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Chemistry, insecticidal and insect neurophysiological activity of some essential oils from Sri Lanka with emphasis on *Piper betle* L. (Piperaceae) leaf oil

A Thesis Submitted for the Doctor of Philosophy to the Faculty of Sciences of the University of Neuchâtel

By

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IMPRIMATUR POUR LA THESE

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autorise l'impression de la présente thèse.

Neuchâtel, le 21 octobre 2002

Le doyen:

F. Zwahlen

To

My wife, Dayani

and our two natural products,

Dhanuddara and Medhani

And

To

My mother and my late father

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Susantha B. Mohottalage

CONTENTS

Part 1: Chemistry and insecticidal activity of *Piper betle* L. leaf oil

Part 2: Chemistry and insect neurophysiological activity of some Sri Lankan essential oils

Page

ABSTRACT

The first part of this thesis describes an investigation on the chemistry and insecticidal activity of the essential oil of *Piper betle* L. leaf oil.

The essential oil of *P. betle* leaf obtained by pilot plant steam distillation was tested against adult *Musca domestica* (housefly) for insecticidal activity. LC_{50} values at the end of 24 and 48 hr exposure period were 10.8 and 9.2 mg/dm^3. Ceylon citronella oil (*Cymbopogon nardus*) used as standard showed LC_{50} of 25.6 and 24.6 mg/dm^3 for the same exposure periods. Bioassay guided fractionation revealed safrole (25) and eugenol (21) as the active principles against *M. domestica,* safrole (25), showing LC_{50} of 4.8 and 4.7 mg/dm^3 and eugenol (21), 6.8 and 6.2 mg/dm^3 for the periods of 24 and 48 hr, respectively, while citronellal (54) (standard) showed equal LC_{50} values of 14.3 mg/dm^3 for the same exposure periods respectively.

Five inactive phenyl propanoids were also isolated from these fractions as methyl eugenol (24), eugenyl acetate (24a), allylpyrocatechol diacetate (16), allylpyrocatechol monoacetate (17) and hydroxychavicol (22). Inactive phenyl propanoids with 3rd and 4th substituent groups attached to the aromatic rings are compared with two active compounds, safrole (25) and eugenol (21) to discuss the reason for particular activity. Since safrole (25) possesses a methylenedioxy moiety, allyl benzene (53) was tested to observe whether this methylenedioxy moiety is essential for the activity.

Using safrole (25) as the starting compound, eight analogues were prepared to study structure activity relationship. Among the 8 analogues, dihydrosafrole (46) gave almost equal mortality of LC_{50} 4.7 mg/dm^3 as that of the parent compound safrole (25) after 24 and 48 hr exposure. But isosafrole (45) was two times more active than safrole (25) showing LC_{50} values of 2.3 and 2.2 mg/dm^3 for the periods of 24 and 48 hr exposure. 3-(3´,4´-methylenedioxyphenyl)-propan-2-on (48) showed a moderate LC_{50} activity of 33.7 and 29.1 mg/dm^3 after 24 and 48 hr exposure periods, respectively.

Comparisons of the isolated and synthesized compounds were done with citronellal (54), which is reported to have insecticidal activity. It was also found that safrole (25) was three times, and isosafrole (45) six times, more active than citronellal (54).

Chemical constituents of pilot plant distillation of Sri Lankan *P. betle* leaf oil and its eight chromatographic fractions were also analyzed by GC-MS. A total of 97 compounds were identified from these fractions. This detailed study is the first report to the Sri Lankan *P. betle* leaf oil.

The second part of the thesis describes the electroantennographic detection of essential oil compounds by the tsetse fly, *Glossina pallidipes* and mosquito, *Aedes aegypti*. Eleven Sri Lankan essential oils were tested on tsetse antenna. Gas chromatography-electroantennogramme detection (GC-EAD) was used to locate active compounds. 35 compounds evoked strong (GC-EAD) responses from tsetse antenna. These compounds were identified by means of mass spectrometry. They were identified as, limonene (30) as a monoterpene hydrocarbon, *cis* pinocarveol (224), isopulegol (185), citronellal (54), borneol (42), terpin-4-ol (73), α-terpineol (1), citral b (neral) (139), piperitone (147), geraniol (37), citral a (geranial) (138), methyl geranate (170), geranyl acetate (141), neryl isovalerate (254) as oxygenated monoterpenes, cyperene (243), γ-caryophyllene (251), β-caryophyllene (27), 2-*epi*-caryophyllene (247), α-gurjunene (174), δ-elemene (87), α-humulene (101), γ-muurolene (104), germacrene D (105) as sesquiterpenes, (*E*)-nerolidol (120), spathulenol (121) as oxygenated sesquiterpenes, safrole (25), eugenol (21), methyl eugenol (24), myristicin (44), elemicin (43), 1,3 diisopropyl benzene (248), *p*-cymen-8-ol (206) as phenolic compounds, 1-octen-3-ol (133a), 1-octen-3-yl-acetate (253) as oxygenated short chain hydrocarbons and 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4. 3. 0]-non-1(6)-ene-2, 4-dione (154) as a bicyclic diketone. Among these 35 active compounds 1-octen-3-ol (133a) and β-caryophyllene (27) appeared to be the best stimulants found from these oils.

Fifteen essential oils were also tested on mosquito (*A. aegypti*) antenna for electroantennogramme activity. They are as follows; *Cymbopogon flexuosus* (lemon grass), *Lantana camara*, *Myristica fragrans* (mace), *C. nardus* (citronella), *Pinus cembra*, *Callistemon citrinus*, *Eucalyptus globulas*, *Myristica fragrans* (nutmeg), *Tagetes erecta*, *Plectranthus zeylanicus*, *Ocimon graticimom*, *P. betle*, *Eupatorium odoratum*, *Hemidesmus indicus*. All the tested essential oils at 1 mg source dose gave significant responses ranging from 0.477 mV to 0.264 mV and the highest response was to the *C. flexuosus* (lemon grass) oil and the lowest to the *H. indicus*. The average response to the blank control was 0.101 mV.

Since the reported data on chemical composition of *L. camara* was different from the samples collected from different geographical areas, eight *L. camara* samples collected from Asia (Sri Lanka), Africa (Kenya) and Europe (Switzerland & France) were also analyzed by GC-MS to investigate the differences of composition. Since β-caryophyllene (27) and 1-octen-3-ol (133a) are present in *L. camara* in considerable amounts, this plant would be a potential plant for resting for tsetse flies.

RESUME

La première partie de ce travail de recherche est consacrée à l'étude chimique ainsi qu'à l'évaluation de l'activité insecticide de l'huile essentielle des feuilles de *Piper betle* L.

L'huile essentielle des feuilles de *Piper betle* obtenue par distillation pilote a été testée sur la mouche *Musca domestica* (mouche des maisons) pour son activité insecticide. Les valeurs LC_{50} pour une période d'exposition de 24 et 48 h sont de 10.8 et 9.2 mg/dm^3. L'huile de citronnelle de Ceylan (*Cymbopogon nardus*) utilisée comme standard a montré une valeur LC_{50} de 25.6 et 24.6 mg/dm^3 pour une même période d'exposition. Le fractionnement biodirigé de l'huile essentielle de *P. betle* a révélé la présence de composés actifs contre *M. domestica*, le safrole (25) et l'eugenol (21), Le safrole (25) a montré une valeur de LC_{50} de 4.8 et 4.7 mg/dm^3 et l'eugenol (21) de 6.8 et 6.2 mg/dm^3 pour une période de 24 et 48 h respectivement, alors que citronellal (54) (standard) n'a montre qu'une valeur égale à 14.3 mg/dm^3 pour une même période d'exposition.

Cinq phenyl-propanoides, n'ayant pas montré une activité spécifique, ont aussi été isolés à partir des fractions étudiées, le methyl eugenol (24), l'acétate d' eugenyle (24a), le monoacetate (17) et diacetate d'allylpyrocatechol (16), et l'hydroxychavicol (22). Ces phenyl-propanoides, substitués en position 3 et 4 du noyau aromatique, ont été comparés aux produits actifs, safrole (25) et eugenol (21), afin d'établir une éventuelle relation structure-activité. Comme le safrole (25) possède en plus un reste dioxyméthylène, l'allyl benzene (53) a été testé pour établir si le groupe dioxyméhylène est essentiel pour l'expression de l'activité.

En utlisant le safrole (25), comme composé de départ, huit composés analogues ont été synthétisés pour étudier la relation structure-activité. Parmi les huit analogues, le dihydrosafrole (46) montre pratiquement la même valeur de mortalité LC_{50}, 4.7 mg/dm^3, que le composé initial, le safrole (25), après 24 et 48 h d'exposition. L'isosafrole (45), par contre, est deux fois plus actif, que le safrole (25), montrant une valeur LC_{50} de 2.3 et 2.2 mg/dm^3 pour une période de

24 et 48 h d'exposition. La 3-(3',4'-methylenedioxyphenyl)-propan-2-one (48) a montré une valeur de LC_{50} modérée de 33.7 et 29.1 mg/dm^3 après 24 et 48 h d'exposition respectivement.

La comparaison des composés isolés et synthétiques a été effectuée par rapport au citronellal (54), connu pour son activité insecticide. Nous avons ainsi mis en évidence que le safrole (25) est trois fois plus actif, et l'isosafrole (45) six fois, plus actif que le citronellal (54).

La composition chimique de l'huile essentielle des feuilles de *P. betle* du Sri Lanka et ses huit fractions chromatographiques ont été aussi analysées par GC-MS. Au total, 97 composés ont été identifiés à partir de ces fractions. Ceci constitue la première étude détaillée de l'huile essentielle de *P. betle* du Sri Lanka.

La deuxième partie de la thèse décrit la détection électro-antennographique de plusieurs huiles essentielles du Sri Lanka contre la mouche tsé-tsé, *Glossina pallidipes*, et le moustique , *Aedes aegypti*. L'electroantennographie couplée à la chomatographie en phase gaseuse (GC-EAD) a été utilisée pour la reconnaissance des produits actifs. 35 composés ont provoqué une forte réponse GC-EAD chez les antennes de tsé-tsé. Ces composés sont essentiellement identifiés par spectrométrie de masse, et sont un hydrocarbure monoterpènique, le limonène (30), des monoterpènes oxygénés : *cis*-pinocarveol (224), isopulegol (185), citronellal (54), borneol (42), terpin-4-ol (73), α-terpineol (1), citral b (neral) (139), piperitone (147), geraniol (37), citral a (geranial) (138), methyl geranate (170), geranyl acetate (141), neryl isovalerate (254) des sequiterpènes: cyperene (243), γ-caryophyllene (27), 2-*épi*-caryophyllène (247), α-gurjunène (174), δ-élemène (87), α-humulène (101), γ-muurolène 8104), germacrène D (105) des sesquiterpènes oxygénés, (*E*)-nerolidol (120), spathulenol (121), des dérivés phénoliques: safrole (25), eugenol (21), methyl eugenol (24), myristicin (44), élémicin (43), 1, 3 diisopropyl benzene (248), *p*-cymen-8-ol (206), 1-octen-3-ol (133a), 1-octen-3-yl-acetate (253) (hydrocarbones oxygéné) à courte chaîne et la 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4.3.0]-non-1(6)-ene-2,4-dione (154) (dicétone bicyclique). Parmi les composés le plus actifs le caryophyllène et le 1-octen-3-ol semblent être les meilleurs stimulants.

Ces mêmes huiles essentielles: *C. flexuosus* (lemon grass), *Lantana camara*, *Myristica fragrans* (mace), *C. nardus* (citronella), *Pinus cembra, Callistemon citrinus, Eucalyptus globules, Myristica fragrans* (nutmeg), *P. betle, Eupatorium odoratum, Hemidesmus indicus,* ont été testées sur les antennes du moustique (*A. aegypti*) pour leur activité EAG. Elles ont montré une réponse significative à la concentration de 1 mg de dose à la source, entre 0.477 mV à 0.264 mV et la plus forte réponse correspondant à l'huile de *C. fexuosus* (lemon grass) et la plus faible celle à *Hemidesmus indicus*. La réponse moyenne comparée au contrôle était de 0.101 mV.

Comme la composition chimique de *L. camara* diffère d'une culture à une autre en fonction du lieu géographique, huit échantillons de *L. camara* ont été étudiés. Ces échantillons ont été récoltés en Asie (Sri Lanka), en Afrique (Kenya), et en Europe (Suisse et France) et ont été analysés et comparés par GC-MS. Puisque le caryophyllène (27) et le 1-octen-3-ol (133a) sont présents dans l'huile de *L. camara,* cette plante pourrait être un bon site de repos pour la mouche tsé-tsé.

PART 1

CHEMISTRY AND INSECTICIDAL ACTIVITY OF *PIPER BETLE* L. LEAF OIL

1. INTRODUCTION

1. 1. Role of plants for insect control

Plants and their derivatives have always been used for insect control by humans. Currently there is an increasing trend to use plant-derived products in pest management. These plant derivatives also offer safe alternative to synthetic pesticides such as organophosphates like malathion, arathion, ethion, deltamethrin, fenthion, carbamates like carbaryl, aldicarb, bendiocarb and chlorinated compounds like acetochlor, bendiocarb, alachlor.

Plants produce a great variety of secondary metabolites such as phenols, alkaloids, and terpenoids which may act in defense against phytophagous insects and plant pathogens.[1] Azadirachtin, nicotine and pyrethrinoids are the best known compounds obtained from plants. Among plant derived substances, spices and extracts of spices have various effects on insect pests.[2] As spices are commonly used in food preparations, it is believed that they are generally harmless to humans. In addition, many spices possess various insect control properties. Toxic effects of acetone and hexane extracts of peppercorn to several species of stored product insects, larvicidal effects of garlic against several species of mosquitoes, insecticidal effects of non polar extracts of the flower buds of clove, *Syzygium aromaticum* L. and star anise, *Illicium verum* Hook f. to *Tribolium castaneum* and *Sitophilus zeamais* moths are some examples.[2, 3]

Among botanicals, plant derived essential oils play a diverse role in pest management. The effect of the essential oils on insects ranges from attraction to repellency. Table 1 illustrates naturally occurring materials and their responsible compounds for attraction and repulsion of insects.[4] The essential oils of anise, *Pimpinella anisum* L. and peppermint, *Menta piperita* L. were found to have fumigant toxicity on four stored product pests namely *Rhyzopertha dominica, T. castaneum, S. oryzae* and *Orzyaephilus surinamensis.*[2] It is also reported that the essential oil of *Cinnamon aromaticum* is toxic to both *T. castaneum* and *S. zeamais*.[2] Pine needle oil causes avoidance behaviors in the pocket gopher, *Geomys bursarius*.[1] Nutmeg oil has also showed toxic and antifeedent action

Table 1. Naturally occurring materials and compounds that attract or repel insects.[4]

Chemical/Extract	Natural Occurrence	Insect	Attractant/ repellent
Alcohols			
α-Terpineol (1)	Citrus oil	Sand fly	Attractant
3-Methyl-3-buten-1-ol (2)	Raspberry	House fly	Attractant
n-Dodecanol (3)	Eucalyptus oil	House fly	Attractant
Ketones			
Carvone (4)	Caraway oil	House fly/Beetle/ Mosquito	Attractant
Pulegone (5)	Pennyroyal oil	House fly/Beetle	Attractant
(*E*)-β-Damascenone (6)	Rose, Apple	House fly/Moth	Attractant
Esters and Acids			
Ethyl-2-Methyl-3-pentanoate (7)	Strawberry	House fly/Mosquito	Attractant
Benzyl formate (8)	Apple Blossom	House fly/Beetle	Attractant
Isobutyric acid (9)	Strawberry, Hops	House fly	Attractant
Miscellaneous			
Methylisoeugenol (10)	Orange, Nutmeg	House fly/Moth	Attractant
Dimethyl disulfide	Pineapple, Cocoa	Sand fly/House fly	Attractant
Marigold absolute	Merigold	House fly	Attractant
Methyl jasmonate (11)	Jasmin, Boronia	House fly/Mosquito	Repellent
trans Dihydromethyl jasmonate (12)	Tea	House fly/ Mosquito	Repellent
cis-*epi*-Dihydromethyl jasmonate (13)	Tea	House fly/Mosquito	Repellent
α-Damascone (14)	Tea	House fly/ Mosquito	Repellent
β-Damascone (15)	Rose	House fly/Mosquito	Repellent

against *T. castaneum* and *S. zeamais*.[3] The essential oil of *Origanam vulgare* have shown insecticidal and genotoxic activities against *Drosophila melanogaster*.[5] The essential oils obtained from *Ocimum gratissimum* L., *Thymus serpyllum* L., *I. verum* Hook f., *Myristica fragrans* and *Curcuma amada* have shown 100% repellent activity against *Musca domestica* L.[6] Cinnamaldehyde, perillaldehyde, citral, citronelal, octanal, nonanal, decanal, carvone, camphor, benzyldehyde, *p*-methylbenzaldehyde, cuminaldehyde, anisaldehyde, vanillin and furfural which are obtained from various essential oils have shown anti fungal activities.[7]

1. 2. Constituents of essential oils

Terpenoids are a group of substances which are widely distributed in the plant and animal kingdoms.[8] The constituents of the essential oils are mainly monoterpenes and sesquiterpenes which are hydrocarbons with general formula of $(C_5H_8)_n$. Oxygenated compounds derived from these hydrocarbons such as alcohols, aldehydes, esters, ethers, ketons and oxides are frequently exist in oils. It is estimated that there are about 1000 monoterpenes and 3000 sesquiterpenes identified so far from the natural products. Other compounds also present in essential oils include phenolics, sulphur and nitrogen containing compounds. In certain plants, one main constituent may predominate. In other species no single component which predominates. In these species the individual chemicals are represented by about 0.1-10% of total oil volume. The presence of trace components, even those not yet identified may influence the odor, flavor and also the biological activity of the oil. It is also reported that the time of harvest influences the oil composition. Biological and antioxidative activity also influence the chemical composition of the oils.[9, 10, 11, 12] Other factors such as genotype, chemotype, geographical origin and environmental and agronomic conditions can also influence the composition of the final natural product.

1. 3. Mode of action of essential oils

It has been established that volatile oils may have an effect on the humans and animals either by inhalation or penetration through the skin. The reaction of the lipophilic fraction of the volatiles with the lipid parts of cell membranes can modify the activity of sodium and potassium ion channels. At certain levels of dosage, the volatile oils saturate the membranes and show effects similar to those of local anesthetics. They can interact with the cell membranes, which can have an effect on the physiological functions mainly in humans such as stimulation, sedation and antidepressant activities. Buchbauer *et al*[13] described the effects of odors on cognition, memory, and mood. The fragrance compounds can be absorbed by inhalation or/and skin. They can cross the blood-brain barrier and interact with receptors in the central nervous system.[13]

Bioassays for the description and explanation of essential oil actions are usually carried out on mice, rats and toads.

1. 4. The genus *Piper*

The genus *Piper* belongs to the family Piperaceae and has over 700 species distributed all over the world. They are herbs, shrubs or rare trees. The *piper* species have high commercial, economic and medicinal values. The family Piperaceae is economically important as pepper, *P. nigrum* which has high demand in the world spice trade. The ripened fruit (red) of pepper is the source of white pepper and the unripe fruit (green) of the same species is the source of black pepper. A narcotic beverage is prepared in some countries from the roots of *P. methysticum*. Several species of *Piper* are grown domestically as houseplant foliage.[14] *Piper* species are widely distributed in the tropical and subtropical regions of the world.

1. 4. 1. Biological activities of *Piper* species

Plants, which belong to the genus *Piper* are reputed in the Asian system of medicine, and in the folklore medicine of Latin America and West Indies for their medicinal properties. The chloroform extract of the stems of *P. aborescens* was found to have activity against a KB cell culture system and a P-388 lymphocytic leukemia system in cell culture.[15] *P. aduncum* and *P. hispidum* are used for stomach disorders and as insect repellents.[14] *P. amalago* distributed from Mexico to Brazil is used to alleviate chest pains and to act as an anti-inflammatory agent.[16] *P. sylvaticum* roots are used as an effective remedy to snake poison in the indigenous system of Indian medicine. *P. chaba* roots and fruits are used as a treatment for asthma, bronchitis, fever and abdominal pains.[17] The stem of *P. futokadsura* is widely used in the Chinese herbal medicine for the treatment of asthma and arthritis.[18] The fruits of West African black pepper, *P. guineense*, are used as a flavorant, while leaves, roots and seeds are used as medicinal agent for bronchitis, gastrointestinal diseases, venereal diseases and rheumatism. The extracts obtained from the seed kernels are used to counter irritation and for there insecticidal properties.[19] The extract of *P. acutisleginum* has shown aflatoxin B_1-DNA binding inhibitor activity.[14] *P. aduncum*, *P. brachystachyu*, *P. guineense* and *P. falconeri* are reported to have insecticidal properties. The *P. acutisleginum* has insecticidal properties against *M. domestica* (house fly) and *Aedes aegypti* (mosquito), while *P. hispidum* acts as an insect repellent.[14]

1. 4. 2. *Piper betle* L.

P. betle is a evergreen vine, climbing up to a height of 3 m. The stem is slender, twining and rounded, the leaves are oval-acuminate, green and smooth, the flowers are green, occurring in spikes, the fruits are fleshy red berries.

This plant is widely cultivated in tropical countries such as Sri Lanka, India, Malaysia and Philippine. People commonly use the leaves for chewing purposes alone or with other plant materials including the areca nut, *Areca catechu* L.[20] It is believed that this tradition of chewing in India may have existed for more than 2000 years. It is also reported that the anti microbial activity of saliva could be increased by *P. betle* leaves at the time when chewing it in human mouth. This enhancement of anti microbial activity may be due to the presence of phenolic compounds in *P. betle*.[21]

1. 4. 3. Chemistry and activity of *P. betle* L.

Three different classes of compounds have been identified from *P. betle*. They are the phenylpropanoids, terpenes and long chain hydrocarbons and there

Table 2. Some chemical constituents of *P. betle*

No.	Compound	Reference
	Phenylpropanoids	
1	Allylpyrocatechol diacetate (16)	20
2	Allylpyrocatechol monoacetate (17)	23
3	Chavibetol (18)	20
4	Chavibetolacetate (19)	20
5	Chavicol (20)	20
6	Eugenol (21)	23
7	HydroxyChavicol (22)	20
8	Isoeugenol (23)	24
9	Methyl eugenol (24)	23
10	Safrole (25)	24
	Terpenes	
11	Camphene (26)	23
12	β-Caryophyllene (27)	25
13	1,8-Cineol (28)	23
14	*p*-Cymene (29)	23
15	Limonene (30)	23
16	Myrcene (31)	24
17	α-Pinene (32)	23
18	β-Pinene (33)	23
19	α-Terpinene (34)	26
20	α-Terpineol (1)	26
21	α-Terpineol acetate (35)	26
	Miscellaneous compounds	
22	Cepharadione A (35a)	14
23	Piperine (35b)	14

Table 2 (Contd.). Some chemical constituents of *P. betle*

No.	Compound	Reference
24	Piperlonguminine (36)	14
25	Dotriacontanoic acid, $CH_3(CH_2)_{30}COOH$	14
26	Hentriacontane, $CH_3(CH_2)_{29}CH_3$	22
27	Pentatriacontane, $CH_3(CH_2)_{33}CH_3$	22
28	Stearic acid, $CH_3(CH_2)_{16}COOH$	22
29	n-Triacontanol, $CH_3(CH_2)_{28}CH_2OH$	22
30	Tritriacontane, $CH_3(CH_2)_{31}CH_3$	14

derivatives. Ten phenylpropanoids, eleven terpenes, two long chain fatty acids, three long chain hydrocarbons and one long chain hydrocarbon alcohol have been identified from this plant (Table 2).

Evans *et al* reported that chloroform extract of *P. betle* leaves showed fungicidal and nematocidal activities and contraceptive activity in humans.[20] It is also reported that *P. betle* poses antiseptic[22] and antihypertensive activities.[14] Adhikary *et al* observed that ethanol extract of *P. betle* have shown anti fertility effects against male rats.[27]

1. 5. Biogenesis of terpenes [28, 29, 30]

The terpenoids are a group of substances which are widely distributed in the plant and animal kingdom. The structures of all terpenoids are based on the linking together of isoprene (C_5H_8) units to form 2, 3, 4, 5, 6 or 8 multiples of this basic unit. The various groups of terpenes are distinguished according to the number of isoprene units as shown in table 3.

It is now believed that the biosynthesis routes of terpinoids are derived from mevalonic acid and/or deoxyxylulose pathways.

