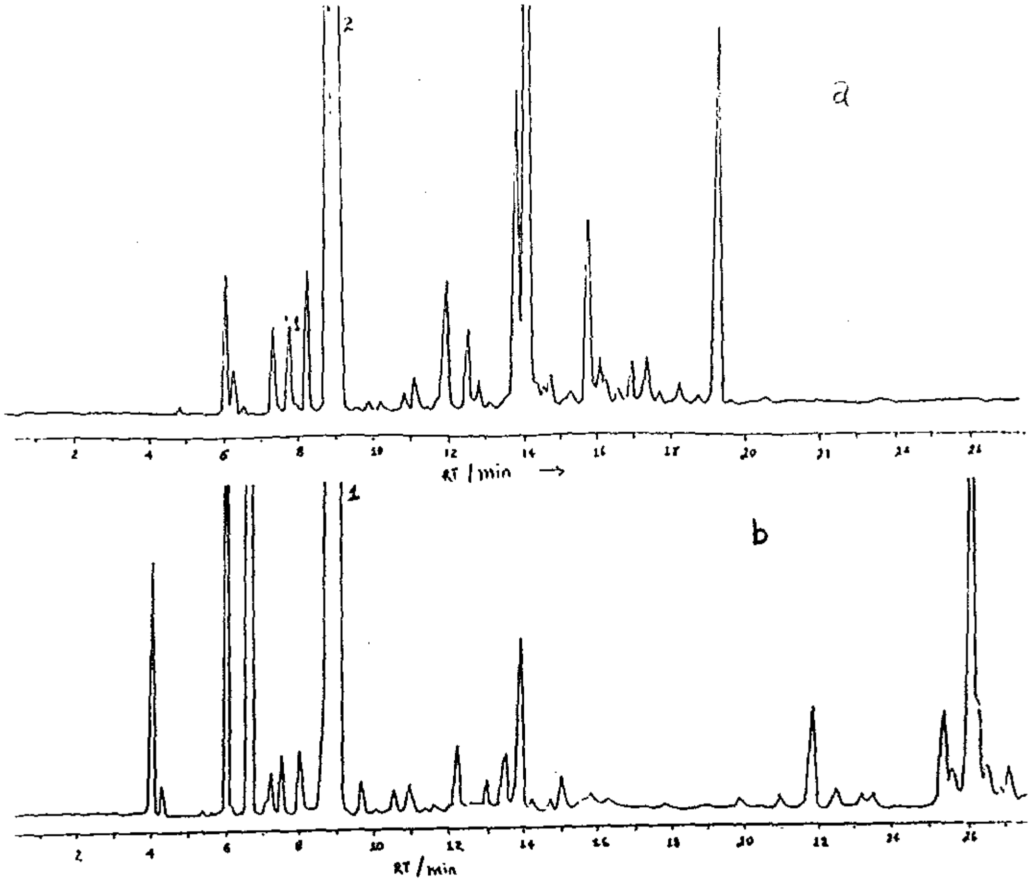

Fig. 3.8 a) Gas chromatogram of oil of *E. tereticornis* leaves: 1, Myrcene; 2, 1,8-Cineole
b) Gas chromatogram of oil of *E. viminalis* leaves: 1, 1,8-cineole

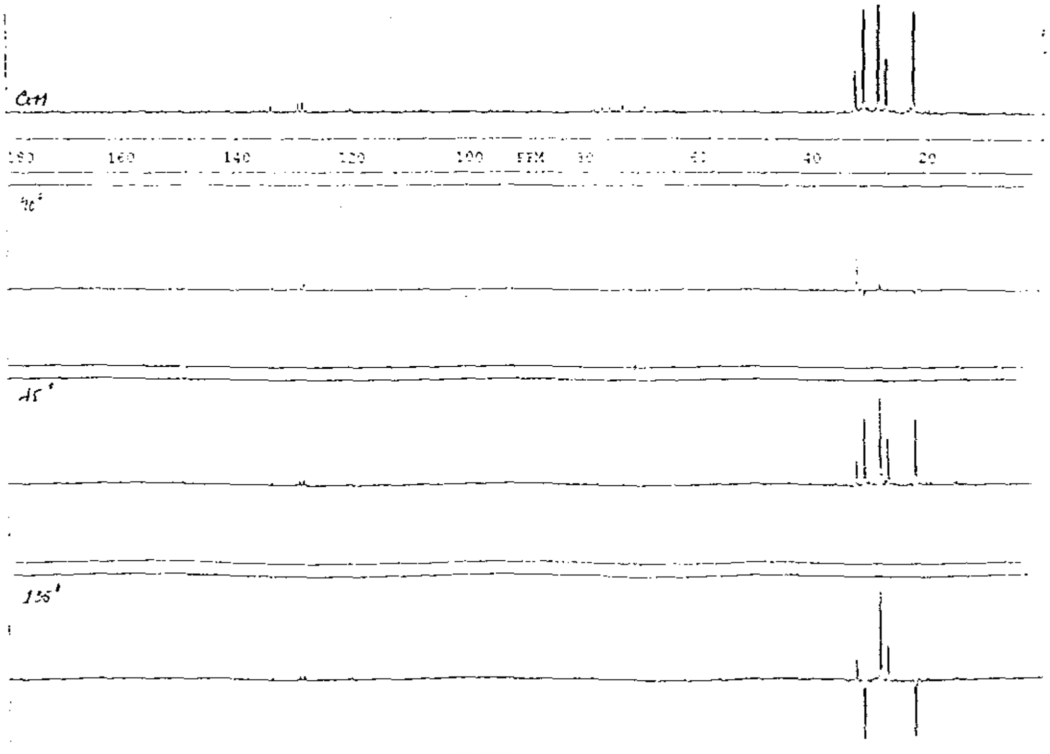

Fig. 3.9 ^{13}C NMR spectrum of crude oil of *E. viminalis* leaves confirming identity of the major component as 1,8-cineole (4).

3.5 *E. viminalis* leaves oil

The oil of *E. viminalis* leaves was obtained by steam distillation. It has light yellow colour. The yield and physicochemical properties of the oil are given in Tables 3.1 and 3.2, respectively. The GLC analysis of this oil revealed the presence of 35 components (see Fig. 3.8). There is only one major component which comprises 73% of the oil. This component was identified as 1,8-cineole (**4**) from the ^{13}C and ^{1}H NMR data of the crude oil. The ^{13}C NMR data of the crude oil of *E. viminalis* leaves is in good agreement with ^{13}C NMR data for 1,8-cineole (**4**) in the literature [9]. It is given in Table 3.12 (see Fig. 3.9).

E. viminalis leaves possessed essential oil rich in 1,8-cineole (>70%). Therefore, it could be used as a source of medicinal *Eucalyptus* oil. This oil also could be redistilled in order to improve its 1,8-cineole content and make its industrial use possible.

Table 3.12: ^{13}C NMR data of crude oil of *E. viminalis* leaves (22.5 MHz, $CDCl_3$) indicating 1,8-cineole (**4**) to be the major component. See also Fig. 3.9

C	δ ppm
1	69.4
2	31.2
3	22.5
4	32.6
5	22.5
6	31.2
7	27.2
8	73.3
9	28.5
10	28.5

3.6 *E. robusta* leaves oil

This oil was obtained from leaves of *E. robusta* by steam distillation . It has yellow colour. The yield and physicochemical properties of the oil are given in Tables 3.1 and 3.2, respectively. The gas chromatogram obtained by running the crude oil of *E. robusta* leaves on DB-5 30 m x 0.23 mm fused silica capillary column showed 25 components (see Fig. 3.10). There is only one major component (53%). The ^1H NMR data of the crude oil was found to be similar with the literature for α-pinene (**23**) [69]. The ^{13}C NMR data of the crude oil also in good agreement with the ^{13}C NMR data of α-pinene (**23**) [7,9].

The ^1H NMR of the crude oil of *E. robusta* leaves showed two singlets of three protons each at δ 0.94 and 1.4 assignable to two methyl groups and multiplet of one proton at δ 5.3 due to the olefinic proton. The quartet at δ 1.8 was assigned to the vinyl methyl group. ^{13}C NMR spectrum of the oil showed the presence of olefinic carbons at δ 115.8 and δ 144.1. The DEPT spectrum revealed the presence of two quaternary carbons, three methine (CH), two methylene groups (CH_2) and three methyl groups (CH_3). One of the quaternary carbons was double bonded quaternary carbon (see Fig. 3.11). From these data the major component of the oil could be identified as α-pinene (**23**). This is in agreement with early finding [15]. The ^{13}C NMR spectral data of the crude oil is given in Table 3.13.

α-Pinene (**23**) is important intermediate in the manufacturing of synthetic aroma compounds and flavouring ingredients [71]. Therefore, the essential oil of leaves of *E. robusta* could be used as a source of this important monoterpenoid hydrocarbon.