Mevalonic acid (MVA) is formed by the reduction of β-hydroxy-β-methylglutaric acid which itself is produced by the addition of acetic acid to acetoacetic acid after the activation of coenzyme A. The subsequent reaction steps in the biosynthesis of terpenes are enzymatically catalysed phosphorylation reactions. Mevalonic acid-5-phosphate and 5-pyrophosphate (MVA-5-PP) are formed successively under the action of ATP and MVA-kinase and phospho-MVA-kinase, respectively. Subsequently, MVA-5-PP is phosphorylated at the tertiary hydroxyl group, and the product is stabilized by the elimination of phosphoric acid and CO_2 to form isopentenyl pyrophosphate (IPP), which is called active isoprene i.e. the true structural unit of all terpenoids. The transformation of IPP to dimethylallyl pyrophosphate (DMAPP) takes place by the influence of isopentenyl pyrophosphate isomerase. This equilibrium reaction is essential for further interlinkage of the terpene units. The linking of IPP and DMAPP to give geranyl pyrophosphate, (GPP) is the starting point for the formation of the majority of plant terpenes.

The precursors for deoxyxylulose pathway are believed to be pyruvate and glyceraldehyde 3-phosphate. The formation of 1-deoxy-D-xylulose 5-phosphate takes place by the enzyme catalyzed reactions of pyruvate and glyceraldehydes 3-phosphate. The further conversion of 1-deoxy-D-xylulose 5-phosphate to 2-C-methyl-D-erythritol 4-phosphate is preceded by intramolecular rearrangement (Scheme 1). The monoterpenes are derived from GPP by the process of cyclization, rearrangement or oxidation (Scheme 2).

The addition of another IPP unit gives farnesylpyrophosphate, which leads to the formation of sesquiterpenes (Scheme 3). The diterpenes are derived from geranylgeranyl pyrophosphate, which is produced by the condensation of two GPP units.

In addition to the head-to-tail condition of the isoprenoid units, which lead to the formation of the above compounds, nature provide a further system of building at the stage of farnesyl-PP and geranylgeranyl-PP. Tri- and tetra-terpenes can be formed by the tail to tail dimerization of the C15 and C20 units respectively. The dimerization of farnesyl-PP forms squalene (C30), from which the cyclic triterpenes and steroids are derived. Tetraterpenes (C40), the dimerization products of geranylgeranyl-PP, are also widely distributed in the plant kingdom, for example the carotenoids (Scheme 4).

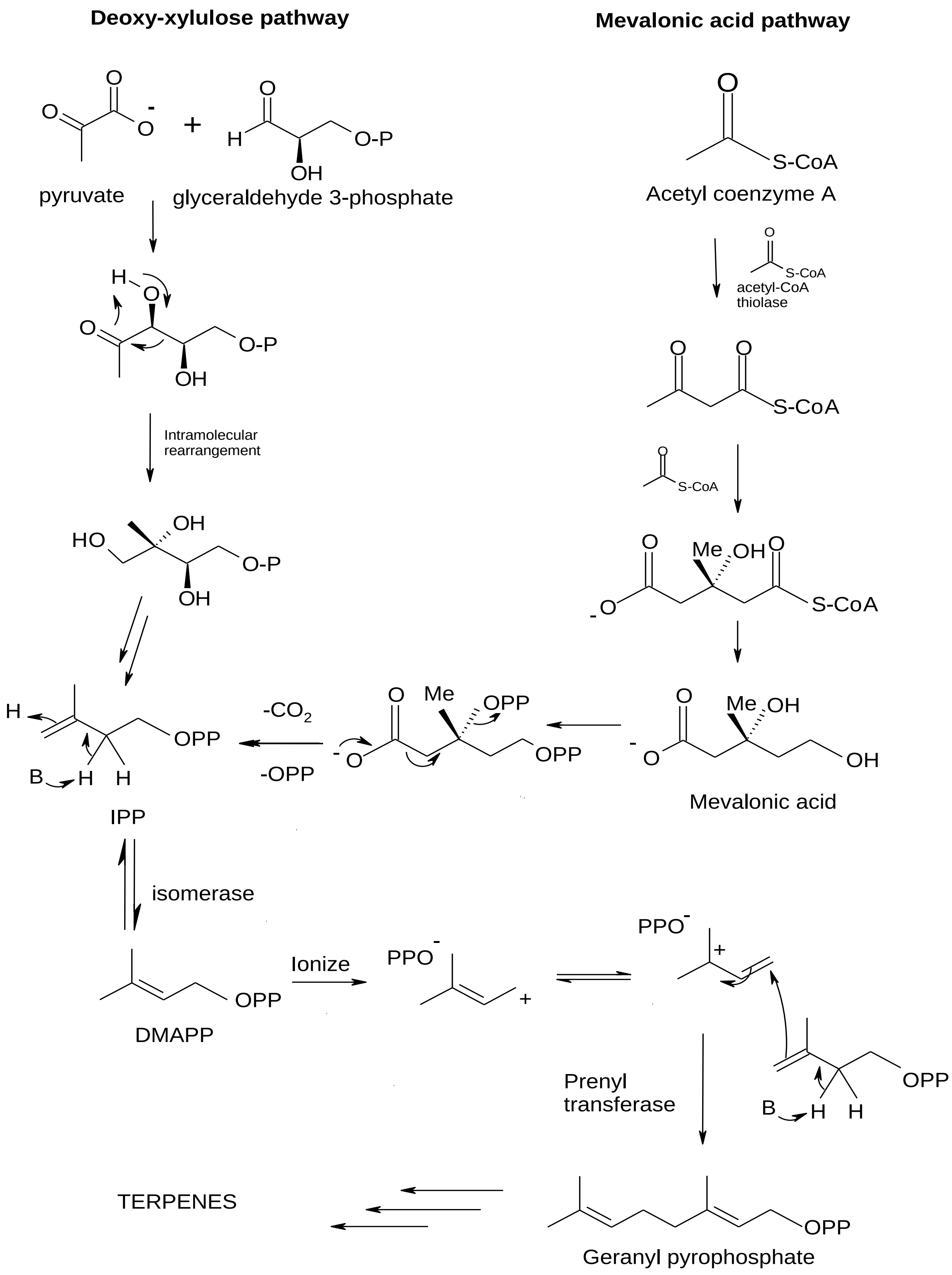
Both terpenoid pathways have been shown to occur in cells of higher plants. Specifically, the mevalonate pathway appears to be operative in supplying the building blocks for the biosynthesis of sterols. The deoxyeylulose pathway appears to be supplying the building blocks for the biosynthesis of hemiterpenes, monoterpenes, diterpenes and caratonoids. Recent studies[30] revealed that the deoxyeylulose pathway can also be involved in biosynthesis of sesquiterpenes.

Table 3. Types of terpenoid compounds

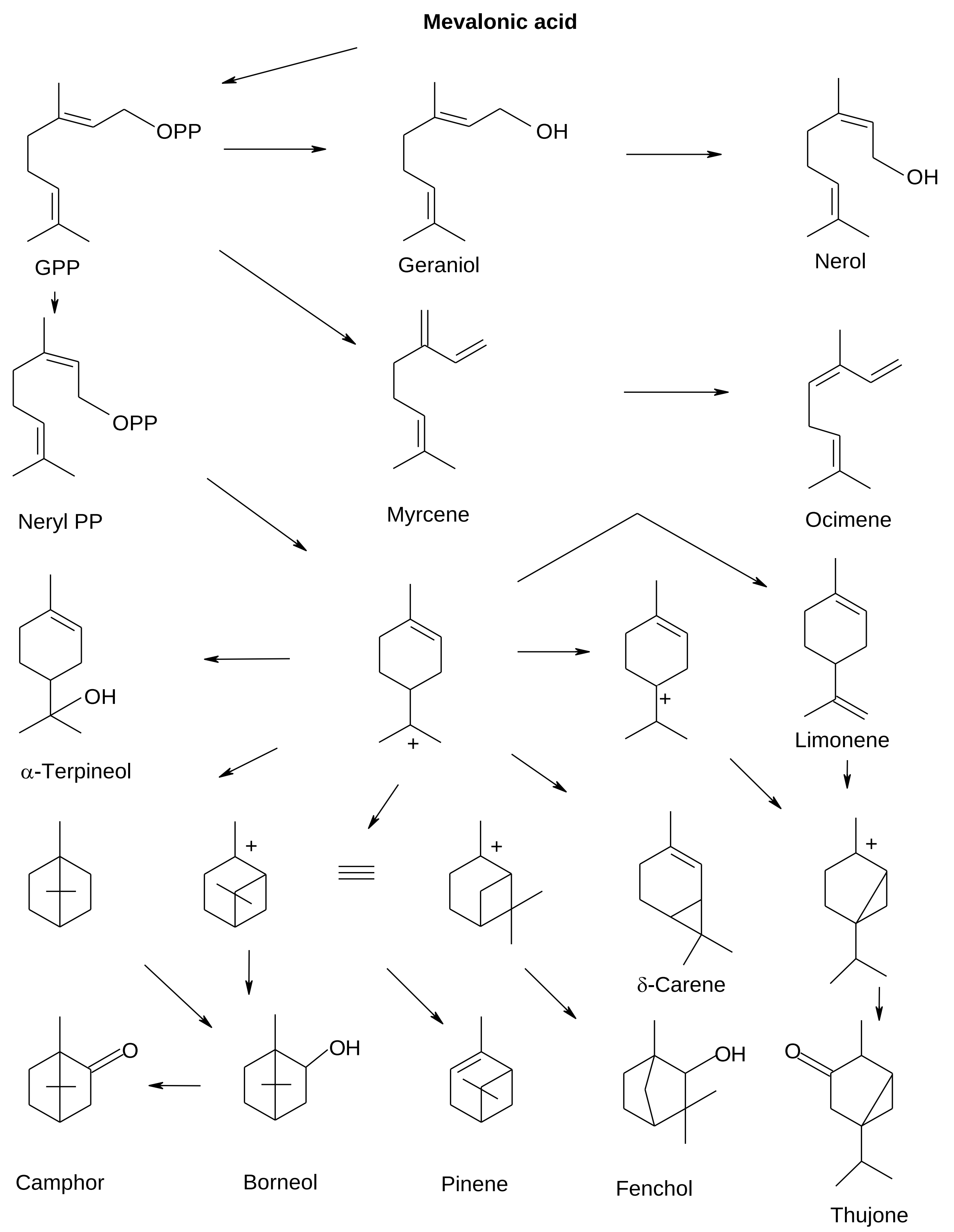
Type	Number of isoprene units
Hemiterpenes	1
Monoterpenes	2
Sesquiterpenes	3
Diterpenes	4
Sesterterpenes	5
Triterpenes	6
Tetraterpenes	8
Polyterpenes	n

1. 6. Biogenesis of phenyl propanoids [31]

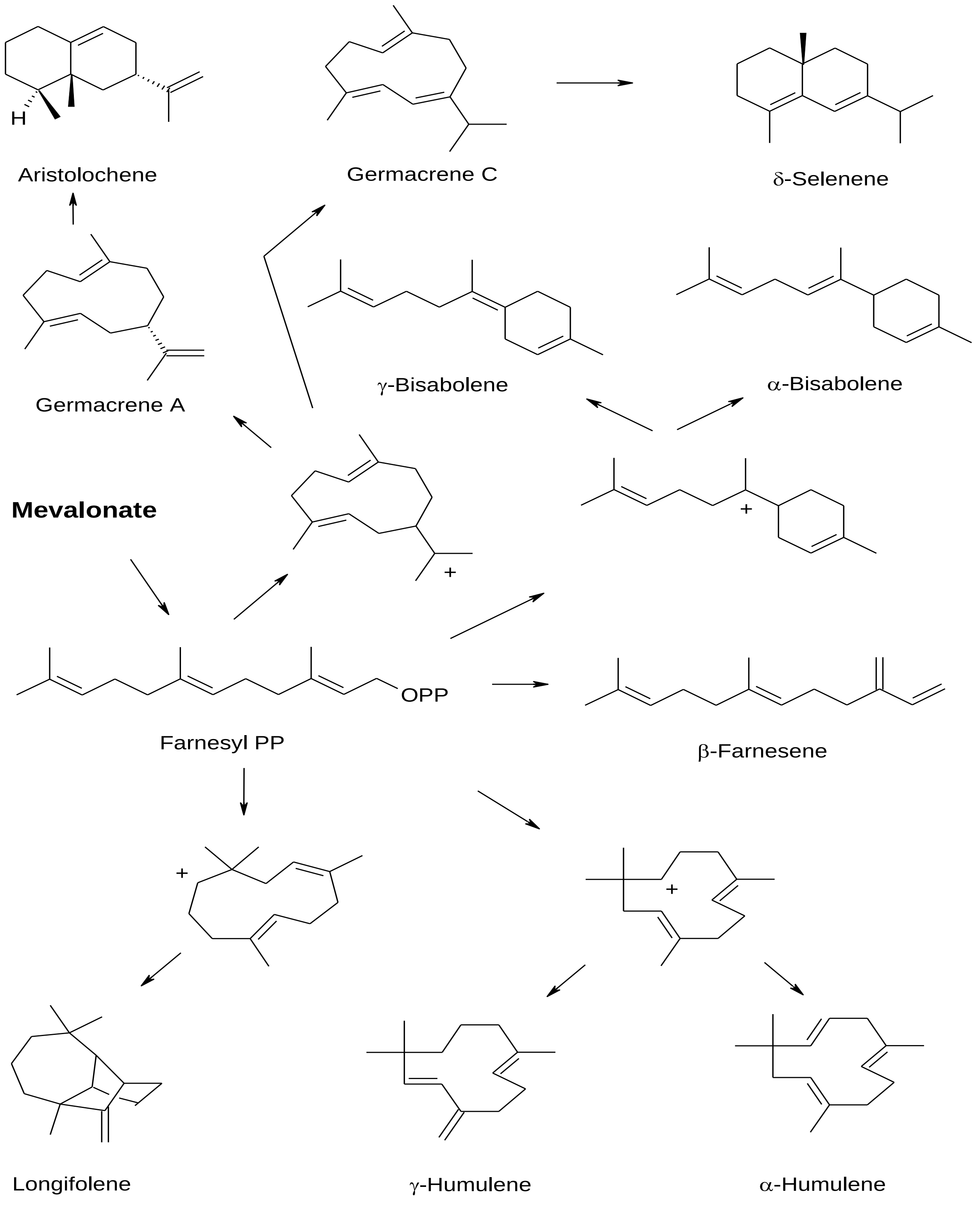
The biosynthesis route of phenyl propanoids is thought to be derived from shikimic acid pathway. The precursors for the phenyl propanoids are believed to be cinnamic acid and *p*-hydroxycinnamic acid (*p*-coumaric acid), which occur naturally in the trans configuration. Cinamic acid is formed by direct enzymatic deamination of phenylalanine. Scheme 5 describes the phenyl propanoid constituents and their possible biogenetic relationships.



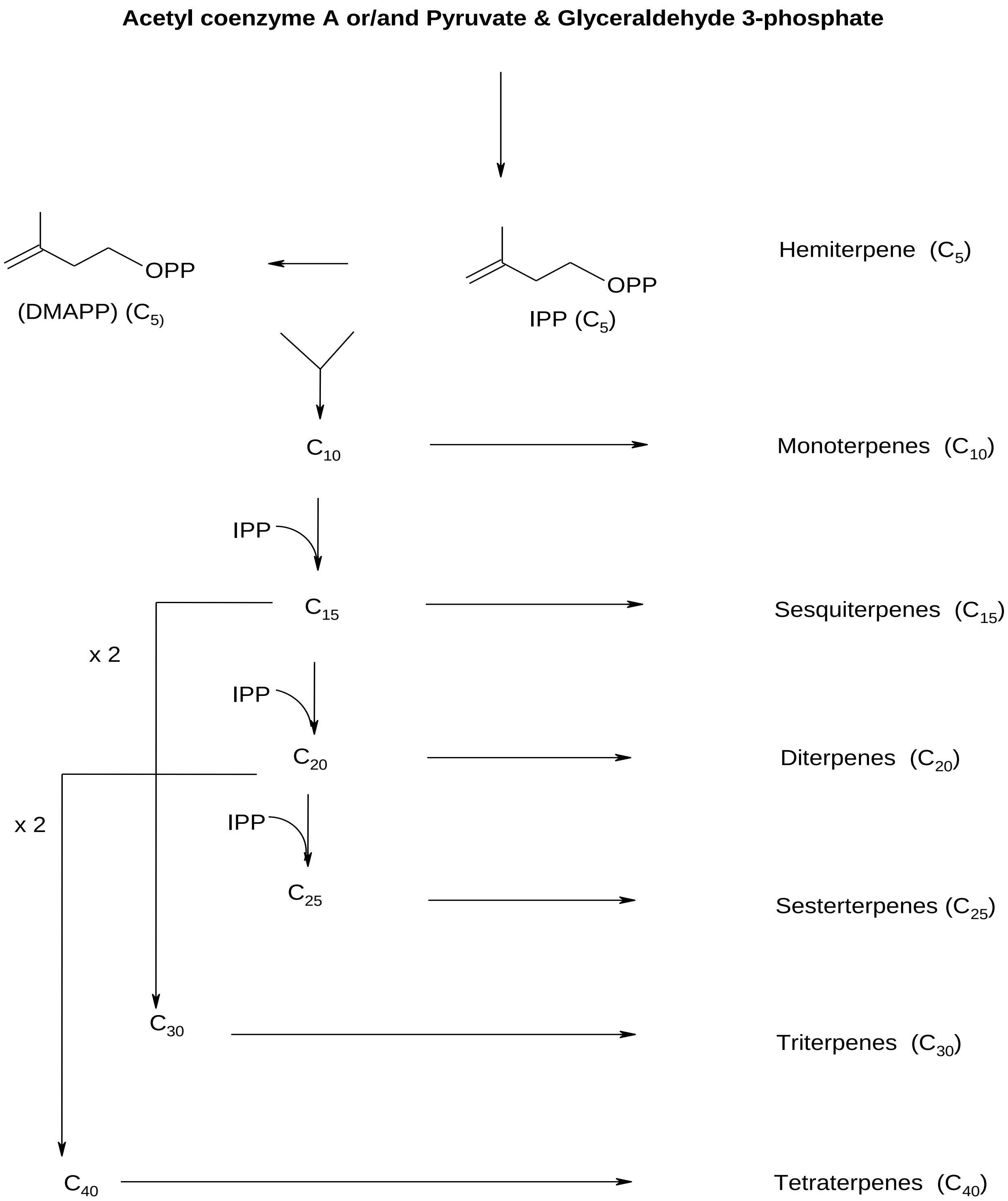
Scheme 1: General biosynthetic pathway for terpenes



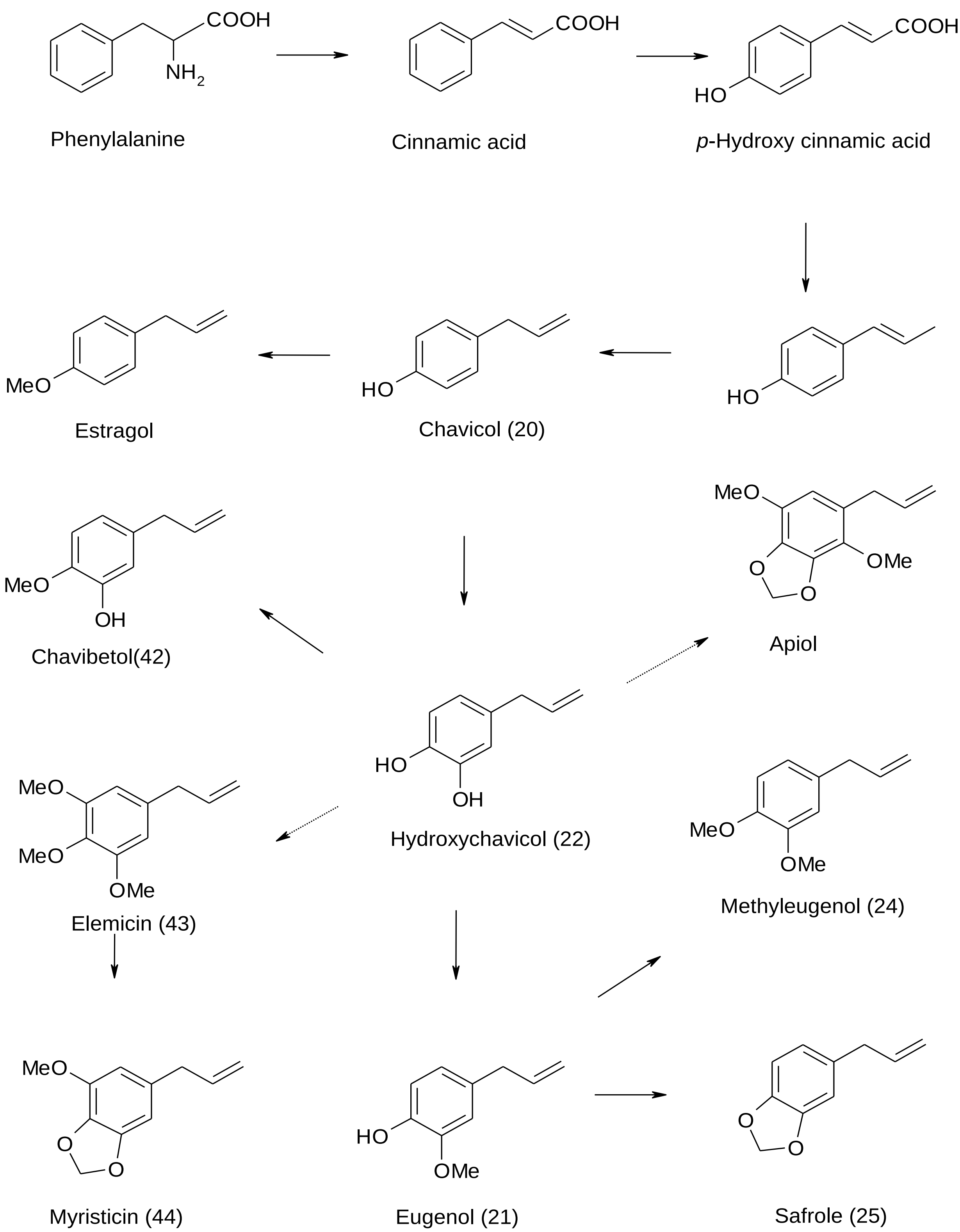
Scheme 2: Biosynthetic pathway for some common monoterpenes



Scheme 3: Biosynthetic pathway for some common sesquiterpenes



Scheme 4: Basic building blocks for terpene biosynthesis



Scheme 5: Biosynthetic pathway for Phenylpropanoids

1. 7. Insects[32]

Insects are the most eminently successful of all animals when compared with the number of species and individuals for adaptability and world-wide distribution. They dominate the present day land fauna, have about 800,000 species, that have been described, and represent about three-fourths of known animal life. The orders that contain the great numbers of species are, *Coleoptera* (beetles), *Lepidoptera* (butterflies and moths), *Hymenoptera* (ants) and *Diptera* (true flies). The majority of insects are smaller in size, usually less than 6 mm long. The range in size is wide. Some are almost microscopic, while some tropical forms attain to the considerable size of up to 16 cm. Insects thrive in almost any habitant where life is possible. Some are found only in the Arctic regions and some live only in deserts. Many species of insects can live in freezing or tropical temperatures. Although they are adaptable and versatile as a group, insects are often unable to adjust to unusual weather conditions. Excessive rain, an unusual early frost, an extended drought and other weather extremes can quickly wipe out or drastically reduce insect populations in a region. Insects are also important items for the diet of many other animals. The totality of factors unfavorable to insect survival is overwhelming, thus in some species, out of hundreds of eggs laid by the single female, seldom do more than a few individuals reach adulthood. Therefore it is obvious that the survival of some species is enhanced by the large number of eggs laid.

The insect body can be divided in to three main regions. They are the head, the middle section (or thorax) and the hind section (abdomen). The head bears the antennae, the mouthparts, the eyes and the brain. The thorax has three segments each with a pair of legs. In winged insects the thorax also bears one or two pairs of wings. The abdomen contains the digestive system. In females the ovipositer or egg laying organ is located at the tip of the abdomen.

Antennae play a vital role in insects because sensory organs of taste, touch, smell and hearing may be located on them. The loss of antennae usually leaves the insect in a helpless state.

Insects that attack humans or anything of value to humans are termed pests. About 10,000 species of insects have been classified as pests. Some are disease carriers. As vectors or transmitting agents of disease organisms, insects have cause massive deaths in humans. Some insects are benefactors of humans as they consume the carcasses of dead animals, pollinate orchards, manufacture honey or simply serve as another link in the food chain of the animal kingdom.

1. 7. 1. Insect control[32]

Six principal methods are used in the control of insect pests. These are chemical, mechanical, radiological, cultural & traditional, biological and legal.

1. 7. 1. 1. Chemical methods

The chemical substances used to destroy insects are called insecticides. They may be classified as stomach poisons, contact poisons, sorptive dusts and fumigants. The stomach poisons are more effective against the chewing insects, contact poisons against sucking insects. Fumigants are gaseous poisons that enter the insects breathing system. Sorptive dusts are dry chemical compounds that kill insects by absorbing fatty substances from the exoskeleton, thus causing vital body fluids to evaporate.