Table 3.13 : ^{13}C NMR data of crude oil of *E. robusta* leaves (22.5 MHz, $CDCl_3$) indicating α-pinene (**23**) to be the major component. See also Fig. 3.11

C	δ ppm
1	46.5
2	144.1
3	115.8
4	31.3
5	40.5
6	31.2
7	37.5
8	26.1
9	22.7
10	20.5

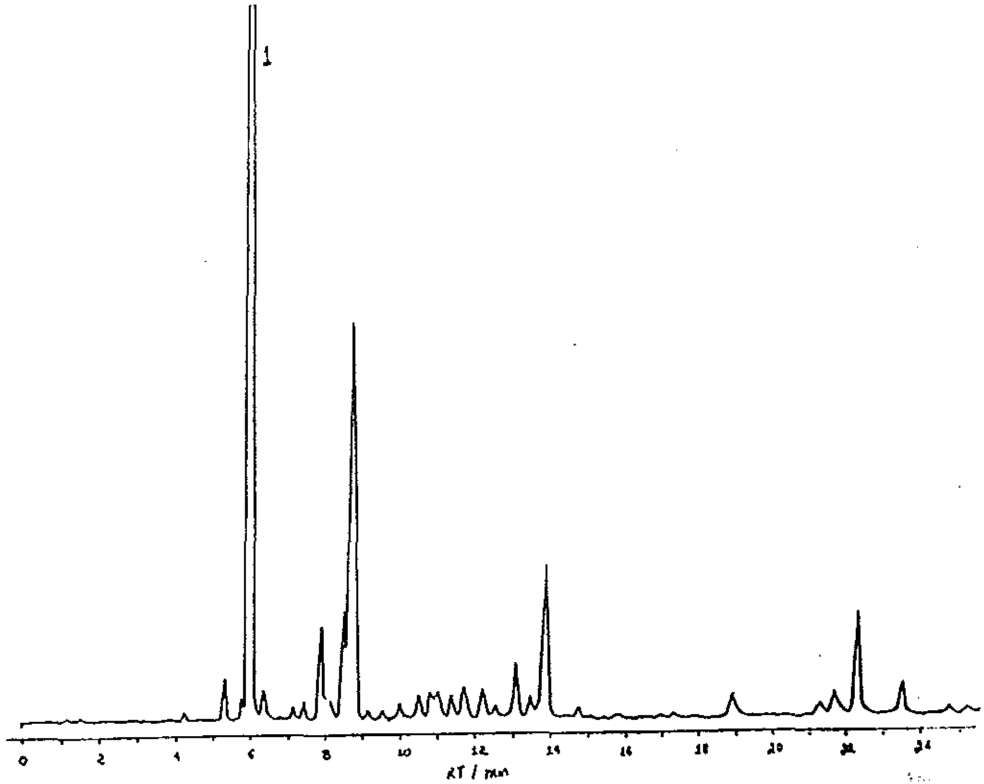

Fig. 3.10 Gas chromatogram of oil of *E. robusta* leaves: 1, α-Pinene

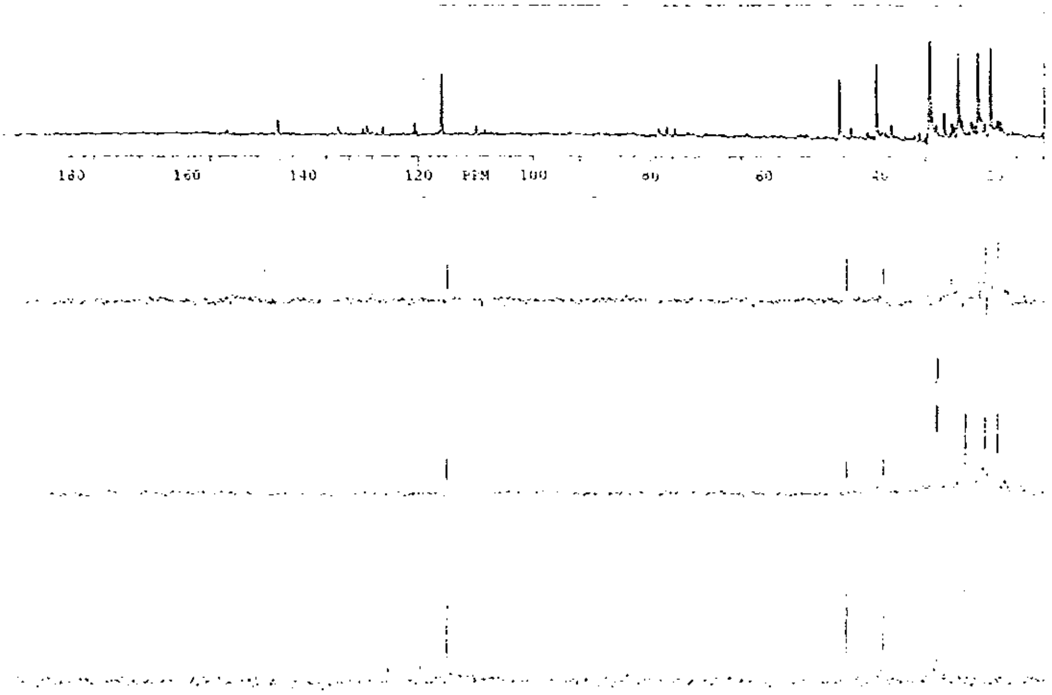

Fig. 3.11 ^{13}C NMR spectrum of crude oil of *E. robusta* leaves confirming identity of the major component as α-pinene (23).

3.7 Oils of fruits of *E. globulus* and *E. citriodora*

The essential oil of fruits of *E. globulus* was obtained by steam distillation at atmospheric pressure. It has yellow colour. The yield and physicochemical properties of the oil can be seen in Tables 3.1 and 3.2, respectively.

The gas chromatogram of the oil showed the presence of about five major (23%, 17%, 15%, 9% and 5%) and 35 minor components (Fig. 3.12). The gas chromatogram of the oil derived from fruits of *E. globulus* was completely different from that of the oil derived from leaves of *E. globulus*. Identification of the major component of the oil from ^1H and ^{13}C NMR data of the crude oil was not possible due to the presence of more than one major components.

3.7.1 Isolation and characterization of aromadendrene (35)

The crude oil of fruits of *E. globulus* was applied on a column of silica gel and eluted with hexane, hexane:EtOAc (7:3), and hexane:EtOAc (1:1). Two fractions for each solvent system and about 50 ml fractions were collected. The first hexane fraction was concentrated and gave one major peak and four minor peaks. The major peak has retention time similar to that of the major component of the crude oil. This component was identified as aromadendrene (**35**) from its ^1H and ^{13}C NMR.

The ^{13}C NMR showed the presence of fifteen carbon atoms indicating the compound to be sesquiterpenoid. DEPT spectrum indicated that there were two quaternary carbons and five methine (CH), five methylene (CH_2) and three methyl (CH_3) groups (see Fig. 3.13). One of the methylene groups was exomethylene and one of the quaternary carbons was double bonded quaternary carbon. The ^1H NMR data of this fraction was found to be similar with the literature for aromadendrene (**35**) [20]. The ^{13}C NMR spectral data assignment for aromadendrene (**35**) is given in Table 3.14.

Table 3.14 : ^{13}C NMR chemical shift assignment for aromadendrene (**35**) (22.5 MHz, $CDCl_3$)

C	δ PPM
1	53.7
2	35.2^b
3	29.5^b
4	35.4
5	44.4
6	29.2^a
7	27.6^a
8	24.9^b
9	39.1^b
10	154.7
11	20.0
12	17.1^c
13	15.7^c
14	105.3
15	28.7

[a,b,c] : Assignments with the same superscript may be interchangeable.

1,8-cineole (**4**) was the second major component of the oil. It was identified by its retention time and co-injection with standard compound.

Table 3.15 : Identified components of essential oil of fruits of *E. globulus*.

peak no.	Compounds	retention time/min	% of oil	Method of identification
1	1,8-Cineole (**4**)	9.0	16	GLC
2	Aromadendrene (**35**)	22.0	23	NMR