1. 7. 1. 2. Mechanical methods

This is a primitive and time consuming method and is generally less effective than chemical methods. It can be applied practically to large populations of insects or over wide area. These methods include swatting, the use of traps and barriers, water control and temperature control.

1. 7. 1. 3. Radiological methods

Sexual sterility is induced to the male insects by treating them with the rays of radioactive materials. Though treated sterile males will mate with fertile females, sterile eggs are produced.

1. 7. 1. 4. Cultural & traditional methods

This method is of special interest to the farmer. This method includes the destruction of plant residues and weeds, crop rotation and the growing of insect resistant strains of crops.

1. 7. 1. 5. Biological methods

This method involves the application of the pest's natural enemies. These enemies may be microbes, mites or other insects.

1. 7. 1. 6. Legal methods

The legal control of insects is the subject of government regulations to prevent the spread of insect pests from one country or region to another.

1. 7. 2. Housefly (*M. domestica*)[32]

M. domestica is a common insect in the family of Muscidae belongs to the order Diptera. About 90 percent of all flies occurring in human habitations are houseflies. The adult housefly is dull gray with dirty-yellowish areas on the abdomen and longitudinal lines on the thorax. Body size ranges from about 5 to 7 mm. The housefly cannot bite because of its sponging or lapping mouthparts. The female deposits more than 100 thin whitish eggs (0.8 to 1 mm long) at a time and producing between about 600 to 1000 eggs in her life. These eggs hatch in 12 to 24 hours. After several molts the dirty-whitish maggots (larvae), transform into pupae. The adults when emerging, expand a pouch resulting in emergence.

Houseflies feed on almost any food and move from person to food or drink. By transferring infective organisms from the skin and intestine, houseflies act as agents for transmitting typhoid, dysentery, cholera, diarrhea and other intestinal diseases caused by viruses and bacteria.

1. 8. Aim of the present work

Since *Piper* species (Piperaceae) have reportedly found widespread applications in medicinal practices and biological activity, most of the times the active compounds have not been specifically defined.[33, 16] Though *P. betle* has also showed various biological activities,[34, 35, 36] studies have not been made on the insecticidal activities up to the stage of identifying the active compounds. We studied the insecticidal properties of the Sri Lankan *P. betle* leaf oil and identified compounds responsible for insecticidal activity against *M. domestica.* We also studied structure activity relationships of analogs of the most active compound isolated from the oil.

Since published data has not been found for Sri Lankan essential oil of *P. betle* leaves, we further studied the chemical constituents of this oil and its chromatographic fractions.

2. EXPERIMENTAL

2. 1. General

Silica gel 60 (Fluka) mesh size 230-400 was used for medium pressure and flash chromatography (FC). Analytical thin layer chromatography (TLC) was carried out (0.1 mm thickness) on Merck kieselgel 60 on pre-coated aluminum sheets. Spots were located by heating after spraying with 1% H_2SO_4 spray reagent.

Melting points were determined using Buchi 510 melting point apparatus. IR spectra were obtained for KBr discs and NaCl plates for crystalline compounds and liquids respectively on a Perkin elmer FT-IR, 1720 X Spectrometer. Cary 1E, UV-Visible spectrophotometer was used for UV absorption spectra. Either Varian 200 MHz or Bruker 400 MHz spectrometers were used for ^{1}H- Nucler magnetic resonance (^{1}H-NMR) spectroscopy. ^{13}C-Nuclear magnetic resonance (^{13}C-NMR) spectroscopy was carried out on Bruker 100 MHz spectrometer. $CDCl_3$ solutions were used for both ^{1}H-NMR and ^{13}C-NMR. Chemical shifts are given in ppm units relative to $CHCl_3$ set to 7.26 (^{1}H-NMR) and 77.0 (^{13}C-NMR) (multiplicity: s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, br-broad). The identity of compounds was established by comparison of spectral and physical data.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on Varian 3400-Saturn 3 instrument and Trace GC-Ploaris Q instruments. Ionization mode; electron impact 70 eV. Analyzer; Quadrupole ion trap (QIT). Carrier gas; helium. Gas chromatographic analyses were performed on Varian model 3400 and Polaris Q instruments for essential oil analysis.

Capillary Column: ZB-5, Phenomenex, USA, (stationary phase-5%diphenyl-95%dimethyl polysiloxane), 30 m, 0.25 mm ID, film thickness 0.25 µm. Temperatures- injector; 220°C, transfer line; 240°C, oven; isotherm 5 min at 60°C, 4 °C/min gradient to 220°C then 20 min isotherm at 220 °C, for GC-MS analysis. High performance liquid chromatography (HPLC), Instrument: Hewlett Packard 1050 series, column; Nucleosil SNG, C_{18}, semi preparative, diameter 250 mm & Preparative GC, column; Carbowax, length 3 m, was used for isolation of compounds after performing initial purification using FC.

2. 2. Collection of plant materials and distillation

2. 2. 1. Pilot plant distillation of *P. betle* L. leaf oil

Fresh leaves (10 Kg) were collected at Kottawa in the district of Colombo, Sri Lanka, air dried for 2 days and subjected to pilot plant steam distillation for 4 hr (yield 40 ml, 0.40 v/w %).

2. 3. Insects

Adult *M. domestica* (housefly) were used for the studies. The housefly maggots (WHO strain) were obtained from laboratory cultures reared at the Novartis Center, St Aubin, Switzerland and maintained at 30°C, 80% RH till eclosion. The emerged adults were fed on casein and sucrose till they were used for the experiments.

2. 4. Bioassay

3-4 day old active houseflies were used for tests with all the treatments. The experiments were carried out at 22 °C, 45-55% RH and a 10-14 hr L:D period. Serial dilutions of the oil were prepared in acetone to obtain different concentrations. Acetone alone constituted the control. Each concentration was tested over 5 insects with 6 replicates. Aliquots of 0.5 ml were spread evenly on Whatman filter paper disks (7 cm dia.) and the solvent was allowed to evaporate for about 10 minutes under the fume hood. After evaporation, filter papers were kept in aluminium plates (7 cm dia., height 3 cm) and the insects were placed on each filter paper and immediately covered with the transparent cups (interior volume 248 cm^3, diameter 7 cm) to prevent the insects from escaping. Mortality was counted after at 24 and 48 hr exposure periods.

2. 4. 1. Bioassay guided fractionation of *P. betle* L. leaf oil

The oil (25.20 g) obtained from pilot plant distillation was subjected to medium pressure column chromatography using hexane, toluene and ethyl acetate in a polarity increasing order. Eight fractions were collected (named as PBL/1 to

PBL/8). These fractions were tested for insecticidal activity against *M. domestica* and active fractions were re-fractionated by bioassay-guided fractionation.

2. 5. Chromatographic isolation

2. 5. 1. Isolation of 3-(3´,4´-methylenedioxyphenyl)-propan-1-en (safrole) (25)

Fraction PBL/3, (8 g) was subjected to flash chromatography (FC) using toluene-hexane, (1:5) as eluent, gave a light yellow oil as safrole (25) (7.80 g). ^{1}H-NMR (400 MHz) δ: 6.78 (1H, d, *J*=7.87 Hz, 5´-H), 6.72 (1H, d, *J*=1.40 Hz, 2´-H), 6.67 (1H, dd, *J*=7.87, 1.69 Hz, 6´-H), 5.96 (1H, m, 2-H), 5.95 (2H, s, O-CH_2-O), 5.10 (2H, m, 1-H), 3.34 (2H, d, *J*=6.71 Hz, 3-H); ^{13}C-NMR (100 MHz) δ: 148.04 (C-3´), 146.23 (C-4´), 138.02 (C-2), 134.28 (C-1´), 121.72 (C-6´), 116.20 (C-1), 109.52 (C-2´), 108.58 (C-5´), 101.21 (O-CH_2-O), 40.33 (C-3); IR, ν_{max} (cm^{-1}): 3077, 2977, 2894, 2776, 1715, 1639, 1502, 1489, 1443, 1247, 1041, 934, 807; λ_{max} (nm, log ε): 287 (3.60) and 232 (3.53); m/z (rel. int., %): 162 (M^+) (100), 135 (11), 131 (11), 119 (2), 103 (14), 79 (2), 53 (1).

2. 5. 2. Isolation of 3-(4´-hydroxy-3´-methoxyphenyl)-propan-1-en (eugenol) (21)

Fraction PBL/5, (2.4 g) was subjected to FC with EtOAc-hexane, (3:100) and obtained 3-(4´-hydroxy-3´-methoxyphenyl)-propan-1-en, eugenol (21) (2.10 g) as a pale yellow oil. ^{1}H-NMR (200 MHz) δ: 6.83 (1H, d, *J*=8.80 Hz, 5´-H), 6.66 (1H, dd, *J*=8.43, 1.83 Hz, 6´-H), 6.65 (1H, d, *J*=1.83 Hz, 2´-H), 5.94 (1H, m, 2-H), 5.50 (1H, br.s, D_2O exchangeable, OH), 5.05 (2H, m, 1-H), 3.86 (3H, s, OCH_3), 3.30 (2H, dt, *J*=6.59, 1.47 Hz, 3-H); ^{13}C-NMR (100 MHz) δ: 146.87 (C-3´), 144.31 (C-4´), 138.26 (C-2), 132.34 (C-1´), 121.60 (C-6´), 115.44 (C-1), 114.70 (C-5´), 111.54 (C-2´), 56.27 (OCH_3), 40.32 (C-3). IR, ν_{max} (cm^{-1}): 3513, 3004, 2937, 2844, 1714, 1638, 1613, 1514, 1464, 1451, 1432, 1365, 1268, 1233, 1149, 1122, 1035, 914, 795; λ_{max} (nm, log ε): 282 (3.52) and 229 (3.78); m/z (rel. int., %): 164 (M^+) (100), 149 (18), 137 (10), 133 (11), 131 (12), 121 (10), 103 (16), 91 (6), 79 (4), 63 (5), 55(10).

2. 5. 3. Isolation and identification of phenylpropanoids from PBL/7 and PBL/8

GC-MS analysis of PBL fractions showed that fraction numbers seven and eight also contained a high percentage of phenylpropanoids as with fractions three and five which contained safrole (25) and eugenol (21), respectively. Though fraction numbers seven and eight contained high percentages of phenyl propanoids, they were inactive unlike fraction numbers three (safrole) and five (eugenol) to *M. domestica* (Table 6). Therefore fraction numbers seven and eight were also subjected to column chromatograph to isolate and identify these inactive phenylpropanoids to study the structural differences between active and inactive compounds.

2. 5. 3. 1. Fractionation of PBL/7

Fraction PBL/7, (0.5 g), subjected to preparative column gas chromatography, resulted in isolation of 3 fractions (PBL/7/1 to PBL/7/3) as pure compounds.

2. 5. 3. 1. 1. Isolation of 3-(3´-4´-dimethoxyphenyl)-propan-1-en (methyl eugenol) (24)

Fraction number PBL/7/1 was identified as Methyl eugenol (24) (80 mg) as a pale yellow oil. ^{1}H-NMR (400 MHz) δ: 6.83 (1H, d, *J*=7.94 Hz, 5´-H), 6.78 (1H, dd, *J*=8.49, 1.99 Hz, 6´-H), 6.76 (1H, d, *J*=1.99 Hz, 2´-H), 5.98 (1H, m, 2-H), 5.11 (2H, m, 1-H), 3.89 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.36 (2H, d, *J*=6.69 Hz, 3-H); ^{13}C-NMR (100 MHz) δ: 149.27 (C-3´), 147.76 (C-4´), 138.10 (C-2), 133.04 (C-1´), 120.79 (C-6´), 116.02 (C-1), 112.24 (C-5´), 111.62 (C-2´), 56.34 (OCH_3), 56.20 (OCH_3), 40.22 (C-3). IR, ν_{max} (cm^{-1}): 3006, 2930, 2848, 1613, 1518, 1440, 1430, 1365, 1260, 1233, 1140, 1110, 1030, 940; λ_{max} (nm, log ε): 283 (3.60) and 228 (3.60); m/z (rel. int., %): 178 (M^+) (100), 163 (16), 151 (7), 147 (18), 135 (8), 131 (4), 115 (4), 107 (16), 103 (14), 91 (6), 79 (4), 63 (4), 51 (4).

2. 5. 3. 1. 2. Isolation of 3-(4´-acetoxy-3´-methoxyphenyl)-propan-1-en (eugenyl acetate) (24a)

Fraction number PBL/7/2 was identified as eugenyl acetate (24a) (142 mg) as a pale yellow oil. ^{1}H-NMR (400 MHz) δ: 6.97 (1H, d, *J*=7.96 Hz, 5´-H), 6.80 (1H, dd, *J*=8.21, 1.58 Hz, 6´-H), 6.78 (1H, d, *J*=1.34 Hz, 2´-H), 5.99 (1H, m, 2-H), 5.13 (2H, m, 1-H), 3.84 (3H, s, OCH_3), 3.40 (2H, d, *J*=6.73 Hz, 3-H), 2.33 (3H, s, $COCH_3$); ^{13}C-NMR (100 MHz) δ: 169.66 ($CH_3\underline{C}O$), 151.25 (C-3´), 139.42 (C-4´), 138.37 (C-1´), 137.44 (C-2), 122.92 (C-6´), 121.07 (C-5´), 116.58 (C-1), 113.11 (C-2´), 56.21 (OCH_3), 40.50 (C-3), 21.10 ($\underline{C}H_3CO$). IR, ν_{max} (cm^{-1}): 3076, 3005, 2938, 2847, 1766, 1639, 1605, 1510, 1466, 1420, 1370, 1269, 1217, 1150, 1123, 1035, 1011, 908; λ_{max} (nm, log ε): 274 (3.12) and 226 (3.53); m/z (rel. int., %): 207 $(M+H)^+$ (8), 164 (100), 149 (20), 137 (6), 133 (10), 131 (14), 121 (8), 103 (12), 91 (11), 77 (8), 65 (5), 55 (6), 43 (28).

2. 5. 3. 1. 3. Isolation of 3-(3´-4´-diacetoxyphenyl)-propan-1-en (allylpyrocatechol diacetate) (16)

The last fraction number of PBL/7/3 was identified as allylpyrocatechol diacetate (16) (234 mg) as a pale yellow oil. ^{1}H-NMR (400 MHz) δ: 7.13 (1H, d, *J*=8.14 Hz, 5´-H), 7.09 (1H, dd, *J*=8.31, 1.91 Hz, 6´-H), 7.03 (1H, d, *J*=1.80 Hz, 2´-H), 5.96 (1H, m, 2-H), 5.13 (2H, m, 1-H), 3.41 (2H, d, *J*=7.33 Hz, 3-H), 2.31(3H, s, CH_3CO), 2.30 (3H, s, CH_3CO); ^{13}C-NMR (100 MHz) δ: 168.90 ($CH_3\underline{C}O$), 168.84 ($CH_3\underline{C}O$), 142.28 (C-3´), 140.68 (C-4´), 139.39 (C-1´), 136.81 (C-2), 127.11 (C-6´), 123.80 (C-5´), 123.57 (C-2´), 117.08 (C-1), 39.87 (C-3), 21.06 (2 x $\underline{C}H_3CO$); IR, ν_{max} (cm^{-1}): 3079, 3005, 2979, 2934, 1771, 1640, 1613, 1596, 1505, 1428, 1471, 1259, 1211, 1113, 1013, 909, 830, 799; λ_{max} (nm, log ε): 272 (3.23) and 226 (3.52); m/z (rel. int., %): 235 $(M+1)^+$ (10), 192 (18), 150 (100), 149 (6), 131 (13), 132 (14), 123 (4), 104 (14), 103 (9), 91 (6), 77 (4), 65 (3), 43(53).

2. 5. 3. 2. Fractionation of PBL/8

Fraction PBL/8, (621 mg) was subjected to FC with CH_2Cl_2-MeOH (9.5:0.5) followed by repeated flash chromatography with Toluene-EtOAc (9:1) gave two fractions as PBL/8/4/1-2.

2. 5. 3. 2. 1. Isolation of 3-(4´-acetoxy-3´-hydroxyphenyl)-propan-1-en (allylpyrocatechol monoacetate) (17)

Fraction number PBL/8/4/1 was subjected to reverse phase semi preparative HPLC (solvent gradient, 95% MeOH:5% H_20 to 100% MeOH in 18 min, flow rate 0.800 ml/min, Detector: UV λ_{max} 320 nm), gave 3-(4´-acetoxy-3´-hydroxyphenyl)-propan-1-en (17) (10 mg) as pale yellow oil. ^{1}H-NMR (400 MHz) δ: 6.99 (1H, d, *J*=8.18 Hz, 5´-H), 6.92 (1H, dd, *J*=8.02, 2.01 Hz, 6´-H), 6.80 (1H, d, *J*=2.02 Hz, 2´-H), 5.94 (1H, m, 2-H), 6.12 (1H, br.s, D_2O exchangeable, OH), 5.09 (2H, m, 1-H), 3.32 (2H, d, *J*=6.75 Hz, 3-H), 2.32 (3H, s, CH_3CO); ^{13}C-NMR (100 MHz) δ: 171.01 ($CH_3\underline{C}O$), 147.42 (C-3´), 145.82 (C-4´), 137.41 (C-1´), 136.83 (C-2), 122.80 (C-6´), 121.25 (C-5´), 117.94 (C-1), 116.68 (C-2´), 40.12 (C-3), 21.45 ($\underline{C}H_3CO$); IR, ν_{max} (cm^{-1}): 3401, 3005, 2977, 2927, 1746, 1640, 1608, 1506, 1436, 1371, 1270, 1113, 1015, 968, 914, 821; λ_{max} (nm, log ε): 272 (3.23) and 226 (3.52); m/z (rel. int., %): 192 (M^+) (4), 150 (100), 135 (12), 133 (22), 131 (28), 123 (14), 104 (33), 103 (18), 91 (12), 77 (22), 65 (10), 55(8).

2. 5. 3. 2. 2. Isolation of 3-(4´-acetoxy-3´-hydroxyphenyl)-propan-1-en (hydroxychavicol) (22)

The fraction number PBL/8/4/2 was identified as hydroxychavicol (22) (40 mg) as a pale yellow oil. ^{1}H-NMR (400 MHz) δ: 6.81 (1H, d, *J*=8.05 Hz, 5´-H), 6.73 (1H, d, *J*=1.99 Hz, 2´-H), 6.65 (1H, dd, *J*=8.05, 1.48 Hz, 6´-H), 5.95 (1H, m, 2-H), 5.54 (1H, br.s, D_2O exchangeable, OH), 5.46 (1H, br.s, D_2O exchangeable, OH), 5.08 (2H, m, 1-H), 3.29 (2H, d, *J*=6.81 Hz, 3-H); ^{13}C-NMR (100 MHz) δ: 143.90 (C-3´), 142.12 (C-4´), 138.05 (C-2), 133.64 (C-1´), 121.41 (C-6´), 116.14 (C-2´), 115.98 (C-1), 115.79 (C-5´), 39.91 (C-3); IR, ν_{max} (cm^{-1}): 3380, 3078, 2925, 2853, 1638, 1605, 1518, 1445, 1351, 1290, 1192, 1113, 914, 758; λ_{max} (nm, log ε): 274 (3.24) and 228 (3.56); m/z (rel. int., %): 150 (M^+) (100), 149 (12), 135 (14), 133 (23), 131 (48), 123 (36), 103 (65), 91 (16), 77 (62), 63 (18), 51 (56).

Scheme 6 describes the fractionation and isolation procedure of *P. betle* leaf oil.

2. 6. Preparation of analogs of safrole (25)

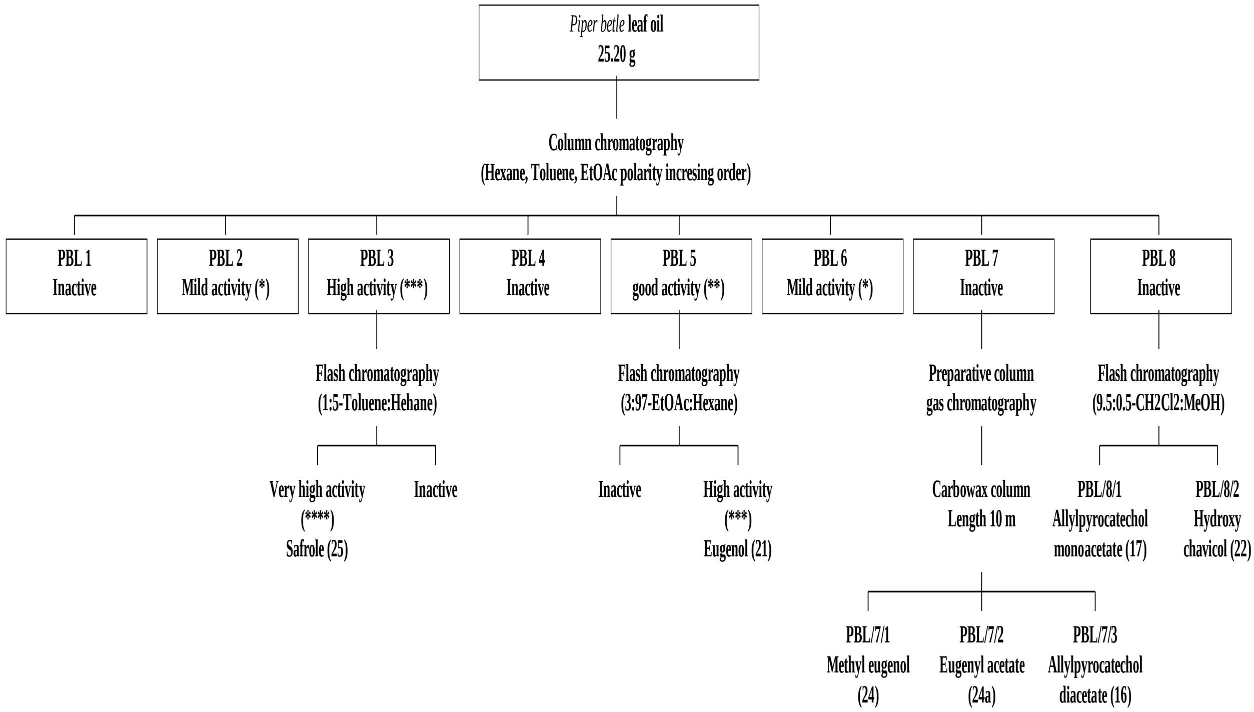
As safrole (25) has allylic moiety attached to the aromatic ring, 8 analogues (45-52) were prepared starting from safrole (25) to study the structure activity relationships. Since safrole (25) also has a methylenedioxy moiety, allyl benzene (53) was tested to observe whether this methylenedioxy moiety is essential for the insecticidal activity.

2. 6. 1. 3-(3´,4´-methylenedioxyphenyl)-propan-1-en, (isosafrole) (45)[37]

Safrole (25) (2.00 g), CaO (10 g) and KOH (1.4 g) were mixed together and heated at 245 °C for 30 min. After cooling, CH_2Cl_2 (50 ml) was added and mixture was filtered. Usual workup followed by evaporation of solvent yielded isosafrole (45) (1.63 g, yield, 82 %). ^{1}H-NMR (200 MHz) δ: 6.87-6.72 (3H, m, Ar-H), 6.30 (1H, dd, *J*= 15.75, 1.83 Hz, 3-H), 6.03 (1H, m, 2-H), 5.91 (2H, s, O-CH_2-O), 1.83 (3H, dd, *J*= 6.23, 1.47 Hz, 1-H); ^{13}C-NMR (100 MHz) δ: 148.31 (C-3´), 146.90 (C-4´), 132.91 (C-1´), 130.94 (C-3), 124.35 (C-2), 120.47 (C-6´), 108.61 (C-2´), 105.74 (C-5´), 101.31 (O-CH_2-O), 18.76 (C-1); IR, ν_{max} (cm^{-1}): 2959, 2939, 2886, 1629, 1501, 1485, 1438, 1336, 1244, 1189, 1095, 1044, 942, 930, 806; m/z (rel. int., %): 162, (M^+) (100), 135 (8), 131 (12), 119 (1), 103 (18), 91 (2), 77 (4), 63 (5), 51(2).

2. 6. 2. 3-(3´,4´-methylenedioxyphenyl)-propan, (dihydrosafrole) (46)[38]

Safrole (25) (500 mg) was stirred with MeOH (10 ml) and $NiCl_2.6H_2O$ (1.09 g) at O°C. A small portion of $NaBH_4$ (1.173 g) was then added while stirring and maintaining the temperature at 0°C for 30 min. Usual work up gave dihydrosafrole (46) (450 mg, yield, 90 %) ^{1}H-NMR (200 MHz) δ: 6.71 (1H, d, *J*=7.51 Hz, 5´-H), 6.66 (1H, d, *J*=1.11 Hz, 2´-H), 6.60 (1H, dd, *J*=7.81, 1.71 Hz,



Mild insecticidal activity	- *	Against *M. domestica*
Good insecticidal activity	- **	
High insecticidal activity	- ***	
Very high insecticidal activity	- ****	

Scheme 6- Bioassay-guided fractionation and isolation procedures of phenylpropanoids from *P. betle* leaf oil

6´-H), 5.90 (2H, s, O-CH_2-O), 2.49 (2H, t, *J*=7.60 Hz, 3-H), 1.58 (2H, m, 2-H), 0.91 (3H, t, 1-H); ^{13}C-NMR (100 MHz) δ: 147.85 (C-3´), 145.82 (C-4´), 136.99 (C-1´), 121.52 (C-6´), 109.32 (C-2´), 108.41 (C-5´), 101.09 (O-CH_2-O), 38.20 (C-3), 25.24 (C-2), 14.12 (C-1); IR, ν_{max} (cm^{-1}): 2959, 2930, 1504, 1489, 1442, 1364, 1247, 1187, 1100, 1040, 941, 927; m/z (rel. int., %): 164 (M^+) (88), 135 (100),121 (1), 105 (6), 79 (6), 53 (2).

2. 6. 3. 3-(3´,4´-methylenedioxyphenyl)-propan-2-ol (47)[39]

Safrole (25) (622 mg) was gradually added to a stirred solution of $Hg(OAc)_2$ (1.25 g) in THF (11.5 ml):H_2O (3.8 ml). After 1 hr at room temperature the yellow color was discharged itself and then the reaction mixture was alkalinized with 3M NaOH (3.75 ml) followed by addition of a solution of $NaBH_4$ (73 mg) in 3M NaOH (3.75 ml). After 1 hr the mixture was decanted to separate Hg and then saturated with NaCl, the organic layer separated and the aqeous layer was extracted with EtOAc (3x75 ml). Combined organic layers were then washed successively with H_2O (3x75 ml) and saturated brine (75 ml), dried over anhydrous Na_2SO_4 and evaporated to give alcohol (47) as a clear viscous oil (568 mg, yield, 91 %). ^{1}H-NMR (200 MHz) δ: 6.70 (1H, d, *J*=7.69 Hz, 5´-H), 6.66 (1H, d, *J*=1.47 Hz, 2´-H), 6.60 (1H, dd, *J*=7.69, 1.83 Hz, 6´-H), 5.86 (2H, s, O-CH*2*-O), 3.89 (1H, m, 2-H), 2.59 (2H, m, 3-H), 2.20 (1H, br.s, D_2O exchangeable, OH), 1.16 (3H, d, *J*= 6.23, 1-H); ^{13}C-NMR (100 MHz) δ: 148.16 (C-3´), 146.61 (C-4´), 132.61 (C-1´), 122.67 (C-6´), 110.07 (C-2´), 108.72 (C-5´), 101.29 (O-CH_2-O), 69.32 (C-2), 45.82 (C-3), 23.11 (C-1);); IR, ν_{max} (cm^{-1}): 3391, 2968, 2927, 2778, 1608, 1503, 1490, 1442, 1371, 1247, 1189, 1122, 1099, 1039, 939, 804, 772; m/z (rel. int., %): 180 (M^+) (98), 163 (18), 135 (100), 121 (2), 105 (12), 91 (2), 77 (14), 63 (4), 51(10).

2. 6. 4. 3-(3´,4´-methylenedioxyphenyl)-propan-2-on (48)[39]

To a well stirred suspension of pyridinium dichromate (PDC) (680 mg) in CH_2Cl_2 (20 ml) under N_2. A solution of 2-hydroxy dihydrosafrole (47) in CH_2Cl_2 (5 ml) was added. After stirring for 12 hr at room temperature Et_2O (40 ml) was added, vigorous stirring maintained for 2h. Purification via filtration followed by FC (EtOAc: Toluene, 1:20) gave the ketone 48 (160 mg, yield, 80 %). ^{1}H-NMR (200 MHz) δ: 6.75 (1H, d, *J*=8.06 Hz, 5´-H), 6.65 (1H, d, *J*=1.47 Hz, 2´-H), 6.61 (1H, dd, *J*=7.80, 1.47 Hz, 6´-H), 5.92 (2H, s, O-CH_2-O), 3.58 (2H, s, 3-H), 2.12 (3H, s, 1-H); ^{13}C- NMR (100 MHz) δ: 206.99 (C-2), 148.31 (C-3´), 147.09 (C-4´), 128.22 (C-1´), 122.92 (C-6´), 110.16 (C-2´), 108.88 (C-5´), 101.47 (O-CH_2-O), 50.94 (C-3), 29.54 (C-1); IR, ν_{max} (cm^{-1}): 2898, 2779, 1711, 1504, 1490, 1444, 1356, 1249, 1160, 1101, 1039, 938, 926, 813, 776; m/z (rel. int., %): 178 (M^+) (68), 162 (5), 135 (100), 121 (1), 105 (8), 91 (1), 77 (4), 63 (2), 43(10).

2. 6. 5. 3-(3´,4´-methylenedioxyphenyl)-propan-1-ol (49)[40]

Hydroboration of safrole (25)

$NaBH_4$ (285 mg) was stirred at 0°C with dry THF (15 ml) placed in a flask with a reflux condenser under N_2. $Hg(OAc)_2$ (1.194 g) was then added slowly to the mixture and stired for 1 hr at 0°C. Then the mixture was brought to room temperature and safrole (25) (1.824 g) was added drop wise. The mixture was stirred for a further 16 hr.

Oxidation

3M NaOH (3 ml) was added slowly to the hydro borated mixture at 0°C. H_2O_2 (3 ml, 30%) was then added drop wise and the temperature was raised slowly. The mixture was heated at 70 °C for 1 hr, during which the mercury coagulated. Then contents were brought to room temperature, decanted to separate mercury, saturated with NaCl, usual work up followed by FC (EtOAc:Toluene, 1:5) gave the alcohol 49 (1.370 g, yield, 75%). ^{1}H-NMR (200 MHz) δ: 6.71 (1H, d, *J*=8.06 Hz, 5´-H), 6.67 (1H, d, *J*=1.47 Hz, 2´-H), 6.62 (1H, dd, *J*=7.69, 1.83 Hz, 6´-H), 5.90 (2H, s, O-CH_2-O), 3.64 (2H, t, *J*=6.41, 1-H), 2.61 (2H, t, *J*=7.69 Hz, 3-H), 1.82 (2H, m, 2-H), 1.48 (1H, br.s, D_2O exchangeable, OH); ^{13}C-NMR (100 MHz) δ: 147.98 (C-3´), 146.03 (C-4´), 136.06 (C-1´), 121.52 (C-6´), 109.29 (C-2´), 108.57 (C-5´), 101.17 (O-CH_2-O), 62.48 (C-1), 34.82 (C-2), 32.20 (C-3); IR, ν_{max} (cm^{-1}): 3368, 2931, 1503, 1489, 1442, 1246, 11188, 1099, 1038, 933, 810;

m/z (rel. int., %): 180 (M^+) (100), 162 (6), 147 (6), 135 (96), 131 (8), 121 (4), 105 (8), 91 (8), 77 (11), 63 (5), 51(10).

2. 6. 6. 3-(3´,4´-methylenedioxyphenyl)-propan-1-al (50)[41]

To a well stirred suspension of pyridinium dichromate (PDC) (680 mg) in CH_2Cl_2 (20 ml) under N_2. A solution of 1-hydroxy dihydrosafrole (49) in CH_2Cl_2 (5 ml) was added. After being stirring for 12 hr at room temperature Et_2O (40 ml) was added and vigorous stirring maintained for 2h. Purification via filtration followed by FC (EtOAc: Toluene, 1:20) gave the aldehyde 50 (191 mg, yield, 96 %). ^{1}H-NMR (200 MHz) δ: 9.78 (1H, t, *J*=1.40 Hz, 1-H), 6.71 (1H, d, *J*=7.69 Hz, 5´-H), 6.66 (1H, d, *J*=1.83 Hz, 2´-H), 6.61 (1H, dd, *J*=7.79, 1.74 Hz, 6´-H), 5.90 (2H, s, O-CH_2-O), 2.86 (2H, m, 3-H), 2.71 (2H, m, 2-H); ^{13}C-NMR (100 MHz) δ: 201.99 (C-1), 148.15 (C-3´), 146.39 (C-4´), 134.51 (C-1´), 121.48 (C-6´), 109.17 (C-2´), 108.72 (C-5´), 101.30 (O-CH_2-O), 45.95 (C-2), 28.28 (C-3); IR, ν_{max} (cm^{-1}): 2896, 1724, 1503, 1491, 1444, 1246, 1189, 1100, 1037, 928, 811; m/z (rel. int., %): 178 (M^+) (100), 150 (8), 135 (67), 121 (6), 105 (4), 91 (4), 79 (4), 63 (5).

2. 6. 7. 3-(3´,4´-methylenedioxyphenyl)-1,2-epoxy-propan (51)

Metachloro perbenzoic acid (*m*-CPBA) (3.730 g) was added to a cold solution of safrole (25) (815 mg) in $CHCl_3$ (25 ml) and the mixture was left at room temperature for 3 hr. The solution was then washed successively with H_2O (75 ml), 5% $NaHCO_3$ (50 ml) and saturated brine (2x25 ml) dried over Na_2CO_3. Evaporation followed by FC on silica gel with EtOAc-toluene (1:19) gave 3-(3´,4´-methylenedioxyphenyl)-1,2-epoxy-propan (51) (642 mg, yield, 79%). ^{1}H-NMR (400 MHz) δ: 6.78 (1H, d, *J*=7.90 Hz, 5´-H), 6.77 (1H, d, *J*=1.44 Hz, 2´-H), 6.71 (1H, dd, *J*=7.60, 1.40 Hz, 6´-H), 5.96 (2H, s, O-CH_2-O), 3.13 (1H, m, 2-H), 2.87-2.75 (3H, m, 3-H and 1-H), 2.55 (1H, m, 1-H); ^{13}C-NMR (100 MHz) δ: 148.09 (C-3´), 146.71 (C-4´), 131.23 (C-1´), 122.29 (C-6´), 109.87 (C-2´), 108.69 (C-5´), 101.31 (O-CH_2-O), 52.98 (C-2), 47.23 (C-1), 38.82 (C-3); IR, ν_{max} (cm^{-1}): 2992, 2907, 1503, 1490, 1443, 1248, 1189, 1101, 1039, 927, 835; m/z (rel. int.,

%): 178 (M^+) (100), 147(18), 135 (68), 121 (4), 105 (4), 91 (8), 77 (4), 63 (8), 51(3).

2. 6. 8. 3-(3´,4´-methylenedioxyphenyl)-propan-1, 2-diol (52)

3-(3´,4´-methylenedioxyphenyl)-1,2-epoxy propan (51) (200 mg) in aq. THF (15 ml) was stirred with 0.5M H_2SO_4 (3 ml) at room temperature for 1 hr. Work up followed by FC with EtOAc-toluene (4:1) gave 3-(3´,4´-methylenedioxyphenyl)-propan-1,2-diol (52) (160 mg, yield 80 %). ^{1}H- NMR (200 MHz) δ: 6.73 (1H, d, *J*=8.06 Hz, 5-H´), 6.69 (1H, d, *J*=1.47 Hz, 2-H´), 6.62 (1H, dd, *J*=8.06, 1.65 Hz, 6-H´), 5.90 (2H, s, O-CH_2-O), 3.85 (1H, m, 2-H), 3.79-3.41 (2H, m, 1-H), 2.74-2.56 (2H, m, 3-H), 2.60 (2OH, br.s, D_2O exchangeable, 1-OH and 2-OH); ^{13}C-NMR (100 MHz) δ: 147.67 (C-3´), 146.14 (C-4´), 131.39 (C-1´), 122.14 (C-6´), 109.57 (C-2´), 108.26 (C-5´), 100.84 (O-CH_2-O), 73.06 (C-2), 65.79 (C-1), 39.30 (C-3); m/z (rel. int., %): 196 (M^+) (70), 178 (3), 165 (5), 135 (100), 121 (2), 107 (8), 91 (2), 79 (11), 77 (11), 63 (5), 51(10).

Allylbenzene (53) (~ 97% GC pure) and citronellal (54) (~ 98% GC pure) were purchased from Fluka, Switzerland.

2. 7. Standards

Ceylon citronella oil was used as positive control in the bioassay of *P. betle* leaf oil, whereas citronellal (54) (synthetic) was used in the bioassays involving isolated compounds and prepared analogues.

2. 8. Statistical analysis

The mortality was corrected according to the following equation, (a-b)100/a, where a and b are number of the surviving adults in the control and test experiments respectively. LC_{50} values were determined by “Environmental protection agency (EPA) probit analysis program” version 1.5. Comparison among the treatments were made by one-way ANOVA, after log transforming of the LC_{50} values, and ranked by Duncan’s multiple range test method (DMRT).

3. RESULTS

3. 1. Distillation of *P. betle* L. leaf oil

Collected *P. betle* leaf was subjected to pilot plant distillation for 4 hr resulted in a 40 ml (0.40%) yield of a light essential oil.

3. 2. Bioassay for *P. betle* L. leaf oil

P. betle leaf oil showed LC_{50} values of 10.8 & 9.2 mg/dm^3 after 24 and 48 hr periods respectively, compared to Ceylon citronella oil which registered 25.6 and 24.6 mg/dm^3 values for the same exposure periods (Table 4).

3. 2. 1. Bioassay guided fractionation of *P. betle* L. leaf oil

Among the 8 fractions obtained by flash chromatography, fraction PBL/3 and PBL/5 showed lower LC_{50} values, 5.3 and 8.5 mg/dm^3 after 24 hr exposure. Fractions PBL/2 and PBL/6 also showed low LC_{50} values of 8.8 and 9.9 mg/dm^3 for the same exposure period (Table 5).

GC-MS analysis showed that PBL/3 and PBL/5 contain safrole (25) and eugenol (21) as major components, respectively. The GC-MS analysis also revealed that the fraction PBL/2 contained safrole (25) as a major constituent whereas fraction PBL/6 contained eugenol (21) as one of the major components (Table 7).

3. 2. 2. Bioassay guided re-fractionation

Since the chemical compositions of active fractions of PBL/3 & PBL/5 were different from each other these two fractions were taken for re-fractionation.

3. 2. 3. Isolation of active compounds

Bioassay guided fractions of PBL/3 and PBL/5 yielded Safrole (25) and eugenol (21) as the active components showing LC_{50} value of 4.8, 4.7 mg/dm^3 and 6.8, 6.2 mg/dm^3 after 24 and 48 hr periods, respectively (Table 6).

Table 4. LC_{50}(mg/dm^3) of *P. betle* leaf and Ceylon citronella for *M. domestica*

Name of the oil	LC_{50} (mg/dm^3)*	
	24 h	**48 h**
Piper betle leaf	10.3^b	8.7^b
Ceylon Citronella[#]	26.5^a	24.2^a

[#] Ceylon citronella oil was used as a standard.

* LC_{50} values followed by the same letters in the same column are not significantly different at 5% significance level by Dunkans multiple rang test (DMRT).

Tested highest dose, 80.6 mg/dm^3.

Table 5. LC_{50} (mg/dm^3) values of *P. betle* leaf oil fractions for *M. Domestica*

Fraction Number	Active or Inactive	LC_{50} (mg/dm^3)	
		24h	**48h**
PBL/1	V. mild activity	23.5	23.5
PBL/2	Good activity	8.8	8.8
PBL/3	High activity	5.3	5.3
PBL/4	Inactive*	-	-
PBL/5	Good activity	8.5	8.5
PBL/6	Mild activity	9.9	9.5
PBL/7	Inactive*	-	-
PBL/8	Inactive*	-	-

* LC_{50} value is too high to be determined.

Tested highest dose, 80.6 mg/gm^3.

3. 2. 4. Isolation of inactive phenylpropanoids

Fractions seven and eight were subjected to column chromatography resulted the isolation of five phenyl propanoids, methyl eugenol (24), eugenyl acetate (24a), allylpyrocatechol diacetate (16), allylpyrocatechol monoacetate (17) and hydroxychavicol (22).

Table 6. LC_{50} (mg/dm^3) of eugenol (21), safrole (25) and its analogues for *M. domestica*

Compound	Active or Inactive	LC_{50} (mg/dm^3)	
		24 h*	48 h*
Eugenol (21)	Active	7.3^c	6.2^c
Safrole (25)	Active	4.8^c	4.7^c
Isosafrole (45)	Active	2.3^d	2.2^d
Dihydrosafrole (46)	Active	4.7^c	4.7^c
(48)	Active	37.4^a	29.8^a
(47)	Inactive□	-	-
(49)	Inactive□	-	-
(50)	Inactive□	-	-
(51)	Inactive□	-	-
(52)	Inactive□	-	-
Allylbenzene (53)	Inactive□	-	-
Citronellal (54)#	Active	14.3^b	14.3^b

Citronellal was used as a standard.

* LC_{50} values followed by same letters within a column are not significantly different at 5% significance level by Dunkan's multiple rang test (DMRT).

□ LC_{50} values were too high to be accurately determined.

Tested highest dose, 80.6 mg/dm^3.

3. 3. Bioassays of analogs of safrole (25)

The analogues of safrole (25) showed greater variability in toxicity, isosafrole (45) showing higher toxicity than safrole (25), whereas dihydrosafrole (46) showed almost an equal LC_{50}. After 24 and 48 hr isosafrole (45) showed higher mortality indicating LC_{50} values of 2.3 and 2.2 mg/dm^3 whereas safrole (25) and dihydrosafrole (46) showed LC_{50} values of 4.8 and 4.7 and 4.7 and 4.7 mg/dm^3, respectively. Compound 48 also showed moderate LC_{50} values of 33.7 and 29.1 mg/dm^3 for the 24 and 48 hr periods, respectively. Other analogues and allylbenzene (53) showed no mortality at their highest dose tested, i.e. 80.6

mg/dm^3 even after 48 hr exposure. Citronellal (54) showed LC_{50} of 14.3 mg/dm^3 after 24 hr period and this remained constant up to 48 hr exposure (Table 6).

3. 4. Compositional analysis of *P. betle* L. leaf oil and its fractions

GC-MS analysis revealed that safrole (25) (52.66%) was the major component present in the pilot plant distilled oil followed by allylpyrocatechol diacetate (16) (15.41%), eugenol (21) (6.42%) and eugenyl acetate (24a) (5.82 %).

Fractionation of this oil using hexane, toluene and ethyl acetate in increasing polarity revealed that some of the compounds present in the oil as minor compounds could be enriched by fractionation. Fraction 1 showed sabinine (56) (12.10%), α-selinene (108) (8.24%), β-selinene (106) (6.77%), and β-caryophyllene (27) (6.38%) as major components. Fraction 2 gave safrole (25) (34.32%), α-humulene (101) (9.63%), sabinene (56) (7.12%) and germacrene B (120) (6.47%) as major components. Fraction 3 yielded safrole (25) with 98.7% purity, where as fraction 4 yielded n-eicosane (129) (80%) and n-docosane (130) (8.20%) as high concentrations. Fraction 5 yielded 91.80% eugenol (21). Fraction 6 was rich in methyl eugenol (24) (31.60%), eugenyl acetate (24a) (31.6%) and eugenol (21) (22.10%). Fraction 7 yielded allylpyrocatechol diacetate (16) (26.50%) as a major component followed by eugenyl acetate (24a) (18.40%), terpin-4-ol (73) (17.50%) and methyl eugenol (24) (6.90%). Fraction 8 yielded allylpyrocatechol monoacetate (17) (23.00%), allylpyrocatechol diacetate (16) (13.20%), α-terpineol (1) (9.62%) and α-cadinol (128) (6.41%) as major compounds. Table 7 illustrates the chemical composition of the *P. betle* oil and its fractions.

Table 7. Chemical compositions of *Piper betle* leaf oil (Pilot Plant distillation) and its fractions

No.	Compound	Percentage (%, w/w)									Retention Index (RI)	Retention Index (RI) reference[42]
		PBL Oil	PBL Fr. 1	PBL Fr. 2	PBL Fr. 3	PBL Fr. 4	PBL Fr. 5	PBL Fr. 6	PBL Fr. 7	PBL Fr. 8		
1.	α-Thujene (55)	0.41	2.38	0.61	-	-	-	-	-	-	926	931
2	α-Pinene (32)	0.52	3.57	0.44	-	-	-	-	-	-	933	939
3	Camphene (26)	0.21	1.42	0.22	-	-	-	-	-	-	948	953
4	Sabinene (56)	2.62	12.10	7.12	-	-	-	-	-	-	971	976
5	β-Pinene (33)	0.11	0.31	trace	0.11	-	-	-	-	-	974	980
6	Myrcene (31)	1.12	4.14	2.03	-	-	-	-	-	-	987	991
7	α-Phellandrene (57)	0.12	0.7	0.21	-	-	-	-	-	-	1005	1005
8	(*Z*)-3-Hexenyl acetate (57a)	-	-	-	-	-	1.15	-	-	-	1004	1007
9	1,4-Cineole (58)	-	-	-	-	-	-	0.56	0.01	-	1014	1016
10	α-Terpinene (34)	0.51	4.78	2.28	0.14	-	-	-	-	-	1013	1018
11	*p*-cymene (29)	0.53	2.64	3.19	-	-	-	-	-	-	1025	1026
12	β-Phellandrene (59)	0.71	4.32	1.80	-	-	-	-	-	-	1029	1031
13	Benzylalcohol (60)	-	-	-	-	-	-	-	-	0.10	1031	1032
14	1,8-Cineole (28)	0.10	-	-	-	-	-	0.46	3.62	-	1034	1033
15	(*Z*)-β-Ocimene (61)	-	0.1	0.06	-	-	-	-	-	-	1041	1041

Table 7 (contd.). Chemical compositions of *Piper betle* leaf oil (Pilot Plant distillation) and its fractions

No.	Compound	Percentage (%, w/w)									**Retention Index (RI)**	Retention Index reference[42]
		PBL Oil	PBL Fr. 1	PBL Fr. 2	PBL Fr. 3	PBL Fr. 4	PBL Fr. 5	PBL Fr. 6	PBL Fr. 7	PBL Fr. 8		
16	(*E*)-β-Ocimene (38)	Trace	0.39	0.27	-	-	-	-	-	-	1054	1050
17	γ-Terpinene (62)	0.91	5.31	2.98	0.19	-	-	-	-	-	1065	1062
18	*cis*-Sabinene hydrate (63)	-	-	-	-	-	-	-	0.74	5.12	1070	1068
19	Fenchone (64)	-	-	-	-	-	-	-	5.94	5.62	1090	1087
20	Terpinolene (65)	0.22	1.79	1.14	trace	-	-	-	-	-	1097	1088
21	2-Nonanone (66)	-	-	-	-	-	-	0.50	-	-	1099	1091
22	*trans*-Sabinene hydrate (67)	trace	-	-	-	-	-	-	0.93	1.58	1106	1097
23	Linalool (68)	0.22	-	-	-	-	-	-	-	-	1111	1098
24	*p*-Menta-1,3,8-triene (69)	-	0.02	0.02	-	-	-	-	-	-	1115	1111
25	*cis*-*p*-Menth-2-en-1-ol (70)	-	-	-	-	-	-	-	-	3.58	1132	1121
26	*cis*-Limonene oxide (71)	-	-	-	-	-	trace	0.24	-	-	1140	1134
27	Benzyl acetate (72)	-	-	-	-	-	trace	-	-	-	1155	1163
28	Terpin-4-ol (73)	2.21	-	-	-	-	-	-	17.50	1.85	1178	1177
29	*m*-Cymen-8-ol (74)	-	-	-	-	-	-	-	-	0.39	1180	1180
30	α-Terpineol (1)	0.11	-	-	-	-	-	-	0.11	9.62	1190	1189

Table 7 (contd.). Chemical compositions of *Piper betle* leaf oil (Pilot Plant distillation) and its fractions

No.	Compound	Percentage (%, w/w)									**Retention Index (RI)**	Retention Index (RI) reference[42]
		PBL oil	PBL Fr. 1	PBL Fr. 2	PBL Fr. 3	PBL Fr. 4	PBL Fr. 5	PBL Fr. 6	PBL Fr. 7	PBL Fr. 8		
31	*cis*-Piperitol (75)	trace	-	-	-	-	-	-	0.33	0.15	1193	1193
32	n-Decanal (76)	-	-	-	-	-	trace	-	-	-	1206	1204
33	*trans*-Piperitol (77)	trace	-	-	-	-	-	-	0.56	-	1208	1205
34	*cis*-Sabinene hydrate acetate (78)	-	-	-	-	-	trace	-	0.22	-	1223	1219
35	*trans*-Sabinene hydrate acetate (79)	-	-	-	-	-	1.08	-	-	-	1258	1253
36	Chavicol (20)	-	-	-	-	-	-	-	0.19	-	1259	1253
37	2-Phenyl ethyl acetate (80)	-	-	-	-	-	-	2.02	0.10	-	1264	1256
38	Safrole (25)	52.66	-	34.32	98.7	-	-	-	-	-	1296	1285
39	*p*-Cymen-7-ol (81)	-	-	-	-	-	-	-	-	0.10	1298	1287
40	Thymol (82)	-	-	-	-	-	trace	-	-	-	1301	1290
41	2-Undecanone (83)	-	-	-	-	-	-	1.94	-	-	1303	1291
42	*trans*-Ascaridole (84)	-	-	-	-	-	-	-	-	0.11	1305	1301
43	*cis*-Piperitol acetate (85)	-	-	-	-	-	trace	-	-	-	1332	1330
44	5-Indanol (86)	-	-	-	-	-	trace	-	-	-	1335	1336
45	δ-Elemene (87)	-	0.4	0.12	-	-	-	-	-	-	1339	1339