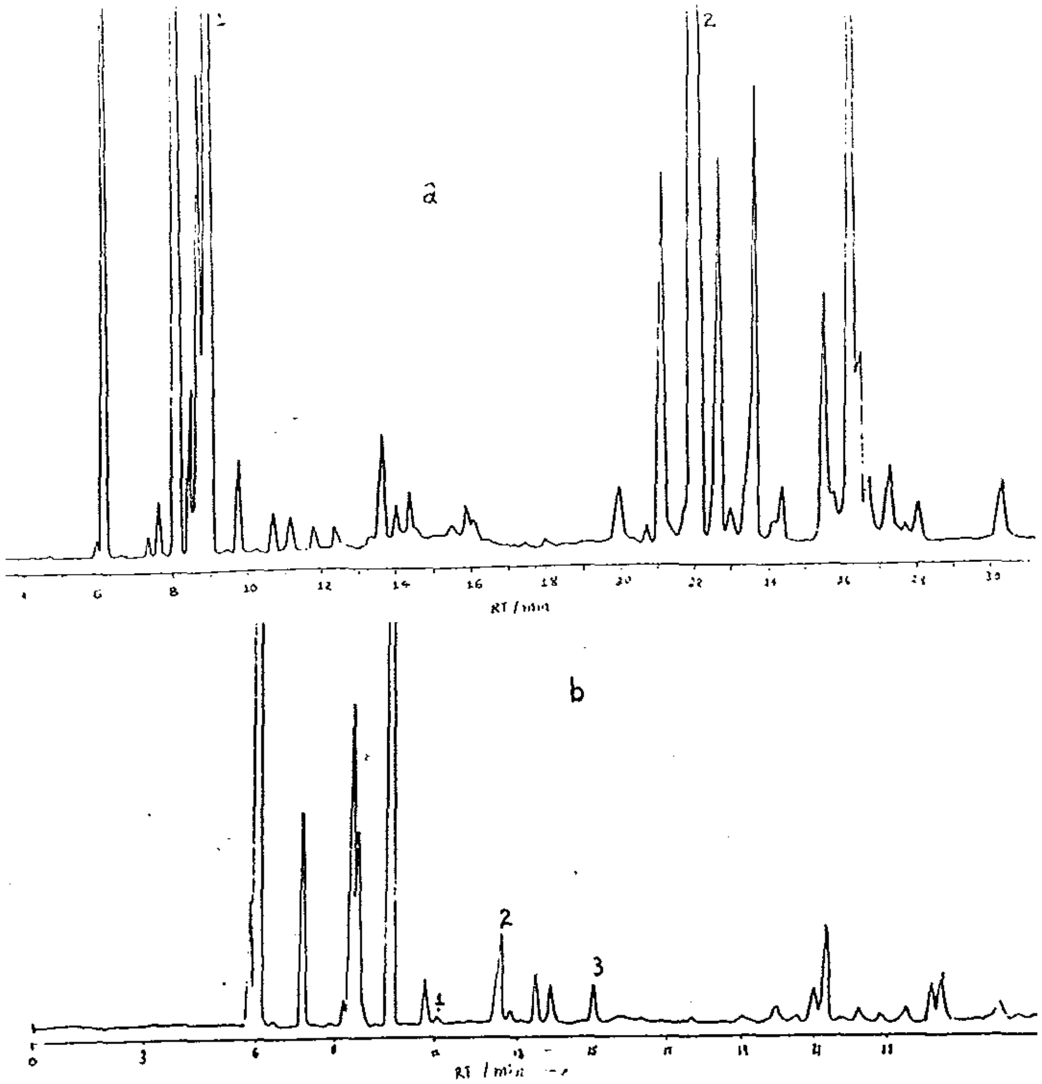

Fig. 3.12 a) Gas chromatogram of oil of *E. globulus* fruits: 1, 1,8-cineole; 2, aromadendrene.

b) Gas chromatogram of oil of *E. citriodora* fruits: 1, Linalool; 2, Citronellal; 3, Citronellol

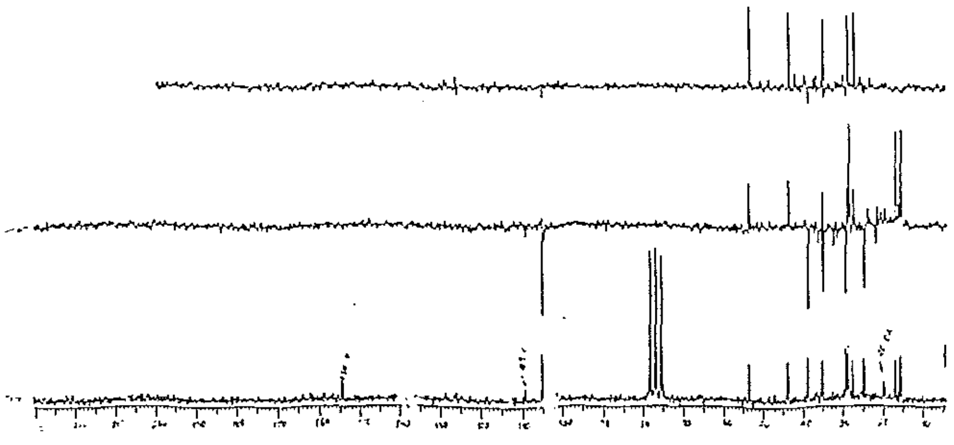

Fig. 3.13 ^{13}C NMR spectrum of aromadendrene (10) obtained from *E. globulus* fruits oil

The essential oil from fruits of *E.citriodora* was also obtained by steam distillation. The oil yield of *E. citriodora* fruits is very small. Fruits of *E. citriodora* with a yield of 0.2% could never be a useful and economical source of essential oil unless it was the source of a very rare and important constituent. The gas chromatogram of the oil showed 30 components (Fig. 3.12). There are two major components (50% and 19%0). The gas chromatogram of the oil derived from fruits of *E. citriodora* was completely different from that of the oil derived from leaves of *E. citriodora*.