Table 7 (contd.). Chemical compositions of *Piper betle* leaf oil (Pilot Plant distillation) and its fractions

No.	Compound	Percentage (%, w/w)									**Retention Index (RI)**	Retention Index (RI) reference[42]
		PBL oil	PBL Fr. 1	PBL Fr. 2	PBL Fr. 3	PBL Fr. 4	PBL Fr. 5	PBL Fr. 6	PBL Fr. 7	PBL Fr. 8		
46	α-Terpinyl acetate (35)	trace	-	-	-	-	1.29	-	-	-	1348	1350
47	α-Cubebene (88)	0.11	0.88	0.11	-	-	-	-	-	-	1349	1351
48	Eugenol (21)	6.42	-	-	-	-	91.80	22.1	1.28	0.40	1361	1356
49	α-Ylangene (89)	-	-	-	-	-	-	-	-	-	1369	1372
50	n-Undecanol (90)	-	-	-	-	-	-	-	-	0.10	1372	1372
51	Isoledene (91)	-	0.06	-	-	-	-	-	-	-	1373	1373
52	(*E*)-Isosafrole (45)	-	-	-	0.24	-	-	-	-	-	1374	1373
53	α-Copaene (92)	0.52	3.59	0.32	-	-	-	-	-	-	1376	1376
54	(*E*)-β-Damascenone (6)	-	-	-	-	-	-	2.2	-	-	1378	1380
55	β-Bourbonene (93)	0.21	1.33	0.17	-	-	-	-	-	-	1381	1384
56	β-elemene (94)	0.23	2.17	1.74	-	-	-	-	-	-	1386	1391
57	Vanillin (95)	-	-	-	-	-	-	-	-	0.10	1388	1391
58	Methyl eugenol (24)	0.72	-	-	-	-	-	31.6	6.90	-	1401	1401
59	β-Caryophyllene (27)	1.23	6.38	3.44	-	-	-	-	-	-	1413	1418
60	(*E*)-α-Ionone (96)	-	-	-	-	-	-	trace	-	-	1424	1426

Table 7 (contd.*)*. Chemical compositions of *Piper betle* leaf oil (Pilot Plant distillation) and its fractions

No.	Compound	Percentage (%, w/w)									**Retention Index (RI)**	Retention Index (RI) reference[42]
		PBL Oil	PBL Fr. 1	PBL Fr. 2	PBL Fr. 3	PBL Fr. 4	PBL Fr. 5	PBL Fr. 6	PBL Fr. 7	PBL Fr. 8		
61	β-Gurjunene (97)	0.12	1.29	0.16	-	-	-	-	-	-	1428	1432
62	γ-Elemene (98)	-	-	0.17	-	-	-	-	-	-	1430	1433
63	Neryl acetone (99)	-	-	-	-	-	-	trace	-	-	1432	1434
64	Aromadendrene (100)	-	0.43	0.10	-	-	-	-	-	-	1436	1439
65	α-Humulene (101)	0.81	2.57	9.63	0.07	-	-	-	-	-	1452	1454
66	(*Z*)-Methyl isoeugenol (102)	-	-	-	-	-	-	trace	-	-	1456	1456
67	*cis*-Muurola-4(14)-5-diene (103)	-	0.19	-	-	-	-	-	-	-	1472	1460
68	γ-Muurolene (104)	0.84	4.37	1.70	-	-	-	-	-	-	1477	1477
69	Germacrene D (105)	0.63	4.64	5.29	-	-	-	-	-	-	1482	1480
70	β-Selinene (106)	1.22	6.77	2.95	-	-	-	-	-	-	1487	1485
71	*epi*-Cubebol (107)	-	-	-	-	-	-	-	0.42	-	1495	1493
72	α-Selinene (108)	1.41	8.24	4.58	-	-	-	-	-	-	1497	1494
73	*trans*-β-Guaiene (109)	-	-	-	-	-	-	-	-	-	1501	1500
74	Cuparene (110)	-	-	-	trace	-	-	-	-	-	1502	1502
75	Germacrene A (111)	-	trace	0.85	-	-	-	-	-	-	1507	1508

Table 7 (contd.). Chemical compositions of *Piper betle* leaf oil (Pilot Plant distillation) and its fractions

No.	Compound	Percentage (%, w/w)									**Retention Index (RI)**	Retention Index (RI) rerence[42]
		PBL Oil	PBL Fr. 1	PBL Fr. 2	PBL Fr. 3	PBL Fr. 4	PBL Fr. 5	PBL Fr. 6	PBL Fr. 7	PBL Fr. 8		
76	Cubebol (113)	-	-	-	-	-	-	-	0.44	-	1518	1514
77	7-*epi*-α-Selinene (114)	0.21	-	0.48	-	-	-	-	-	-	1519	1517
78	δ-Cadinene (115)	0.53	3.98	1.42	-	-	-	-	-	-	1521	1524
79	Hydroxy chavicol (22)	-	-	-	-	-	-	-	-	4.74	1522	-
80	Allylpyrocatechol monoacetate (17)	-	-	-	-	-	-	-	-	23.00	1530	-
81	Eugenyl acetate (24a)	5.82	-	-	-	-	-	31.6	18.40	-	1531	1524
82	Cadina-1,4-diene (116)	-	0.31	0.07	-	-	-	-	-	-	1534	1532
83	α-Cadinene (117)	-	0.39	0.13	-	-	-	-	-	-	1538	1538
84	Selina-3,7 (11)-diene (118)	-	0.29	-	-	-	-	-	-	-	1546	1542
85	Germacrene B (119)	0.31	0.66	6.47	0.19	-	-	-	-	-	1557	1556
86	(*E*)-Nerolidol (120)	-	-	-	-	-	-	1.34	-	-	1560	1564
87	Spathulenol (121)	trace	-	-	-	-	-	-	1.20	-	1577	1576
88	β-Caryophyllene oxide (122)	-	-	-	-	-	-	-	1.06	-	1582	1581
89	Globulol (123)	trace	-	-	-	-	-	-	0.90	4.51	1583	1583
90	Humulene epoxide II (124)	-	-	-	-	-	-	-	0.49	-	1605	1606

Table 7 (contd.). Chemical compositions of *Piper betle* leaf oil (Pilot Plant distillation) and its fractions

No.	Compound	Percentage (%, w/w)									**Retention Index (RI)**	Retention Index reference[42]
		PBL Oil	PBL Fr. 1	PBL Fr. 2	PBL Fr. 3	PBL Fr. 4	PBL Fr. 5	PBL Fr. 6	PBL Fr. 7	PBL Fr. 8		
91	1-*epi*-Cubenol (125)	trace	-	-	-	-	-	-	1.59	-	1626	1627
92	Cubenol (126)	-	-	-	-	-	-	-	trace	-	1642	1642
93	Allylpyrocatechol diacetate (16)	15.41	-	-	-	-	-	-	26.50	13.20	1647	-
94	α-Muurolol (127)	-	-	-	-	-	-	-	-	4.93	1652	1645
95	α-Cadinol (128)	trace	-	-	-	-	-	-	-	6.41	1660	1653
96	n-Eicosane (129)	trace	-	-	-	80.00	-	-	-	-	1994	2000
97	n-Docosane (130)	trace	-	-	-	8.20	-	-	-	-	2193	2200

PBL Oil - *Piper betle* leaf oil (pilot plant steam distillation)

PBL Fr. - *Piper betle* leaf oil (pilot plant steam distillation) fractions

\- - not detected

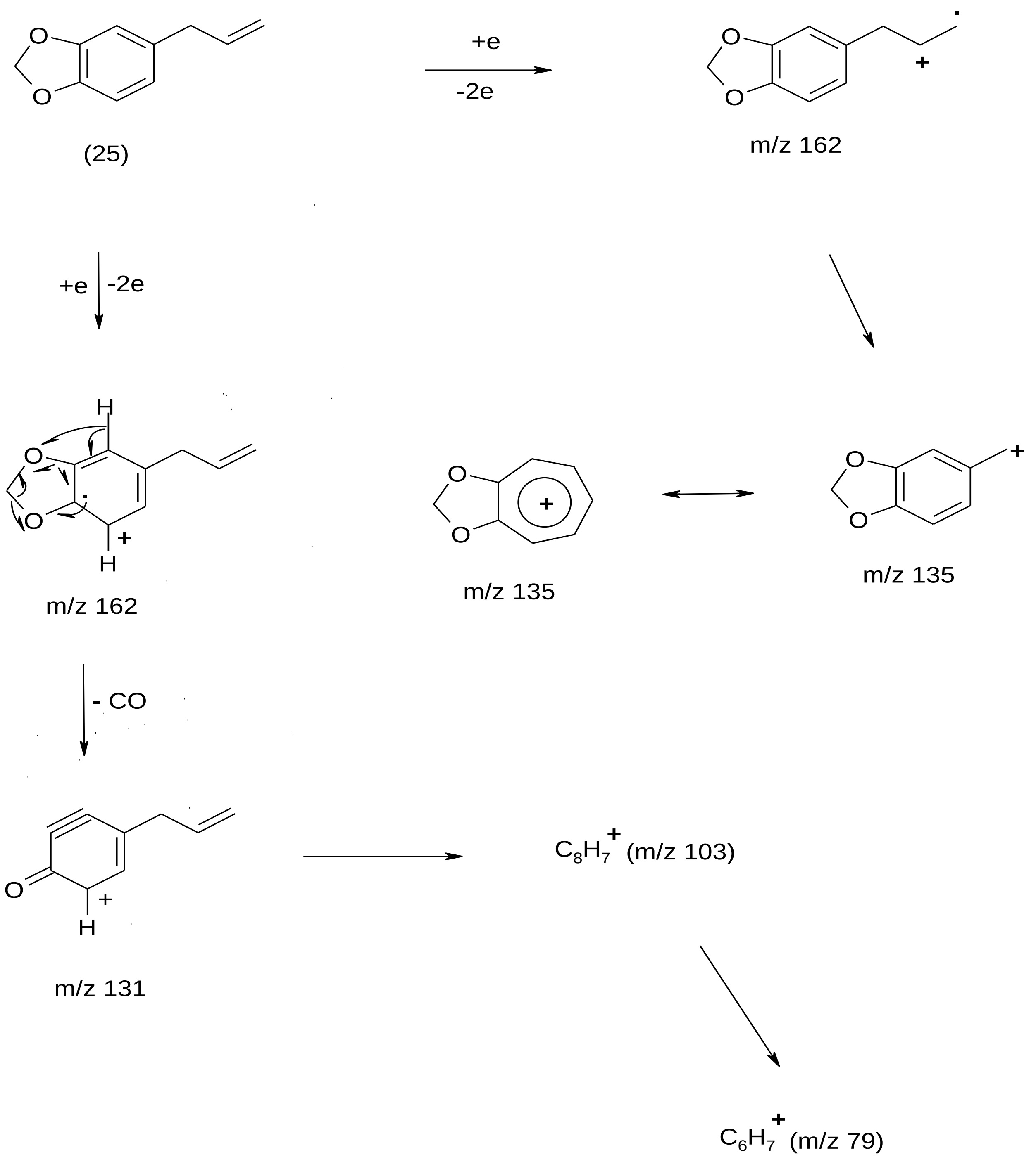
4. STRUCTURE ELUCIDATION AND DISCUSSION

4. 1. Isolation and structure elucidation of PBL/3 and PBL/5

Fraction number three was subjected to flash chromatography (FC) with a mixture of toluene and hexane, (1:5) gave a pale yellow oily compound (25). Its UV absorption at 229 and 282 suggesting it to have a phenyl propanoid skeleton. This was confirmed by its ^{1}H-NMR spectrum[43] and IR spectrum. Its UV absorption pattern also showed its aromatic ring having no conjugation with its propanoid skeleton.[44] Its mass spectrum with a molecular ion at m/z 162 suggested a molecular formula of $C_{10}H_{10}O_2$. Doublets with J=7.89 Hz at δ 6.78 and J=1.40 at δ 6.72 and double doublet with J=7.87 and 1.69 Hz at δ 6.67 in its ^{1}H-NMR spectrum suggested that there were three unsubstituted aromatic positions having a 1, 2, 4-relationship. Two proton multiplet centering at δ 5.10, single proton multiplet at δ 5.96 and two proton doublet with J=6.71 Hz at δ 3.34 suggested an allyl moiety was present. This more down field two proton doublet at δ 3.34 suggested the allyl moiety to be directly attached to the aromatic ring. A low field two proton singlet appearing at δ 5.95 suggested that two methylene protons attached to two electro negative atoms. The molecular formula suggested that these two electro negative atoms were oxygen. Since the aromatic ring is unsubstituted with 1,2,4 relationship, the methylene dioxy moity should be attached to the aromatic ring at 3 and 4 positions with respect to the position where the allyl group is attached to the aromatic ring. This was confirmed by ^{13}C-NMR with the more down field values of δ 148.04 and 146.23 at C-3 and C-4 positions in aromatic ring. Therefore the compound is suggested to be 3-(3´,4´-methylenedioxyphenyl)-propan-1-en (safrole) (25).

Comparison of the spectral data of safrole (25) with those reported for the compound with this structure showed them to be identical, confirming it to be safrole (25).[43, 44] Scheme 7 illustrates mass spectral fragmentations of safrole (25).

Flash chromatography using ethyl acetate-hexane (3:97) of a fraction of PBL/5 gave a compound (21) whose ^{1}H-NMR[45] and IR spectrum suggested it to



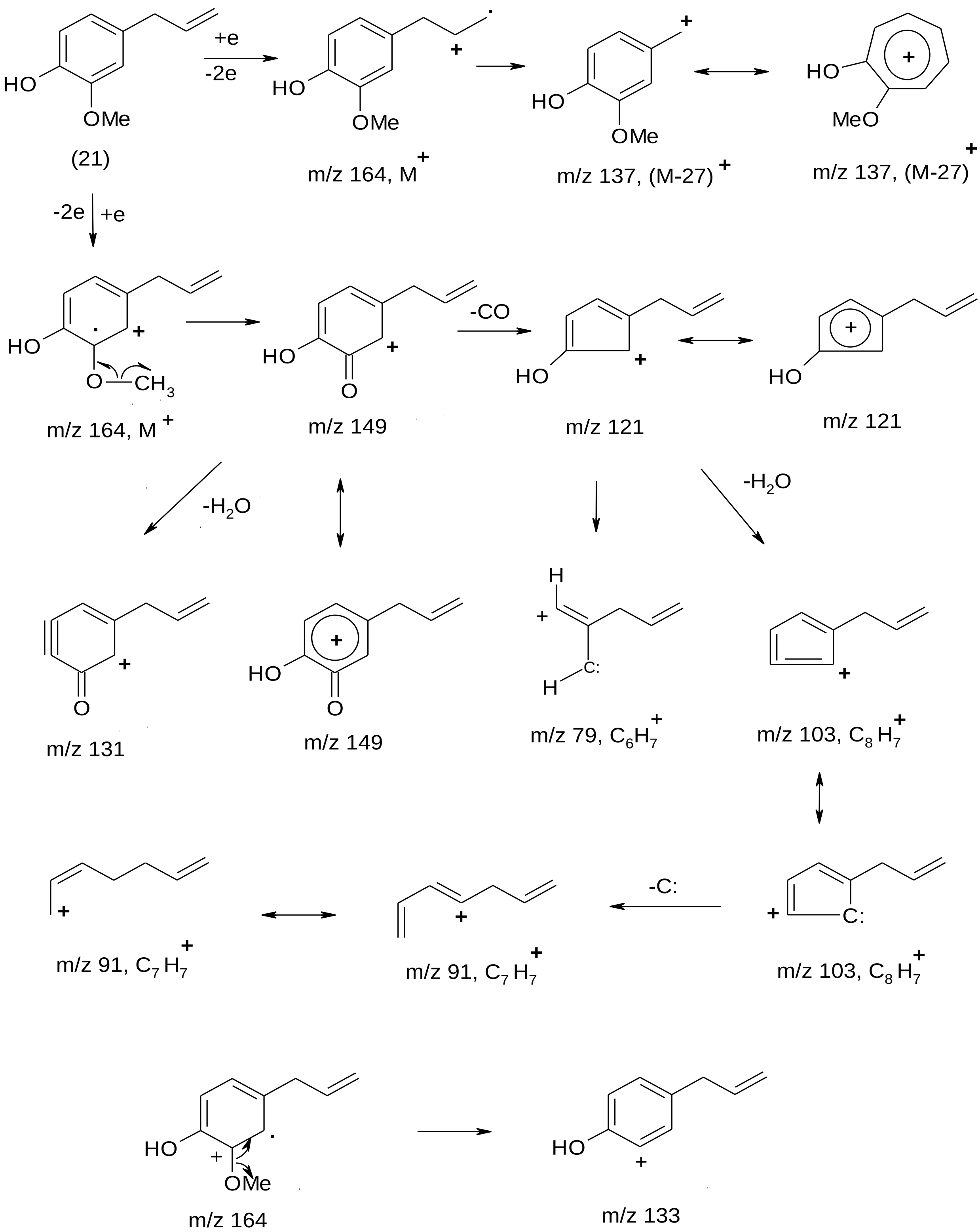
Scheme 7: Mass spectral fragmentations of safrole (25)

be a phenyl propanoid. The similar pattern observed from its UV spectrum to that of safrole (25) confirmed it to be a phenyl propanoid with substitutions at the 3rd and 4th positions in the aromatic ring. Its mass spectrum with a molecular ion at m/z 164 suggested a molecular formula of $C_{10}H_{10}O_2$. Doublets with *J*=8.80 Hz at δ 6.83 and *J*=1.83 Hz at δ 6.65 and a double doublet with *J*=8.43 and 1.83 Hz at δ 6.66 in its ^{1}H-NMR spectrum suggested that there were three unsubstituted aromatic positions having a 1, 2, 4-relationship. Broad peak centering at 3513 cm^{-1} in its IR spectrum and D_2O exchangeable peak at δ 5.50 in its ^{1}H-NMR spectrum suggested it to have an OH group. Two proton multiplet centering at δ 5.05, single proton multiplet at δ 5.94 and two proton doublet of a triplet with *J*=6.59 Hz and 1.47 Hz at δ 3.30 suggested an allyl moiety is present. As in safrole (25), this more down field two proton doublet at δ 3.30 suggested the allyl moiety was directly attached to the aromatic ring. The UV absorption pattern revealed that this allyl moiety is not conjugated with the aromatic ring.[44] Three proton singlet at δ 3.86 in its ^{1}H- NMR spectrum suggested it to have methyl group which was attached to the aromatic ring through an electronegative atom. The molecular formula of $C_{10}H_{10}O_2$ suggested it to be oxygen. The second most abundant ion at m/z 149 in its mass spectrum confirmed the characteristic loss of 15 for an aromatic methyl ether (Scheme 8). These spectral data suggested the compound should be either 21 or 18. Comparison of retention indices obtained from GC capillary chromatography with authentic samples of 21 and 18 with the isolated sample, confirmed it to be structure 21.

The spectral data of this compound agreed with those reported for 3-(4´-hydroxy-3´-methoxyphenyl)-propan-1-en, which has been reported as eugenol and therefore the compound was identified as eugenol (21).[46, 47]

4. 2. Isolation and identification of phenylpropanoids from PBL/7 and PBL/8

Fraction PBL/7, (0.5 g) was subjected to preparative column gas chromatography resulted an isolation of 3 fractions (PBL/7/1 to PBL/7/3) as pure compounds. Above fractionated number PBL/7/1 was identified as methyl eugenol (24).



Scheme 8: Mass spectral fragmentation pattren of eugenol (21)

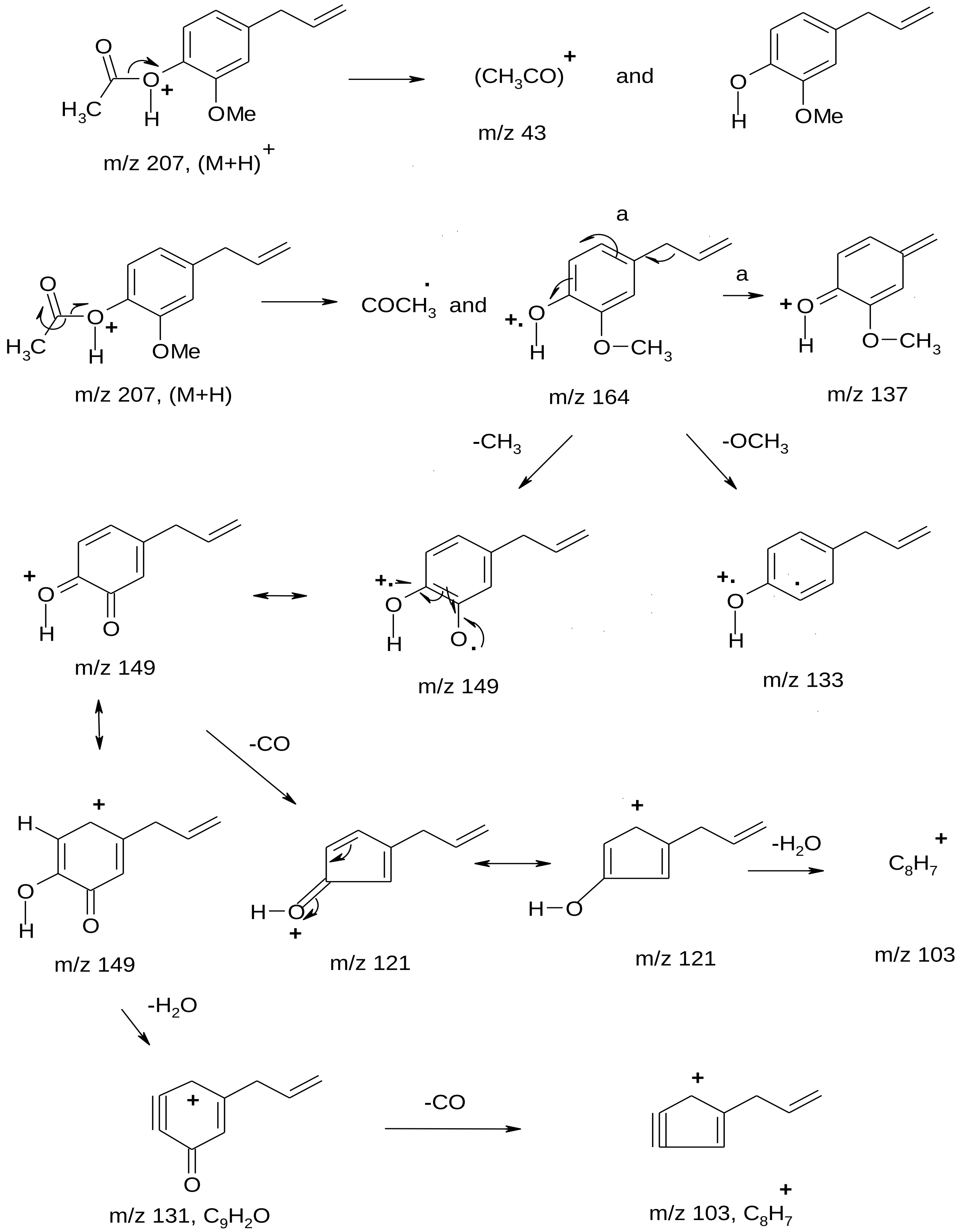
Its UV absorption pattern and its maxima at 228 and 283 suggested it to have a phenylpropanoid skeleton with substitutions at 3rd and 4th positions in the aromatic ring as that of eugenol (21). This was also confirmed by its ^{1}H-NMR spectrum and IR spectrum. Its UV absorption pattern also showed its aromatic ring to have no conjugation with its propanoid skeleton.[44] Its mass spectrum with a molecular ion at m/z 178 suggested a molecular formula of $C_{11}H_{14}O_2$. Doublets with *J*=7.94 Hz at δ 6.83 and *J*=1.99 Hz at δ 6.76 and double doublet with *J*=8.49 and 1.99 Hz at δ 6.78 in its ^{1}H-NMR spectrum suggested that there were three un-substituted aromatic positions having a 1, 2, 4-relationship. This observation also agreed with the substituents at 3 and 4 position in the aromatic ring. Sharp down field three-proton singlets at δ 3.89 and 3.88 in its ^{1}H-NMR spectrum and two peaks at δ 56.34 and 56.20 in its ^{13}C-NMR spectrum suggested that two methyl groups to be present in the aromatic ring through electronegative atoms. This was confirmed with more down field values of δ 149.27 and 147.76 in its ^{13}C-NMR spectrum due to C-3 and C-4 aromatic carbons. A molecular formula of $C_{11}H_{14}O_2$ suggested that these electronegative atoms to be oxygens. Similar pattern of its ^{1}H-NMR signals due to its allylic protons like eugenol (21) suggested that the allyl group was substituted to the aromatic ring through its saturated carbon atom. Therefore the compound was suggested to be 3-(3´,4´dimethoxyphenyl)-propan-1-en, (methyl eugenol) (24).

Comparison of the spectral data of methyl eugenol (24) with those reported[42, 48] for the compound with this structure showed them to be identical, confirming it to be methyl eugenol (24).

PBL/7/2 gave a compound (24a) whose ^{1}H-NMR and IR spectrum suggested it to be a phenylpropanoid. A similar pattern observed from its UV spectrum as that of eugenol (21) confirmed it to be a phenyl propanoid with substitutions at the 3rd and 4th positions in the aromatic ring. Mass spectral analysis suggested its molecular formula to be $C_{12}H_{14}O_3$. A down-field signal at δ 169.66 in its ^{13}C-NMR spectrum suggested it to have carbonyl carbon, which attached to the electronegative group. An up-field three-proton singlet at δ 2.33 in its ^{1}H-NMR and a peak at δ 21.10 in its ^{13}C-NMR suggesting it to have a methyl group and therefore it was believed to be the compound containing a acetate group. Base peak at m/z 164 and fragment peak at m/z 43 in its mass spectrum suggested a loss of a $CH_3C{=}O$ group, confirmed the presence of an acetate group. Another down-field signal appearing at δ 139.42 in ^{13}C-NMR suggesting that the acetate group was attached to the aromatic ring through its oxygen atom. Another three-proton singlet at δ 3.84 in its ^{1}H-NMR and signal at δ 56.21 in its ^{13}C-NMR suggested it as having another methyl group, which attached to the electronegative atom. Molecular formula of $C_{12}H_{14}O_3$ confirmed it to be an oxygen atom. A signal appearing at δ 151.25 in its ^{13}C-NMR spectrum suggested this OCH_3 group to be attached to the aromatic ring. Doublets at δ 6.97 with *J*=7.96 Hz and at δ 6.78 with *J*=1.34 Hz and a double doublet at δ 6.80 with *J*=8.21 and 1.58 Hz in its ^{1}H-NMR spectrum suggested that there were three un-substituted aromatic positions having a 1, 2, 4-relationship. This observation also agreed with the substituents at the 3rd and 4th positions in the aromatic ring namely, acetate and methoxy groups. ^{1}H-NMR and ^{13}C-NMR suggesting it also to have an allyl moiety attached to the aromatic ring as that of eugenol (21). Therefore the structure of the compound was suggested to be either (24a) or (19). Its GC retention index coincided with those reported[42] for eugenyl acetate confirmed it to be 3-(3´-methoxy-4´-acetoxy)-propan-1-en, (24a). A plausible mass spectral fragmentation of compound (24a) is shown in Scheme 9.

Comparison of the spectral data of eugenyl acetate (24a) with those reported[49, 50, 42] for the compound with this structure also showed them to be identical, confirming it to be eugenyl acetate (24a).

The fraction PBL/7/3 was identified as allylpyrocatechol diacetate (16). Its UV absorptions at 226 and 272 suggesting it too have phenyl propanoid skeleton. This was confirmed by its ^{1}H-NMR and IR spectral data. Its UV absorption pattern also showed its aromatic ring to have no conjugation with its propanoid skeleton.[44] Its mass spectrum with a molecular ion at m/z 235 $(M+H)^+$ suggested a molecular formula of $C_{13}H_{14}O_4$. The 400 MHz ^{1}H-NMR spectrum of isolate 16 shows two singlets at δ 2.31 and 2.30 integrating to six protons, suggesting two methyl groups to be present. The 100 MHz ^{13}C-NMR spectrum of compound 16



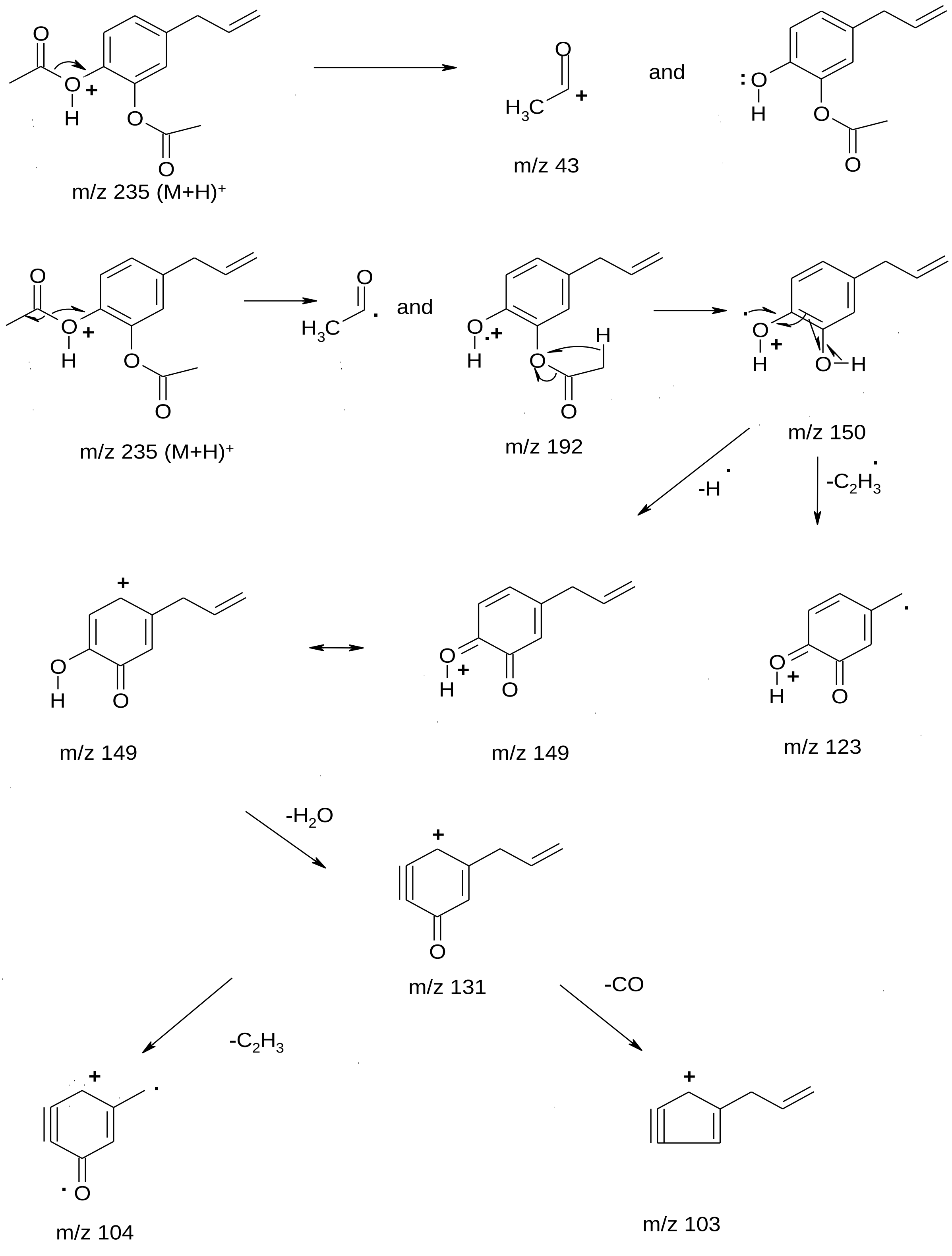
Scheme 9: Mass spectral fragmentations of eugenyl acetate (24a)

showed two down field signals at δ 168.90 and 168.84 indicating two carbonyl groups were present. MS fragment ions at m/z 192 and 43 indicated the loss of acetate. A base peak at m/z 150 revealed the loss of another acetate group. Doublets with *J*=8.14 Hz at δ 7.13 and with *J*=1.80 Hz δ 7.03 and a double doublet with *J*=8.31 and 1.91 Hz at δ 7.09 in its ^{1}H-NMR spectrum suggested that there were three un- substituted aromatic positions having a 1, 2, 4-relationship. Two-proton multiplet centering at δ 5.13, single-proton multiplet at δ 5.96 and the two-proton doublet with *J*=6.71 Hz at δ 3.41 suggested an allyl moiety was present. This more down field two proton doublet at δ 3.41 suggested the allyl moiety was directly attached to the aromatic ring through its saturated carbon atom.

Therefore the compound 16 is suggested to be 3-(3´,4´-diacetyloxy phenyl)-propan-1-en, (allylpyrocatechol diacetate) (16).

Comparison of the spectral data of allylpyrocatechol diacetate (16) with those reported[20] for the compound with this structure showed them to be identical, confirming it to be allylpyrocatechol diacetate (16). Scheme 10 illustrates mass spectral fragmentations of compound 16.

PBL/8/4/1 was obtained from fraction PBL/8 using repeated flash chromatography with dichloromethane:methanol (9.5:0.5) and toluene:ethyl acetate (9:1) followed by HPLC with MeOH-H_2O. Its ^{1}H-NMR and IR spectrum suggested it to be a phenylpropanoid. A similar pattern observed from its UV spectrum as that of allylpyrocatechol diacetate (16) confirmed it to be a phenyl propanoid with substitutions at 3 and 4 positions in the aromatic ring. Its mass spectral analysis suggested its molecular formula to be $C_{11}H_{12}O_3$. A three-proton singlet at δ 2.32 in its ^{1}H-NMR suggested it to have methyl group. A base peak at m/z 150 and fragment peak at m/z 43 in its mass spectrum suggested a loss of CH_3C=O group suggesting a presence of an acetate group. A D_2O exchangeable peak at δ 6.12 in its ^{1}H- NMR spectrum suggesting it too have an OH group. Peaks at 3401 and 1746 cm^{-1} in its IR spectrum confirmed the presence of an OH group and a carbonyl group, respectively. A two-proton multiplet centering at δ 5.09, single- proton multiplet at δ 5.94 and two-proton doublet with *J*=6.75 Hz at



Scheme 10: Mass spectral fragmentations of allylpyrocatechol diacetate (16)

δ 3.32 suggested an allyl moiety to be present. Doublets at δ 6.99 with *J*=8.18 Hz and at δ 6.80 with *J*=2.02 Hz and a double doublet at δ 6.92 with *J*=8.02 and 2.01 Hz in its ^{1}H-NMR spectrum suggested that there were three un-substituted aromatic positions having a 1, 2, 4-relationship. This observation also agreed with the substituents at the 3rd and 4th positions in the aromatic ring namely acetate and hydroxyl groups with respect to the allyl group attached to the aromatic ring.

Therefore the structure of the compound was suggested to be either 17 or 17a. Methylation of the compound with methyl iodide gave a product whose retention index and mass spectral pattern agreed with eugenyl acetate[42] (24a) confirmed the structure of the compound to be 3-(4´-acetoxy-3´-hydroxy)-propan-1-en, allylpyrocatechol monocetate (17).

^{1}H-NMR and IR spectrum of PBL/8/4/2 suggested it also to be a phenyl propanoid. It was confirmed by the UV pattern with its maxima appearing at 228 and 274. Its mass spectral analysis suggested its molecular formula to be $C_9H_{10}O_2$. The characteristic ^{1}H-NMR signals due to allyl moiety were also observed for this compound. Two broad D_2O exchangeable peaks at δ 5.54 and 5.46 in its ^{1}H-NMR suggested it to have two hydroxyl groups. On silica TLC, this compound was more polar than eugenol (21), confirming the presence of additional hydroxyl group to eugenol (21). Down field signals at δ 143.90 and 142.12 in its ^{13}C-NMR spectrum suggested that these two hydroxyl groups were directly attached to the aromatic ring. Doublets at δ 6.81 with *J*=8.05 Hz and at δ 6.73 with *J*=1.99 Hz and a double doublet at δ 6.65 with *J*=8.05 and 1.48 Hz in its ^{1}H-NMR spectrum suggested that there were three un-substituted aromatic positions having a 1, 2, 4-relationship. This observation also agreed with the substituents at 3rd and 4th positions in the aromatic ring with two hydroxyl groups with respect to the allyl group attached to the aromatic ring.

Comparison of the spectral data of hydroxychavicol (22) with those reported[48] for the compound with this structure showed them to be identical, confirming it to be hydroxychavicol (22).

4. 3. Insecticidal activity of *P. betle* L. leaf oil

The essential oil obtained from *Piper betle* leaf using pilot plant steam distillation was tested against adult *M. domestica* for insecticidal activity. These studies demonstrated that the essential oil of *P. betle* leaf exhibits insecticidal activity against *M. domestica* adults. LC_{50} values at the end of 24 and 48 hr exposure periods were 10.3 and 8.7 mg/dm^3 respectively.

Since our idea was to study an insecticidal activity of essential oil of *P. betle* leaf we compared our results also with insecticidally active essential oil as a standard. Since citronella (*Cymbopogon nardus*) oil is used as insecticidal agent against *Sitotroga cerealella* (Olivier)[51] we used citronella oil as a standard showed LC_{50} of 26.5 and 24.2 mg/dm^3 for the same exposure periods. These results indicated that *P. betle* leaf oil is to be more promising than the Ceylon citronella oil, registering 2.6 and 2.8 times (in terms of LC_{50} values) more toxicity at 24 and 48 hr exposure periods to housefly adults (Table 4).

Citronellal (54) which is one of the active constituent of citronella oil is also insecticidal to *M. domestica* and *Tribolium castaneuma* (Herbst) (Red flour betle) and larvisidal to *Diabrotica undecimpunctata howardi* Barber (southern corn rootworm).[52] Therefore we used citronellal (54) as a standard to compare with the compounds isolated from *P. betle* leaf oil and safrole analogs.

Bioassay guided fractionation revealed safrole (25) and eugenol (21) as the active principles against *M. domestica,* safrole (25), showing LC_{50} of 4.8 and 4.7 mg/dm^3 and eugenol (21), 7.3 and 6.2 mg/dm^3 for the periods of 24 and 48 hr, respectively, while citronellal (54) (synthetic standard) showed equal LC_{50} values of 14.3 mg/dm^3 for the same exposure periods. This indicates that safrole (25) and eugenol (21) are almost three and two times active than citronellal (54) against *M. domestica.*

Since safrole (25) (52.7%) and eugenol (21) (6.4%), abundant in the oil both have insecticidal activity, thus accounting for the insecticidal activity of *P. betle* leaf oil. (Table 7).

Since safrole (25) is the most active compound present in this oil analogs of safrole were prepared to get increased insecticidal activity. As safrole (25) has an allylic moiety attached to the aromatic ring, 8 analogs of safrole were prepared starting from safrole (25) to study the structure activity relationships. Since safrole (25) also has a methylenedioxy moiety, allyl benzene (53) was used to observe whether this methylenedioxy moiety is essential for the activity. Among the laboratory prepared safrole analogues, isosafrole (45), dihydrosafrole (46) and compound 48 showed insecticidal properties having LC_{50} values of 2.3, 4.7 & 37.4 mg/dm^3, and 2.2, 4.7 & 29.8 mg/dm^3 for 24 and 48 hr exposures respectively. These observations revealed that isosafrole (45) to be more active than safrole (25). It was also demonstrated by Huang et al., showing isosafrole (45) to be more toxic than safrole (25) against adults of *Sitophilus zeamais* and *Tribolium castaneum.*[53] Their conclusions are in agreement with our investigations here against housefly adults. Our studies also proved that isosafrole (45) was almost 6 times active than citronellal (54). These studies have also shown dihydrosafrole (46) as more toxic agents compared to citronellal (54).

Since dihydrosafrole (46) is almost equally insecticidally active as that of safrole (25), dihydrosafrole (46) will also be a potential substituent as a insecticidal compound for the safrole (25).

The only structural difference between safrole (25) and allylbenzene (53) is the additional presence of methylendioxy moiety in safrole (25). Since allylbenzene (53) was not active even at the highest dose tested (Table 6) revealed that, the presence of substituents at the 3rd and 4th positions in the aromatic ring of the safrole (25) skeleton is an important factor for the insecticidal activity.

In our experiments we have isolated and elucidated the structures of five naturally occurring phenyl propanoids, 16, 17, 22, 24 & 24a from fractions of PBL/7 and PBL/8. Though the basic skeletons of these five compounds are similar to that of active fractions (PBL/3 & PBL/5) i.e. safrole (25) and eugenol (21), none of them are active against the housefly. Therefore it is confirmed that the specificity of the substituents at the 3rd and 4th positions at the aromatic ring play an important role for the insecticidal activity. Considering 3rd and 4th positions of their aromatic rings, safrole (25) and eugenol (21) each contain a total of two oxygen and one carbon atoms. But unlike safrole (25) and eugenol (21), allylpyrochatechol diacetate (16) with four oxygen and four carbon atoms, allylpyrocatechol monoacetate (17) with three oxygens and two carbons, hydroxychavicol (22) with only two oxygens, methyl eugenol (24) with two oxygen and two carbon atoms and eugenyl acetate (24a) with three oxygens and three carbons can be attributed for the inactivity of these five phenyl propanoids.

The mode of action of many insecticides is due to their disturbing effects on the function of nervous system of insects. The primary toxic action of organophosphates and carbamates involve inhibition of an important enzyme in the CNS, called cholinesterase. This enzyme is important in the process where impulses bridge the gap between two nerve cells, and when inhibited, the communication is disrupted causing death.

In our study, the insecticidal action on *M. domestica* of isosafrole, safrole, dihydrosafrole and eugenol may also be due to disturbance of the nervous system of the insect. But to confirm this will be necessary to conduct further experiments.

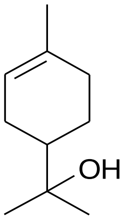
4. 4. Chemical composition of the *P. betle* L. leaf oil

Published data on Philippine *P. betle* leaf oil show that it contains phenolic compounds such as chavibetol (18), chavibetol acetate (19), allylpyrocatechol monoacetate (17), allylpyrocatechol diacetate (16), eugenol (21), methyl eugenol (24) and safrole (25) and terpenes, i.e. camphene (26), β-caryophyllene (27), 1,8-cineol (28), *p*-cymene (29), limonene (30), *α*-pinene (32) and *β*-pinene (33) of which chavibetol (18) (53.1%) and chavibetol acetate (19) (15.5%) are major and secondary components, respectively.[23] Sharma et al reported eugenol (21) (82.2% and 90.5%) and methyl eugenol (24) (6.9% and 4.1%), respectively, as major components from cultivars of Desi Bangla and Ramtek Bangla from India and *p*-cymene (29), α-terpineol (1), α-terpinyl acetate (35) as minor components.[26] The essential oil of *P. betle* flower mainly contained safrole (25) (27.6%) and myrcene (31) (26.4%) along with hydroxychavicol (22), eugenol (24), isoeugenol (23) and methyl eugenol (24) as minor components.[24]

GC-MS analysis of *P. betle* leaf oil revealed that there were compositional differences between the Sri Lankan oil with reported Philippine and Indian oils registering safrole (25) and allylpyrocatechol diacetate (16) as a major and secondary major compounds respectively. We believe the variation of the chemical composition of these two oils was due to the difference in geographical conditions of growth and maturity at harvesting of the materials.

In our studies we have identified 46 compounds from the Sri Lankan essential oil of *P. betle* leaf. In addition to that we were also able to identify a total of 98 compounds from the fractionation obtained from this oil. In these experiments we were able to separate terpene hydrocarbons, oxygenated terpenes and phenyl propanoids from the oil. Also, because of these fractionation experiments, we were able to identify some minor constituents present in the oil (Table 7).

ANNEX 1 (Structures 1 to 130)



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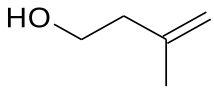

(2)
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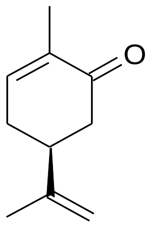
$CH_3(CH_2)_{11}OH$

(3)
CAS # 112-53-8



Carvone (4)
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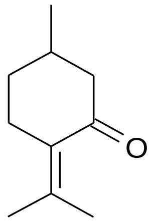

Pulegone (5)
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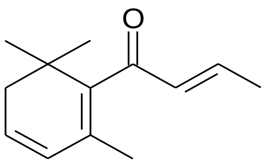

(*E*)–β–Damascenone (6)
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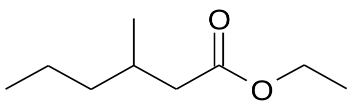
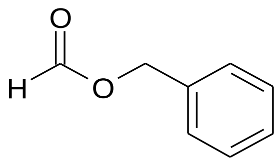


(7)



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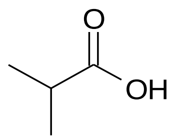
CAS # 104-57-4



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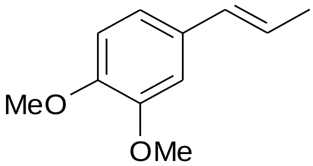
CAS # 79-31-2



(*E*)-Methyl isoeugenol (10)
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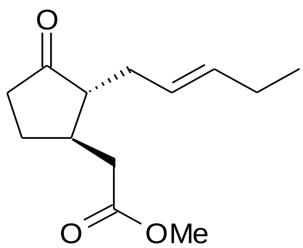

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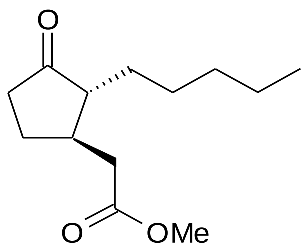

trans-Dihydro methyl jasmonate (12)
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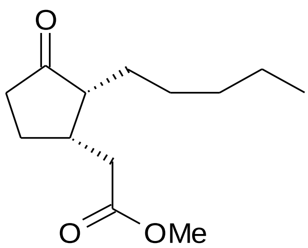
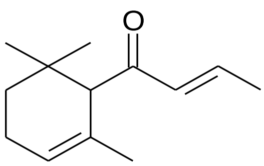


cis-epi-Dihydro methyl jasmonate (13)



α-Damascone (14)
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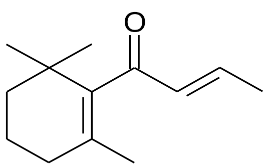
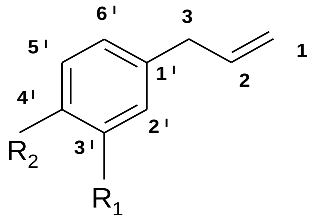


β-Damascone (15)



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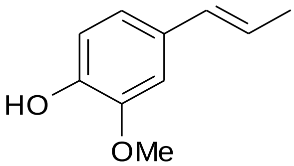

(*E*)-Isoeugenol (23)
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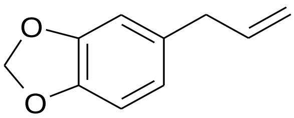

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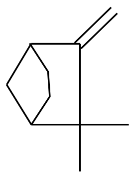

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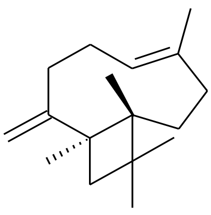

β-Caryophyllene (27)
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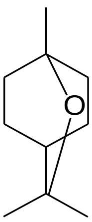

1,8-Cineole (28)
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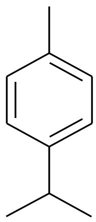
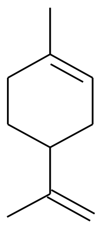


p-Cymene (29)
CAS # 99-87-6



Limonene (30)
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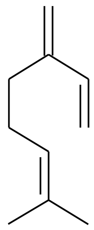

Myrcene (31)
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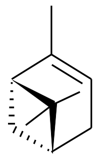
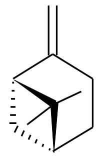
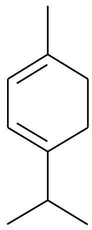


α-pinene (32)
CAS # 127-91-3



β-pinene (33)
CAS # 127-91-3



α-Terpinene (34)
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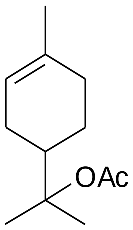

α-Terpineol acetate (35)
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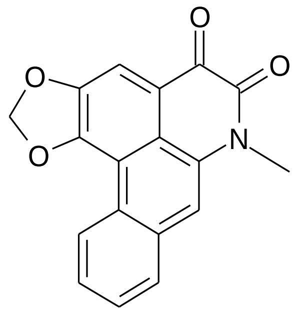