Some minor components of the oil were identified by their retention times and co-injection with pure compounds. The identified components of *E. citriodora* fruits oil are given in Table 3.16.

Table 3.16: Identified components of *E.citriodora* fruits oil.

Peak no.	Compounds	Retention time/min	% of oil	Method of identification
1	Linalool (**16**)	11.01	0.1	GLC
2	Citronellal (**5**)	12.73	2.0	GLC
3	Citronellol (**7**)	15.21	0.7	GLC

4. EXPERIMENTAL

4.1 Plant materials

- Leaves of *E. globulus* from young plant were collected on Sep. 26, 1994 from Addis Ababa (Yeka hill);voucher no. Abera A. S-697
- Leaves of *E. citriodora* were collected from Wondo Genet EORC farm site On oct. 14, 1994; Voucher no. Abera A. S-698
- Leaves of *E. tereticornis* were collected from Arat Killo science campus on Oct 25, 1994; Voucher no. Abera A. S-760
- Leaves and fruits of *E. globulus* from an old tree were collected on Nov. 8, 1994 from Arat Killo science campus; voucher no. Abera A. S-767
- **Leaves of *E. robusta, E. viminalis* and *E. camaldulensis* were collected from Wondo Genet forestry college Herbarium site on Jan. 10, 1995; voucher no. Abera A. S-806, S-807 and S-808 respectively.**
- Fruits of *E. citriodora* were collected from Wondo Genet EORC farm site on Jan. 10, 1995; voucher no. Abera A. S-809.

All voucher specimens were identified by Dr. Sebesebe Demissew and deposited at National Herbarium of Addis Abeba University under the voucher numbers indicated above.

4.2 General

The essential oils from the plant materials were obtained by steam distillation at atmospheric pressure using 4 lt round bottom flask fitted with clevenger apparatus and a glass condenser. Distillation was continued until no more oil was collected. The oils were separated from the distillate and dried over anhydrous sodium sulphate. Neat optical rotations were measured using P20 polarimeter (code no. 30-01). Refractive indices were determined on Abbe's refractometer. Specific gravity of the oils was determined using a pycnometer.

NMR spectra were obtained in $CDCl_3$ on Jeol FX 90 Q spectrophotometer and δ values are given in ppm relative to TMS internal standard. Neat FTIR spectra of the essential oils were recorded using the Perkin Elmer spectrometer model 1600 with NaCl cell.

Analytical TLC were run on a 0.25 mm thick layer of silica gel GF_{254} (Merck). Components were detected by their UV fluorescence and 1% vanillin H_2SO_4 spray reagent. Flash column chromatography was performed using silica gel 60 (230-400 mesh) Merck. Gas chromatographic analysis of the oil, fractions and standard samples was performed on Varian model 3700 gas chromatograph equipped with flame ionization detector (FID) using DB-5 fused silica capillary column with 30 m x 0.23 mm internal diameter and 1.5 micron film thickness. Analyses were performed by programming the column temperature 60 °C to 200 °C at 4 °C/min. Injector and detector temperatures were 220 °C and 270 °C respectively. N_2 at 25 ml/min was used as a carrier gas. Peak areas were determined with the aid of a Varian 4290 integrator. Samples of 0.02 μl were injected.

4.3 Oil of *E. globulus* leaves (young plant)

Leaves of *E. globulus* obtained on Sep. 26, 1994 from Addis Ababa (Yeka Hill) was cut to suitable size to put into 4 lt round bottom flask and steam distilled for 3 hr.This gave yellow oil in 1.1% yield.

Yellow oil: Specific gravity (d^{20}) 0.9207, Refractive index (n^{20}) 1.4624, Optical rotation $[\alpha]^{20}$ +3.9°. IR ν_{max} cm^{-1} 2967, 1646, 1465,1375, 1360, 1215, 1168, 1079, 985, 843; ^1H NMR (90 MHz, $CDCl_3$) : δ 1.0 (3H, s, H-7), 1.2 (6H, s, H-9, H-10), 1.3-2.2 (H-2, H-3, H-4, H-5, H-6); ^{13}C NMR (22.5 MHz, $CDCl_3$) : see Table 3.3.