Cepharadione A (35a)
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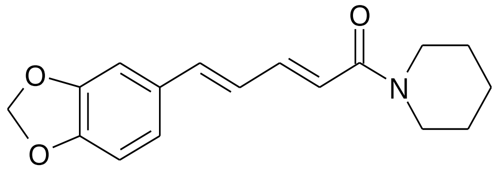

Piperine (35b)
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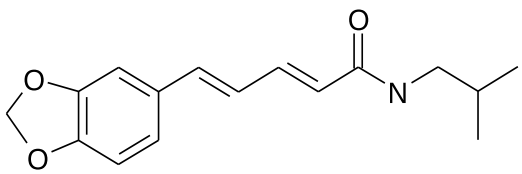
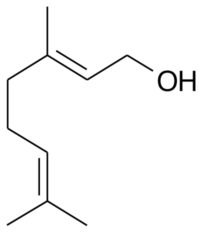


Piperlonguminine (36)



Geraniol (37)
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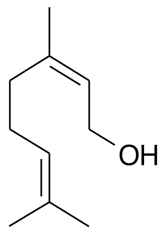

Nerol (38)
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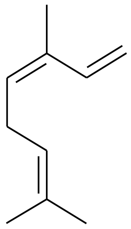

(*E*)-β-Ocimene (39)
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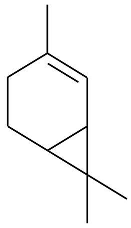
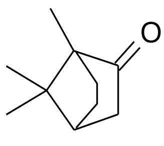
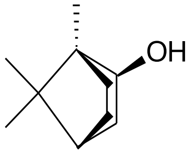


δ-2-Carene (40)
CAS # 554-61-0



Camphor (41)
CAS # 76-22-2



Borneol (42)
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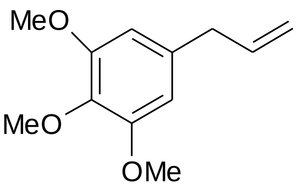

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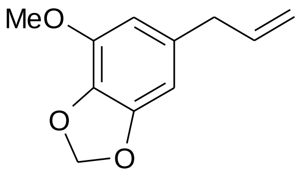

Myristicin (44)
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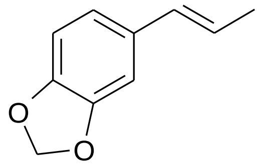

(*E*)-Isosafrole (45)
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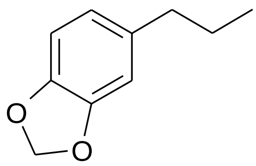

Dihydrosafrole (46)
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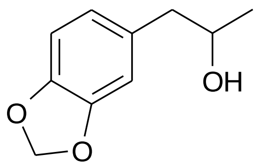
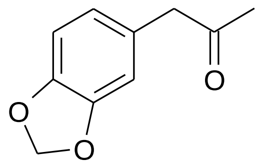
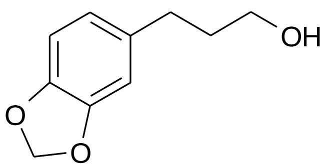


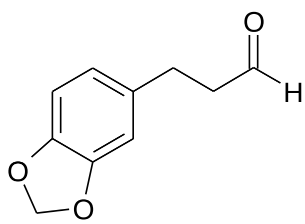
Dihydrosafrole-2-ol (47)
3-(3´,4´-methylenedioxyphenyl)-propan-2-ol
CAS # 6974-61-4



Dihydrosafrole-2-on (48)
Methyl piperonylacetone
CAS # 4676-39-5



Dihydrosafrole-1-ol (49)
2-piperonylethanol
CAS # 7031-03-0



Dihydrosafrole-1-al (50)
2-Piperonylethanal
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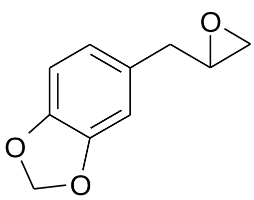
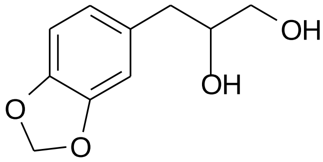
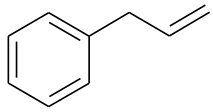


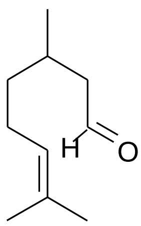
1,2-epoxy Dihydrosafrole (51)
3-(3´,4´-methylenedioxyphenyl)-1,2-epoxy-propan



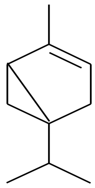
Dihydrosafrole-1,2-diol (52)
3-(3´,4´-methylenedioxyphenyl)-propan-1, 2-diol



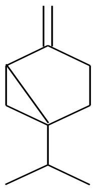
Allylbenzene (53)
CAS # 300-57-2



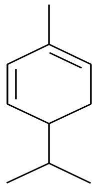
Citronellal (54)
CAS # 106-23-0



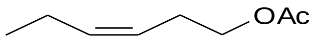
α-Thujene (55)
CAS # 2867-05-2



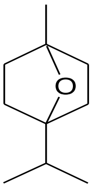
Sabinene (56)
CAS # 3387-41-5



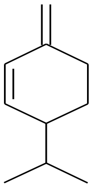
α-Phellandrene (57)
CAS # 99-83-2



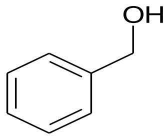
(*Z*)-3-Hexenyl acetate (57a)
CAS # 3681-71-8



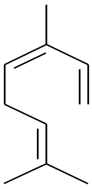
1, 4-Cineole (58)
CAS # 470-67-7



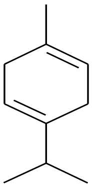
β-Phellandrene (59)
CAS # 555-10-2



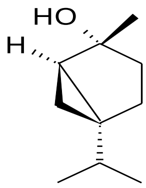
Benzyl alcohol (60)
CAS # 100-51-6



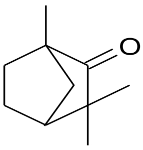
(*Z*)-β-Ocimene (61)
CAS # 27400-71-1



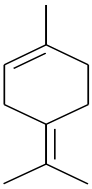
γ-Terpinene (62)
CAS # 555-10-2



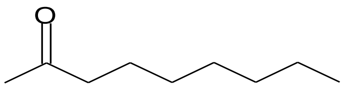
cis-Sabinene hydrate (63)
CAS # 17699-16-0



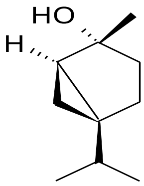
Fenchone (64)
CAS # 1195-79-5



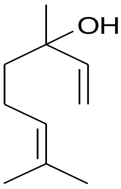
Terpinolene (65)
CAS # 586-62-9



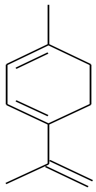
2-Nonanone (66)
CAS # 821-55-6



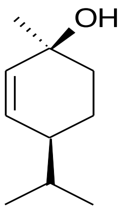
trans-Sabinene hydrate (67)
CAS # 15826-82-1



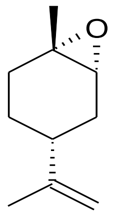
Linalool (68)
CAS # 78-70-6



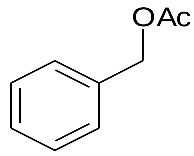
p-Menta-1, 3, 8 triene (69)
CAS # 18368-95-1



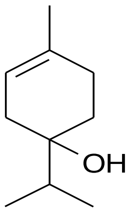
cis-*p*-Menth-2-en-1-ol (70)
CAS # 29803-82-5



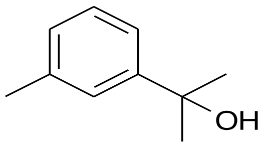
cis-Limonene oxide (71)
CAS # 13837-75-7



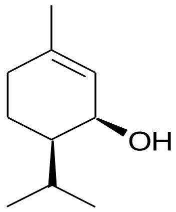
Benzyl acetate (72)
CAS # 140-11-4



Terpin-4-ol (73)
CAS # 562-74-3



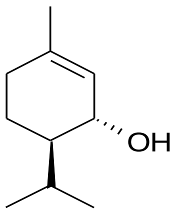
m-Cymen-8-ol (74)
CAS # 5208-37-7



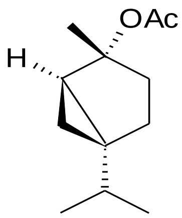
cis-Piperitol (75)
CAS # 16721-38-3

$CH_3(CH_2)_8CHO$

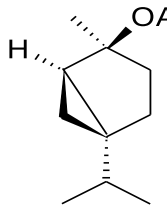
n-Decanal (76)
CAS # 112-31-2



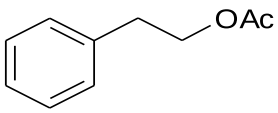
trans-Piperitol (77)
CAS # 16721-39-4



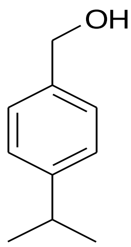
cis-Sabinene hydrate acetate (78)
CAS # 77318-48-0



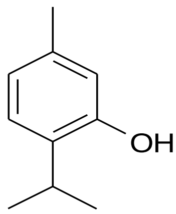
trans-Sabinene hydrate acetate (79)
CAS # 77318-47-9



2-Phenyl ethyl acetate (80)
CAS # 103-45-7



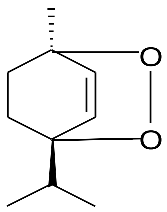
p-Cymen-7-ol (81)
CAS # 536-60-7



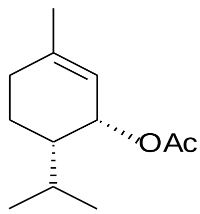
Thymol (82)
CAS # 89-83-8

$CH_3C(=O)(CH_2)_8CH_3$

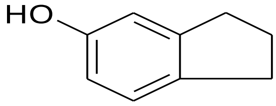
2-Undecanone (83)
CAS # 112-12-9



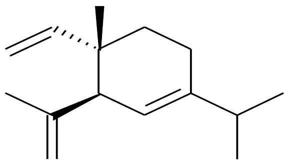
trans-Ascaridole (84)



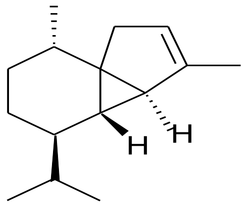
cis-Piperitol acetate (85)
CAS # 112028-22-5



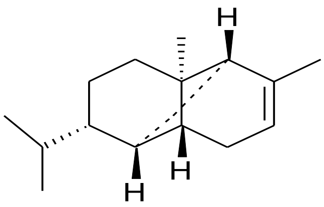
5-Indanol (86)
CAS # 1470-94-6



δ-Elemene (87)
CAS # 20307-84-0



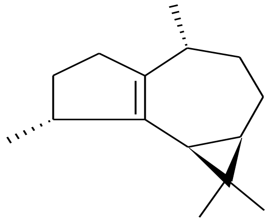
α-Cubebene (88)
CAS-17699-14-8



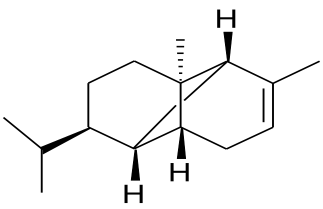
α-Ylangene (89)
CAS # 15917-91-6

$CH_3(CH_2)_{10}OH$

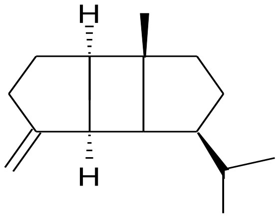
n-Undecanol (90)
CAS # 112-42-5



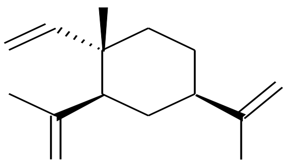
Isoledene (91)
CAS # 95910-36-4



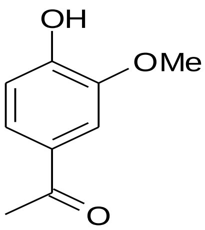
α-Copaene (92)
CAS # 3856-25-5



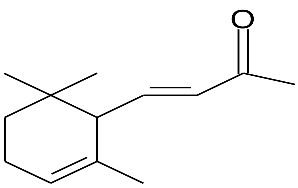
β-Bourbonene (93)
CAS # 5208-59-3



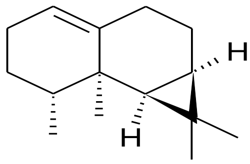
β-Elemene (94)
CAS # 515-13-9



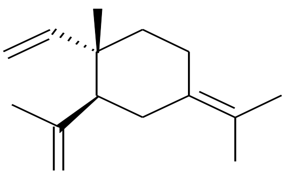
Vanillin (95)
CAS # 121-33-5



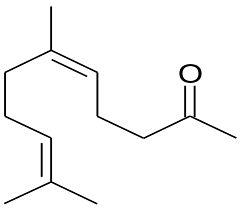
(*E*)-α-Ionone (96)
CAS # 127-41-3



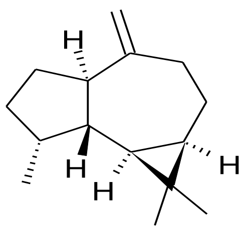
β-Gurjunene (97)
CAS # 17334-55-3



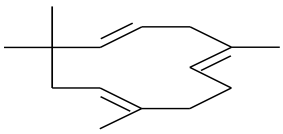
γ-Elemene (98)
CAS # 29873-99-2



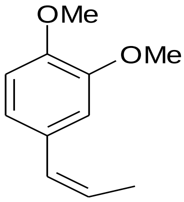
Neryl acetone (99)
CAS # 3879-26-3



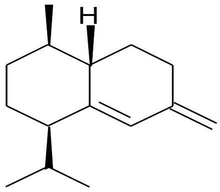
Aromadendrene (100)
CAS # 489-39-4



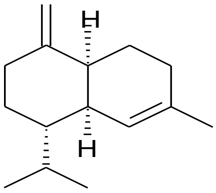
α-Humulene (101)
CAS # 6753-98-6



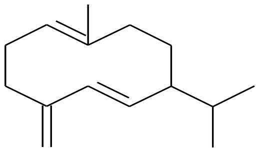
(*Z*)-Methyl isoeugenol (102)
CAS # 6380-24-1



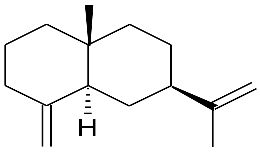
cis-Muurola-4(14),5-diene (103)



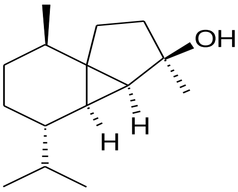
γ-Muurolene (104)
CAS # 30021-74-0



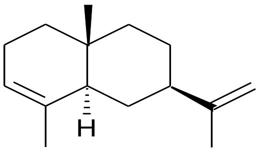
Germacrene D (105)
CAS # 23986-74-5



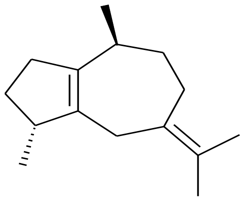
β-Selinene (106)
CAS # 17066-67-0



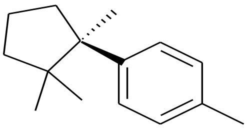
epi-Cubebol (107)
CAS # 23445-02-5



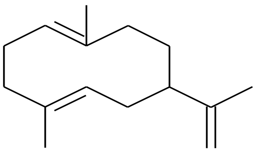
α-Selinene (108)
CAS # 23986-74-5



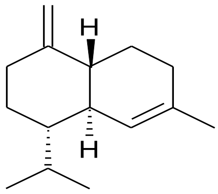
trans-β-Guaiene (109)
CAS # 53863-54-0



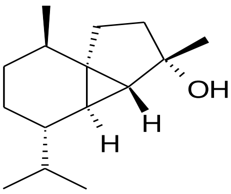
Cuparene (110)
CAS # 16982-00-6



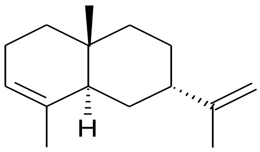
Germacrene A (111)
CAS # 28387-44-2



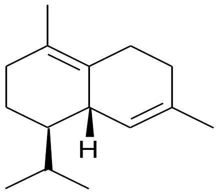
γ-Cadinene (112)
CAS # 39029-41-9



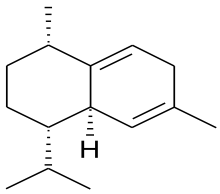
Cubebol (113)
CAS # 23445-02-5



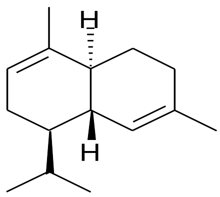
7-*epi*-α-Selinene (114)
CAS # 123123-37-5



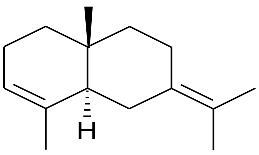
δ-Cadinene (115)
CAS # 39029-41-9



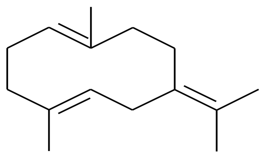
Cadina-1,4-diene (116)
CAS # 29837-12-5



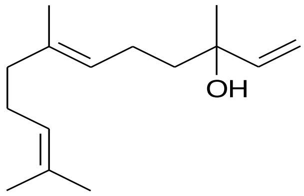
α-Cadinene (117)
CAS # 24406-05-1



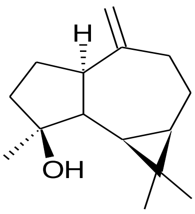
Selina-3,7(11)-diene (118)
CAS # 6813-21-4



Germacrene B (119)
CAS # 15423-57-1



(*E*)-Nerolidol (120)
CAS # 7212-44-44



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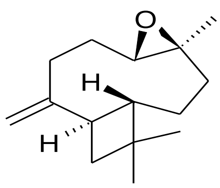
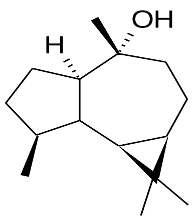


β-Caryophyllene oxide (122)
CAS # 1139-30-6



Globulol (123)
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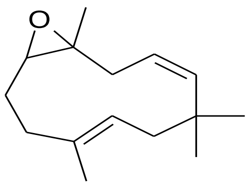

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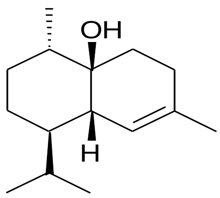
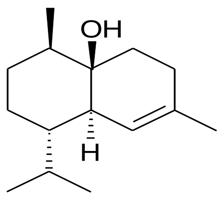


1-*epi*-Cubenol (125)
CAS # 19912-67-5



Cubenol (126)
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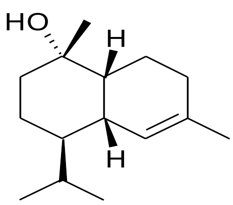
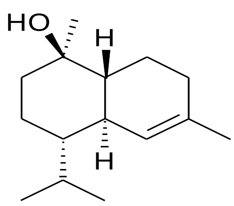


α-Muurolol (127)



α-Cadinol (128)
CAS # 481-34-5

$CH_3(CH_2)_{18}CH_3$

n-Eicosane (129)
CAS # 112-95-8

$CH_3(CH_2)_{20}CH_3$

n-Docosane (130)
CAS # 629-97-0

PART 2

CHEMISTRY AND INSECT NEUROPHYSIOLOGICAL ACTIVITY OF SOME SRI LANKAN ESSENTIAL OILS

5. INTRODUCTION

5. 1. Insect antenna

Insects use their antennae to locate food and to find their partners for mating. The antennae are situated on insect's head. Scapus and pedicellus are the two basal antennal segments to provide strength with muscles to move the antenna as a whole. Olfactory cells of insects are usually positioned on the antennae. The antenna consists of many small sensory units, called sensilla, which are used for the identification of odors and other sensory modalities such as taste, heat, touch, wind and sound. The stimulus molecules first reach to the olfactory sensilla. When the molecules are absorbed by the sensilla then there is a great chance to reach the molecules to the dendrites of the receptor cells in the sensilla lumen.[54, 55, 56]

According to the sensory and other requirements of the insect species, the size and the shape of the antenna can vary. Honeybees fly rather fast and also they have an ability to distinguish a large variety of odors. They live in hive. The hive's architecture is a combination of small cells made of wax. Therefore it is necessary to protect the antennae while passing through the small cells. For these resons honeybees have a rod shaped antenna, with relatively short sensory sensilla and several pore-plates. However male moths are large in size and fly slowly. They are sensitive to their pheromones even at the distance from some kms. Therefore saturniid antennae are large compared with the size of the body and have a feather or comb like structure. These antennae are the best filters to catch odor molecules from an air stream to efficiently access the olfactory receptor cells.[54]

5. 2. Chemosensory cells of insects

Insects have their chemosensory receptor cells in particular structures called sensillae. A cross section of sensillum with its sensory cells is shown in Figure 1a. Olfactory sensilla have many pores or grooves in the walls through which odor molecules pass. Stimulants meet receptors on the dendritic membranes of the sensory cells of the chemosensilla. The number of sensory cells per sensilum can differ from two to several dozen. The branched or un-branched dendrites float in the sensillum lymph, which is isolated from the haemolymph by shield cells.

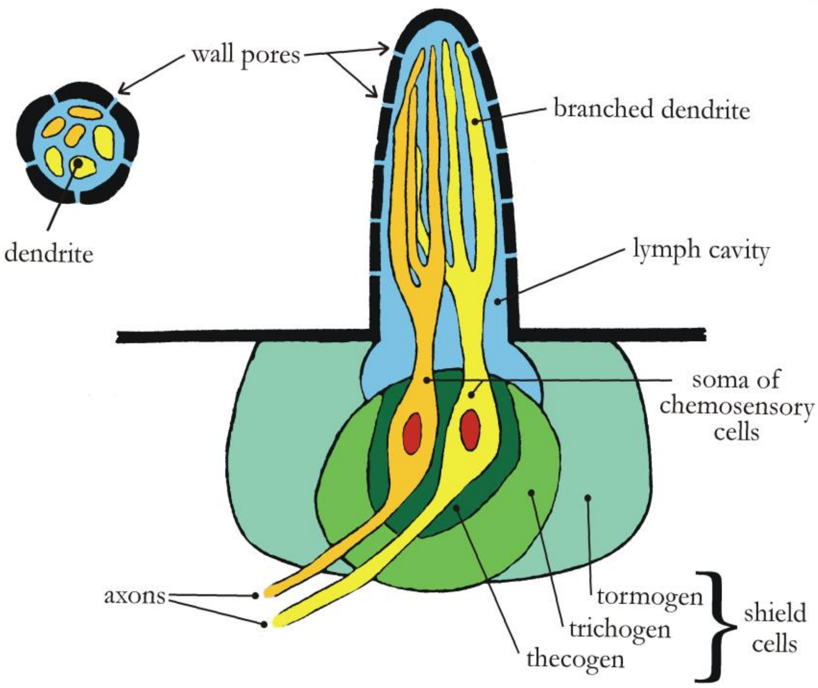


Figure 1a: Cross section of sensillum with its sensory cells (obtained from, Guerin *et al* [57])

The sensillum lymph is rich in odor binding proteins and cations, especially K^+ the latter secreted by the trichogen and tormogen cells. The thecogen cell serves to isolate the sensory units.[57]

A difference in electrical potential is maintained across the plasma membrane of the sensory cell, the inside being negatively charged with respect to the outside, which results in a resting potential of some 120 mV. The sensory cell depolarizes following stimulation through the opening of ion channels in the dendritic membrane. The resulting receptor potential serves to generate action potentials that travel along the axons of the sensory cells to the brain. This occurs primarily via the antennal nerve in insects.[57, 58]

5. 3. Electroantennography (EAG)

Electroantennography (EAG) is a useful method to study the insect-plant and insect-insect interactions for identification of semiochemicals.[59-68] This technique[58a] can be used to identify candidate compounds which may be attractants or repellent to the insects. This method provides a quick method for screening odors. EAG is the technique to measure the electrical potential generated by olfactory cells along the antenna of an insect following stimulation with an adequate stimulus. Although the EAGs measures the globle electrical potential of the antenna of insects, it doed not represent the summed receptor potentials of the population of olfactory cells on the antenna. By measuring a globle response from the population of olfactory cells, a broader response spectrum and faster detection of biologically important odors is possible. Substances, which do not induce any electrophysiological response can be disregarded. High electroantennogramme responses prove that an odor is detected by the insects. Figure 1b illustrates schematic representation of electroantennographic preparation system.