4.4 Oil of *E. globulus* leaves (old plant)

Fresh leaves of *E. globulus* from an old plant were collected on Nov. 8, 1994 from Addis Ababa (Arat Killo science campus) and subjected to steam distillation for 3 hr. This gave essential oil in 1.2% yield.

Yellow oil : Specific gravity (d^{20}) 0.9130, Refractive index (n^{20}), Optical rotation $[\alpha]^{20}$ +3.9°; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.0 (3H, s, H-7), 1.2 (6H, s, H-9, H-10) 1.3-2.2 (H-2, H-3, H-5, H-6); ^{13}C NMR (22.5 MHz, $CDCl_3$) : see Table 3.4.

4.5 Oil of *E. citriodora* leaves

Fresh leaves of *E. citriodora* were collected from Wondo Genet Essential oils Research Centre (EORC) farm site on Oct. 14, 1994 and steam distilled for 3 hr. This gave essential oil in 1.4% yield.

Light yellow oil : Specific gravity (d^{20}) 0.8618, Refractive index (n^{20}) 1.4525, Optical rotation $[\alpha^{20}]$; IR ν_{max} cm^{-1} 2963, 2917, 2873, 2716, 1725, 1456, 734; ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.96 (3H, d, CH_3-3), 1.2-1.5 (H-4, H-3), 1.6 (3H, s, H-8), 1.7 (3H, s, CH_3-7), 1.9-2.4 (H-2, H-5), 5.1 (1H, m, H-6), 9.75 (1H, t, H-1); ^{13}C NMR (22.5 MHz, $CDCl_3$): See Table 3.6.

4.5.1 Isolation of citronellal (5)

500 mg oil of *E. citriodora* leaves was applied on a column of silica gel (7.5 g) and eluted with hexane-EtOAc mixtures of increasing polarity. 22 fractions each 10 ml were collected. Fraction 3-10 were combined based on TLC and gave one major peak (87%) on GC. The peak was identified as citronellal by its retention time. This fraction (300 mg) was further chromatographed on silica gel eluting with hexane. About 50 ml fractions were collected. Fractions 4, 5 and 6 gave citronellal in 93%, 98% and 99% purity on GC, respectively.

4.5.2 Reduction of citronellal (5) in *E. citriodora* leaves oil

1 g of oil of *E. citriodora* leaves was dissolved in 50 ml of EtOH. The solution was stirred magnetically at room

temperature and 0.07 g of sodium borohydried was added to the solution in small portions. The mixture was allowed to be stirred at room temperature over night and then it was poured into 250 Erlenmeyer flask containing 50 ml of ice water. 10 ml of dilute hydrochloric acid was added to the mixture slowly and stirred for 1 hr. The product was extracted with 50 ml diethyl ether. The ether extract was dried over anhydrous sodium sulphate and concentrated on rota vapour. The oil product was analyzed using NMR.

> ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.89 (3H, d, CH_3-3), 1-1.8 (H-2, H-3, H-4), 1.6 (3H, s, H-8), 1.68 (3H, s, CH_3-7), 1.9 (2H, m, H-5), 3.67 (1H, t, OH), 5.08 (1H, m, H-6); ^{13}C NMR (22.5 MHz, $CDCl_3$): See Table 3.8.

4.5.3 Oxidation of citronellal (5) in *E. citriodora* leaves oil

To a solution of 1.63 g of $AgNO_3$ in 3 ml of water a solution of 0.5 g of *E. citriodora* leaves oil in 3 ml of EtOH was added followed by a solution of 1.092g of KOH in 3 ml of water and the mixture was stirred intensively. After the mixture had been stirred at room temperature for 2.5 hr the deposited silver was filtered and the filtrate was cooled and acidified with dilute sulphuric acid. The mixture was extracted with diethylether. The ether extract was dried over sodium sulphate anhydrous and concentrated. The oil product obtained was analyzed using NMR.

> ^{1}H NMR (90 MHz, $CDCl_3$): 0.96 (3H, d, CH_3-3), 1.6 (3H, s, H-8), 1.68 (3H, s, CH_3-7), 1.1-2.9 (H-2, H-3, H-4, H-5), 5.09 (1H, m, H-6), 9.07 (1H, br, COOH); ^{13}C NMR (222.5 MHz, $CDCl_3$): see Table 3.8.

4.6 Oil of *E. camaldulensis* leaves

Fresh leaves of *E. camaldulensis* obtained from Wondo Genet forestry college campus was steam distilled for 3 hr. This gave essential oil in 0.7% yield.

Yellow oil : Specific gravity (d^{20}) 0.9049, Refractive index (n^{20}) 1.4904, Optical rotation $[\alpha]^{20}$ +28°.

4.6.1 Isolation of *p*-cymene (10)

1.5 g of oil of *E. camaldulensis* leaves was applied on a column of silica gel (32 g) and eluted successively with hexane and EtOAc. The hexane fraction exhibited one major peak (73%) and a few minor peaks on GC. The retention time of the major peak was identical with the retention time of the major peak of the crude oil and this major component of the oil was identified as *p*-cymene (**10**) based on its ^{1}H and ^{13}C NMR.

p-Cymene (**10**) : ^{1}H NMR (90 MHz, $CHCl_3$): δ 1.25 (6H, d, H-9, H-10), 2.3 (3H, s, H-7), 2.8 (1H, m, H-8), 7.0 (4H, s, H-2, H-3, H-5, H-6); ^{13}C NMR (22.5 MHz, $CHCL_3$): See Table 3.9

4.7 Oil of *E. tereticornis* leaves

Fresh leaves of *E. tereticornis* were collected from Addis Ababa (Arat Killo science campus) on Oct. 25, 1994 and steam distilled for 3 hr. This gave essential oil in 1.1% yield.

Yellow oil : Specific Gravity (d^{20}) 0.8827, Refractive index (n^{20}) 1.4844, Optical rotation $[\alpha]^{20}$ -32°.

4.8 Oil of *E. viminalis* leaves

Fresh leaves of *E. viminalis* were collected from Wondo Genet forestry college campus and steam distilled for 3 hr. This gave essential oil in 0.7% yield.

Light yellow oil: Specific gravity (d^{20}) 0.9122, Refractive index (n^{20}) 1.4691, Optical rotation $[\alpha]^{20}$ +0.5°, IR ν_{max} cm^{-1} 2960, 2923, 1724, 1463, 1378, 1216, 1163, 1080, 1050, 983, 886; ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.0 (3H, s, H-7), 1.2 (6H, s, H-9, H-10), 1.3-2.2 (H-2, H-3, H-4, H-5, H-6); ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 3.12.

4.9 Oil of *E. robusta* leaves

Fresh leaves of *E. robusta* obtained on Nov. 8, 1994 from Wondo Genet forestry college campus was steam distilled for 3 hr. This gave essential oil in 0.5% yield.

Light yellow oil: Refractive index (n^{20}) 1.4711; IR ν_{max} cm^{-1} 2967, 2925, 1706, 1464, 1375, 1215, 1167, 1079, 1053, 985, 843; ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.94 (3H, s, H-9), 1.4 (3H, s, H-8), 1.8 (3H, q, H-10), 1.9-2.6 (H-4, H-5, H-6), 5.3 (1H, m, H-3); ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 3.13

4.10 Oil of *E. globulus* fruits

Fruits of *E. globulus* obtained on Nov. 8, 1994 from Addis Ababa (Arat Killo science campus) was ground and steam distilled for 4 hr. This gave oil in 0.6% yield

Yellow oil : Specific gravity (d^{20}) 0.8930, Refractive index (n^{20}) 1.4836, Optical rotation $[\alpha]^{20}$ -33°;

4.10.1 Isolation of aromadendrene (35)

The oil of fruits of *E. globulus* (502 mg) was run on column of silica gel. This was eluted successively with hexane, hexane-EtOAc (7:3) and hexane-EtOAc (1:1). Two fractions for each solvent system and about 50 ml frictions were collected. The first fraction gave a major component and other fractions exhibited several peaks on GLC. The major component was analyzed using NMR and was identified as aromadendrene (**35**).

Aromadendrene (**35**) : ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.5-0.76 (2H, m, H-6, H-7), 0.95 (3H, d, H-15), 0.98 (3H, s, H-12), 1.04 (3H, s, H-13), 1.1-2.5 (11H, m, H-1, 2, 3, 4, 5, 8, 9) 4.6 (2H, m, H-14); ^{13}C NMR (22.5 MHz, $CDCL_3$): see Table 3.14.

4.11 Oil of *E. citriodora* fruits

Fruit of *E. citriodora* obtained from Wondo Genet on Nov. 8, 1994 was ground and steam distilled for 3 hr. This gave oil in 0.2% yield.

Colourless oil : Refractive index (n^{20}) 1.4725.

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