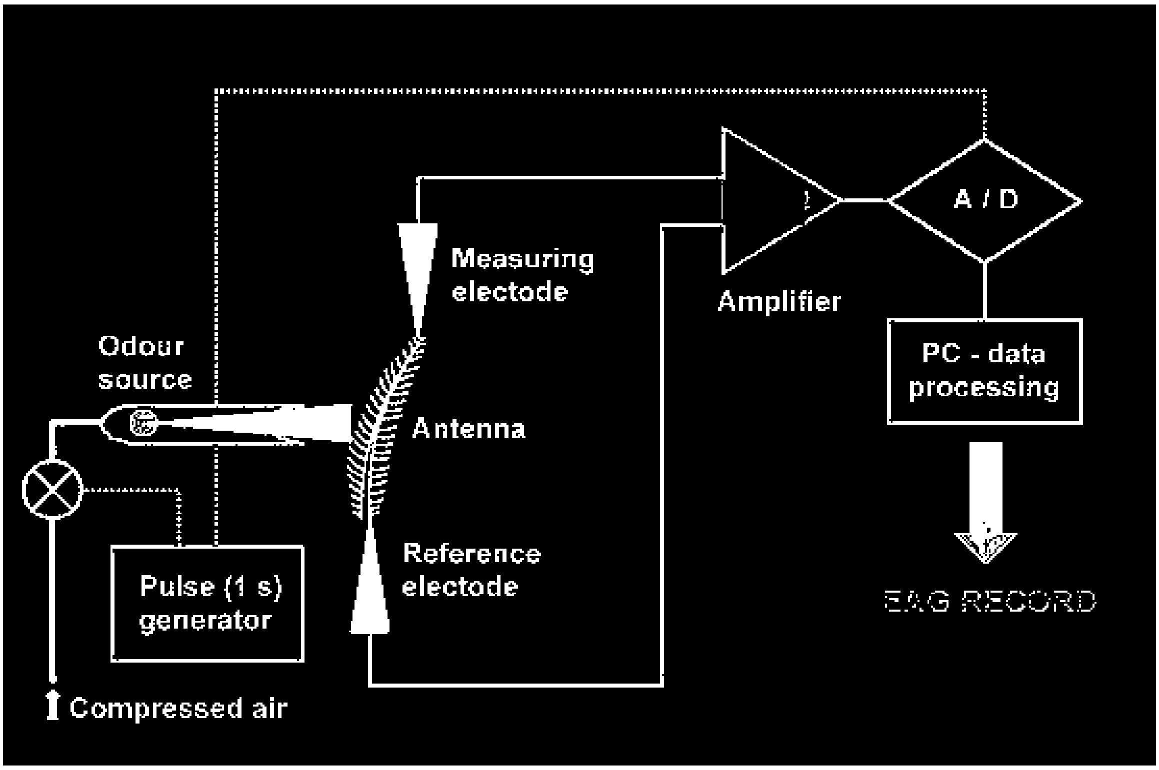


Figure 1b. Schematic representation of the typical EAG system

5. 3. 1. Gas chromatography-electroantennographic detection (GC-EAD)

Electroantennogram (EAG) recording of insect olfactory responses is used for identifying semiochemicals of importance for insects from complex samples of biological origin. To identify single chemostimuli in a substance, it is necessary to use a separation method like gas chromatography (GC). When the EAG is used separately from GC effluent, it is necessary to isolate and transfer the purified compounds to the EAG preparation. This does not take full advantage of the GC resolution and it may also result in a loss of detection for essential minor components. It is also time consuming. Coupling the GC and EAG is an extremely sensitive method for detecting semiochemicals. In this method volatile extracts can be first separated to individual components, on a high resolution capillary column half of which can pass to the FID and the other half to the EAG preparation as the components elute from the column to detect and record FID and EAG responses simultaneously.[69, 70] Finally the recorded EAG active peaks can be identified by GC-MS analysis.

5. 4. Mosquito[31]

Mosquito (family: Culicidae) with many species, numbering about 2500, is a well known insect to humans because of the blood sucking habits of the female. Mosquitoes are known to transmit serious diseases such as yellow fever, malaria, filariasis and dengue.

Adult mosquito have a slender and elongated body. Its long fragile legs and mouthparts, which contains an elongated proboscis are used for its characterization. Its antenne are threadlike appandage and in general the male’s antenna is bushier than that of the female. The males, and sometimes females also feed on nectar and other plant juices. The females need blood meals for their eggs to mature and, which are laid on the surface of water. The laid eggs hatch into aquatic larvae and swim with a circular movement and feed on organic waste. The pupae breathe through tubes on the thorax. The duration of life cycle mainly depends on the species. The noise emitted by the mosquito is a result of high frequencies of its wing beats. Female mosquito releases slightly lower frequency for the purpose of attracting the opposite sex.

There are three important genera of mosquitoes that transmit diseases to humans. *Anopheles* is the malaria vector carrier also transmits filariasis and encephalitis. *Anopheles* mosquitoes are easily recognized in their resting position. At rest the proboscis, head and body are held on a straight line to each other with at an angle to the surface. This mosquito usually selects places for breeding in water containing heavy vegetation. The life cycle is from 18 days to several weeks. Another important mosquito belongs to the genus *Culex*. It is a carrier of encephalitis and filariasis. The adults hold there body parallel to the resting surface and its proboscis is bent downward relative to the surface. Larvae hold their head downward at an angle of 45° from the water surface. The life cycle of this species is usually 10 to 14 days. The other important genus is *Aedes* who carries yellow fever, dengue and encephalitis. It also keeps its body like *Culex* parallel to the resting surface with the proboscis bent downward to the surface. Floodwater, rain pools or salt marshes are usually used by the *Aedes* for their breeding. The larvae also like those of *Culex*, hang its head down at a 45° angle from the water surface. Its life cycle may be as short as 10 days.

N, N-diethyl-3-methylbenzamide (DEET) is the most effective synthetic mosquito repellent currently on the market. This substance has a remarkable safety profile after 40 years of worldwide use. Citronella oil is commonly used as a plant based mosquito repellent. Pyrethrum is a powerful rapidly acting insecticide derived from the crushed and dried folwers of *Chrysanthemum cinerariifolium.* Pyrethrum does not repel insects but works as a contact insecticide, affecting nervous system that leads to the death or knockdown of the insect.[70a]

5. 4. 1. Host and oviposition attractant by mosquitoes

Heat and vision are two important stimuli utilized by mosquitoes to locate hosts. But olfaction is probably the most important stimulus. Volatiles help mosquitoes to locate hosts using their antennae by means of detecting compounds released by the host and following these chemical odors upstream towards the host source. L-lactic acid and carbon dioxide are the two compounds released by humans that are found to attractant to the mosquito, *A. aegypti* L.[71] DU *et al* identified nine stimulants, phenol, *p*-cresol (131), 4-ethylphenol, indole (132), 3-methylindole (133), nonanal, 2-undecanone, 2-tridecanone, naphthalene by GC-EAD analysis with gravid *C. quinquefasciatus* and *C. tarsalis* mosquito antenna of compounds from extracts of fermented infusions of Bermuda grass.[72] It is also reported that methyl butyrate, methyl propionate, ethyl acetate, isopropyl acetate, ethyl propionate, and ethyl butyrate evoked strong responses from antenna of gravid *A. aegypti*.[73]

5. 5. Tsetse flies[31]

Tsetse flies, an African blood-sucking insect belongs to the genus *Glossina*, family Glossinidae (order Diptera) with 21 species. It transmits sleeping sickness to humans and a similar disease called nagana (trypanosomiasis) to domestic animals. Tsetse flies readily feed on humans, domestic and wild animals. This pest is a serious threat for the people specially living in sub-Saharan region of Africa. It is reported that tsetse flies use *Lantana canara*, coffee and banana plantations as resting sites.[73a]

The appearance of all tsetse flies is similar. They are robust, larger in size than houseflies and range from 6 mm to 16 mm in length. Tsetse flies are rather dull in appearance varying from yellowish brown to dark brown. The wings are held over the back at rest. A long branched hair type arista on each antenna, which is different from other flies can can be used to differentiate tsetse flies. Its lifetime may extend for one to three months. In general, tsetse flies grow up in woodlands, but they may fly out a short distance into open grassland for their blood meal. Both sexes suck blood every 2-4 days, especially during the warmest part of the day. Most of the tsetse species stop their activities soon after sunset or at temperatures bellow 15.5 °C.

Trapping of flies and spraying with insecticides are the usual methods to control the tsetse population. Introduction of sterilized male flies sit into the wild population is a modern method for controlling tsetse flies.

5. 5. 1. Host attraction by tsetse flies

Tsetse fly odor mediated responses are efficient to locate hosts even at the distance of 60-120 m. They use their eyes to home in on the host when they are about 10 meters closer.[74-76] Thermal, short range- and contact-chemicals induce a sequence of thstse behaviors for landing and feeding on hosts.[77-80] It is also known that host breath constituents, such as carbon dioxide, acetone and 1-octen-3-ol (133a),[60, 61] and phenolic break down products such as *p*-cresol (131) and 3-n-propylphenol from host skin secretions and urine, are the chemostimuli components used by tsetse fly species to locate their hosts.[62-67] Nicholas *et al*[68] also analyzed Buffalo and Ox skin odor extracts by GC-EAD on tsetse antenna and found that, *p*-cresol (131), 3-n-propylphenol, (*E*)-2-heptenal, octanal, nonanal, decanal, undecanal, (*E*)-2-undecenal and dodecanal evoled responses for tsetse antenne. It has also been reported that different species of tsetse fly are attracted by the specific groups of vertebrate hosts.[81]

5. 6. Chemistry and biological activity of essential oils

5. 6. 1. *Callistemon citrinus*

The genus *Callistemon* belongs to the family Myrtaceae, having about 25 species and is indigenous to Australia.[82, 83]The plant is an ornamental and commonly known as bottlebrush. The trees are about 2-4 meter in height, leaves 3-6 cm long, 0.5-1.0 cm wide, red when young.[83]

The essential oil of *C. citrinus* (*C. lanceolatus*) is reported to have nematocidal and fungicidal activities.[84, 85] The essential oil composition of the plant varies according to its geographical location. Published data[86] on Pakistani *C. citrinus* leaf oil show that it contains 1,8-cineole (28) (44.6%), α-terpineol (1) (31.0%) and δ-cadinene (115) (4.2%) as a major components, whereas flower oil contains α-pinene (32) (42.7%) as a major component followed by linalool (68)

(18.3%) and δ-cadinene (115) (6.9%). Indian scientists, Bhagat *et al*,[83] also reported that the major component of this oil to be 1,8-cineole (28) (47.8 %) followed by α-pinene (32) (28%), α-terpineol (1) (10.6%) and limonene (30) (5.8%). The essential oil composition of the leaves of *C. rigidus* R. Br. is reported[87] to be 1,8-cineole (28) (89.9%) followed by α-terpineol (1) (5.8%), the major components of *C. polandii* are also reported[82] as 1,8-cineole (28) (31.93%) followed by myrcene (31) (14.69%), methyl cinnamate (134) (13.03%), humulene (101) (8.30%) and β-caryophyllene (27) (3.82%).

5. 6. 2. *P. nigram* leaf oil

The species *P. nigram* belongs to the family Piperaceae. It is a perennial climbing vine or shrub with a smooth woody stem with dark green, ovate, acuminate and thick leaves. The vines reach heights of 4.5 to 7.5 m climbing on supports (tree trunks or artificial supports). Fruits, botanically called drupes, but generally called berries, are dark green ovoid or globes. They turns bright orange and red when ripe.[88] The chemistry of the essential oil of *P. nigram* berries has been thoroughly investigated by Govindarajan.[88] It has been reported that Indian *P. nigram* leaf oil contains mostly sesquiterpene alcohols, elemol (135) (11.52 %) followed by caryophyllene alcohol (4.85 %) and (*Z*, *E*)-farnesol (137) (4.59%).[89]

5. 6. 3. *Cymbopogon flexuosus* D. C. (Lemon grass)

C. flexuosus, generally known as lemon grass is a member of the family Gramineae. *Cymbopogan* contains about 60 species, which often contain essential oils. Lemon grass also is one of the economically important essential oil bearing plants among the genus. Two types of lemon grasses are commonly named *C. flexuosus* (East Indian type) and *C. citratus* (West Indian type). *C. flexuosus* is indigenous to India and is cultivated in Ethiopia, Guatemala, India, Indonesia, Japan, Madagascar, Sri Lanka and Thailand. *C. citrates* is found in Anglo Argentina, Brazil, Cameroon, Cuba, Egypt, Guatemala, Indonesia, Jamaica, Madagascar, Mexico, Malaysia, Thailand, Sri Lanka, New Guinea and Philippines.[90] Published data showed that the major constituents of the essential oil of *C. flexuosus* are oxygenated monoterpenes, citral a (geranial) (138) (47 %) and citral b (neral) (139) (31 %) with 9.3 % of the monoterpene hydrocarbons, myrcene (31) and limonene (30).[90] It is also reported that this oil is larvicidal to the mosquito species *A. stephensi* while *C. citratus* also showes larvicidal effects against *A. aegypti*.[91]

5. 6. 4. *C. nardus* (Ceylon citronella)

The species *C. nadrus* belongs to the family Gramineae. There is another existing verity called *C. winterianus* known as Java citronella. Both verities of citronella are commercially important for making low cost perfumes.[90] It is reported that Ceylon citronella oil contains geraniol (37) (18.77 %) as major component. Methyl isoeugenol (9.74 %), limonene (30) (8,81 %) and camphene (26) (8.81 %) were also reported to be present at high amounts in this oil.[91]

Regnault-Roged *et al* reported that *C. nardus* oil contains citronellal (54) (33.8 %), geraniol (37) (21.6 %), citronellol (140) (9.2 %) and geranyl acetate (141) (3.4 %) as major constituents.[92] It has also been reported that the oil has larvicidal and repellent effects against *Aedes* sp., *C. quinquefasciatus* and *A. stephensi*[91] and insecticidal against *Sitrotoga cereallela*.[51]

5. 6. 5. *Myristica fragrans* L. (Nutmeg and Mace)

M. fragrans belongs to the family Myristicaceae. This plant is native to Amboina and Ternute in the East Indies. The plant was introduced to Sri Lanka in 1804. This is well known as a spice for food preparations. There are two types of spices produced by nutmeg call nutmeg and mace: the dried ripe seed named as nutmeg and the dried aril which surrounds the seed called mace, hence the two types of essential oils produced called nutmeg oil and mace oil. Sometimes nutmeg essential oil is used in after-shave lotion preparations.[90] Sabinene (56) (28.60 %), α-pinene (32) (13.90 %), terpinolene (65) (11.48 %), terpin-4-ol (73) (8.10 %) myristicin (44) (6.50 %) and elemicin (43) (3.40 %) are reported to be major compounds present in the nutmeg oil.[90] Regnault-Roged *et al* reported that *M. fragrans* L. oil contains β-pinene (33) (23.2 %), sabinene (56) (22.6 %)and myristicin (44) (7.9 %) as major constituents.[92] Huang *et al* illustrated that nutmeg essential oil showed contact toxicity, fumigant toxicity and antifeedent activity against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* moth.[93] It has also been reported that nutmeg oil showed repellent activity against *M. domestica*.[6]

Mace oil is reported to be contain sabinene (56) (24.45 %), terpin-4-ol (73) (14.63 %), β-pinene (33) (10.89 %), α-pinene (32) (10.00 %), myristicin (44) (5.18 %) and elemicin (43) (3.37 %) as major compounds.[90]

5. 6. 6. *Eucalyptus globules*

The genus *Eucalyptus* belongs to the plant family Myristicaceae. It is native to Australia and with more than 700 species. Reported data on chemical composition of *E. globules* indicated 1, 8-cineloe (28) (74.80 %) as a major followed by α-pinene (32) (16.9 %).[90] The essential oil has also showes larvicidal and repellent effects to the mosquitoes *C. quinquefasciatus* and *A. steohensi*.[91]

5. 6. 7. *Pinus* Species

The genus *Pinus* belongs to the family Pinaceae. It distributes throughout the world but native to northern temperate regions. Young trees are normally conical having horizontal branches. Most of the species have thick, rough bark. It can survive drought but needs sunlight and clean air for good growth.

The major importance of pines is for the construction and paper-product industries. It is also a source of turpentine, rosin, oils, and wood tars. Pine leaf oil is also has some medicinal value. Edible pine seeds are sold commercially as pine nuts.

P. sylvestris is known as Scots pine is tall ranging from 20-40 m, has a straight trunk up to one meter in diameter. This plant is distributed from Finland and Sweden to the mountains of Spain.[31] Manninen *et al* reported that pine needle oil and pine wood oil of *P. sylvestris* obtained from Finland contain α-pinene (32) (37.3 % and 44.1 %), Δ-3-carene (142) (10.7 % and 25.6 %) as a major compounds respectively.[94] Reported data show that *P. cembra* foliage contain α-pinene (32) (45.7 %), β-pinene (33) (25.1 %), and limonene (30) and β-phellandrene (59) (18.4 %) as a major constituents.[95] It has also been reported that industrial pine oil which is a by product of the wood pulp industry showed feeding deterrent activity for snowshoe hare and Townsend's vole (*Microtus townsendii*).[96]

5. 6. 8. *Tagetes erecta* (Marigold)

T. erecta belongs to the family Asteraceae. It is a herb, whose height ranges from 6 inches to 4 feet. Its flowers are orange, yellow, red, cream and maroon having rounded or flat heads. The plant has a rapid growth rate. It takes only 45-50 days to flower after seeding. There are many verities of Marigolds and new ones are introduced each year. It is reported that tagetone (143), dihydrotagetone (144), ociminone (145) are present in *T. minuta* and *T. tenuifolia*. The oil of *T. lucida* has alone about 80 % of methyl chavicol (146). *T. patula* and *T. erecta* usually contain limonene (30), α-terpinolene (65), piperitone (147) and caryophyllene components.[97] It has also been reported that head space extract of live flowers of *T. erecta* analyzed by gas chromatography linked to female *Helicoverpa armigera* electroantennograph (EAG) showed benzaldehyde, (*S*)-(-)-limonene, linalool (68), (*E*)-myroxide (148), (*Z*)-β-ocimene (61), phenylacetaldehyde, and (*R*)-(-)-piperitone as a EAG active compounds.[98]

5. 6. 9. *Eupotorium odoratum* L.

E. odoratum belongs to the family Compositae. The plant is known as Christmas bush or bitter bush. It may reach a height of 1 m or more when climbing into trees or shrubs. The stem is about 2 cm in diameter. The plants are maintained by a system of abundant fine lateral roots. The individual branches are long with relatively few branches. The leaves are aromatic when crushed. There are about 15 to 25 tubular flowers per head, white, lavender, pink, or blue in color. The seeds are brownish gray to black. Christmas bush blooms annually and is an abundant producer of seeds. Flowering and fruiting begins at the age of 1 year. The flowers are pollinated by insects. This plant grows from near sea level to over 1,000 m in elevation. The plant is native to Florida through the West Indies and from Texas through Central and South America to Argentina. It has been accidentally or deliberately introduced and has naturalized throughout much of the tropics.[99] Its leaf extracts with salt are used as a gargle for sore throats and colds. It is also used as scent aromatic baths.[100] Extracts of this plant are shown to inhibit or kill *Neisseria gonorrhoeae* in vitro[101] and to accelerate blood clotting.[102]

5. 6. 10. *Ocimum gratissimum* L.

The plant *O. gratissimum* L. belongs to the family Lamiaceae is widely distributed in tropical and warm temperature regions. The plant is used in folk medicine for treatment of many diseases such as for upper respiratory tract infections, diarrhea, headache, skin diseases, pneumonia, cough and fever.[103, 104] Previous studies on the essential oils of *O. canam*, *O. gratissimum*, *O. trichodon* and *O. urticifolium* obtained from Rwanda, exhibited antimicrobial activity.[105] It has also been reported that the volatile oil of this plant has mostly phenols, particularly thymol (82)[106, 107]which are most probably responsible for its reported antimicrobial activity.[104]

5. 6. 11. *Hemidesmus indicus* L.

H. indicus belongs to the family Asclepiadaceae. Its root is dull red to dark brown with fine longitudinal wrinkles. The leaves are thin and long. The plant has been reported to be used against syphilis, chronic rheumatism, urinary diseases and skin infection in folk remedies.[108] It is also reported to contain biologically active pregnane glycosides.[109]

5. 6. 12. *Lantana camara* L.

The *L. camara* belongs to the family Verbenaceae and comprises 150 species. It is a perennial shrub with a number of flower colors of red, white, yellow, pink and violet, native to tropical America, north to lower Texas and southern Georgia. It has been reported that the leaf tea is used to relieve anemia, cold, cough, fever and dysentery, also as a tonic, stimulant, and antibiotic.[110, 111] It is known to be poisonous too, and has caused fatalities in cattle, horses, sheep, dogs and humans.[110, 111] The toxic activity may be due to the presence of the triterpenes, lantadene A and B.[112] The essential oil of aerial parts of three samples of *L. camara* collected from the different places in the Amazon region of north Brazil showed different chemical compositions, of which the oil obtained from the state of Amapa showed limonene (30) (16.5 %), α-phellandrene (57) (16.4 %), germacrene D (105) (13.2 %) and β-caryophyllene (27) (10.8 %) as a major compounds and the sample collected from Roraima state showed germacrene D (105) (28.4 %) followed by germacrene B (119) (9.1 %), β-caryophyllene (27) (5.6 %), bicyclogermacrene (149) (3.9 %), whereas the sample from Para state showed γ-curcumene (150) + *ar*-curcumene (151) (27.6 %), α-zingiberene (152) (19.2 %), α-humulene (101) (10.7 %), β-caryophyllene (27) (5.9 %) and β-curcumene (153) (4.7 %) as major compounds.[111] The essential oil obtained from the plant materials of leaves and flowers collected from Cameroon showed major compound of *ar*-curcumene (151) (24.7 %) followed by β-caryophyllene (27) (13.3 %) and β-caryphyllene oxide (122) (7.06 %) whereas leaves and flowers collected from Madagascar showed davanone (295) (15.94 %) to be the major component followed by β-caryophyllene (27) (11.98 %) and sabinene (56) (9.02 %).[113] Commercial Brazilian lantana oil contains about 65 % bisabolene derivatives, and (-)-γ-curcumen-15-al (14.9 %) is reported to be the major compound present in this oil.[114] Mollenbeck *et al* reported that essential oils obtained from aerial parts of *L. camara* from Madagascar contains mainly β-caryophyllene (27) (18.8 %) followed by Δ-3-carene (142) (10.4 %).[115] These data on chemical composition of *L. camara* reveale that the oil composition varies dramatically according to geographical origin.

5. 7. Aim of the work

It is known that tsetse flies use their antenna to locate hosts. It is also known that compounds from the host breath, phenolic break-down products from the host skin secretions and urine are the volatile chemostimuli components used by tsetse flies to locate their hosts.[62-67]

In addition, tsetse flies fly only about 30 minutes per day to find a host for their blood meal. The rest of the day is spent under trees.[116] Therefore there may be some interactions with plant volatiles and tsetse flies to help them chose specific plants as resting sites during the day. Hence our aim is to screen different plant volatiles (essential oils) by GC-EAD on tsetse antenna (*G. pallidipes*) in order to identify the GC-EAD active compounds present in the essential oils.

We also analyzed the essential oil compositions of the oils, which we used for these studies.

It is known that the compounds relesed from humans attract mosquitoes. It is also known that the oviposition attractants are for mosquitoes occur in Bermuda grass infusion. These semiochemicals were identified by the help of electroantennography (EAG). EAG response studies of mosquito antenne with essential oil compounds has been studied only once.[124] Therefore we used 15 different essential oils to study the EAG responses on female *A. aegypti* L. mosquito to plant volatiles.

6. EXPERIMENTAL

6. 1. Collection of plant materials

Laboratory hydrodistilled essential oils were used for the experiments. Essential oils of 15 plants were used for the present study. *P. cembra* was collected from Engadin, Switzerland whereas *L. camara* was collected from Neuchatel (Papiliorama and Botanical garden), Switzerland; Papignon, France; Nairobi, Kenya and Kottawa, Sri Lanka. All the other plants were collected from different places in Sri Lanka (Table 8).

6. 2. Distillation of essential oils

6. 2. 1. Laboratory distillation of essential oils

Fresh leaves were air dried for 2 days and subjected to hydro distillation for 6 hr using the Clevenger arm distillation apparatus (Table 8).

6. 3. Sample preparation for GC-MS and GC-EAD analysis

6. 3. 1. Essential oils: 1 µl of each oil was dissolved in a 1 ml of CH_2Cl_2. 1 µl of solution was injected on the GC.

6. 3. 2. Headspace extracts: *L. camara* headspace of fresh leaves was trapped on Porapak Q (60-80 mesh) tubes using purified air pumping system and eluted by 1ml of CH_2Cl_2.

6. 3. 3. Headspace vapor analysis: The cut leaves of *L. camara* (5 g) were placed in a sealed glass bottle (50 ml) with a rubber septum. A syringe needle was then inserted into the bottle through the septum and odour saturated vapor was obtained (0.2 ml) from the bottle and injected into the GC-MS with a gas tight syringe.

6. 4. Insects

6. 4. 1. *Aedes aegypti*

3-4 days adult female, *A. aegypti* (mosquitoes), reared at Institute of Zoology, Animal Physiology Division, Neuchatel were used for the experiments.

6. 4. 2. *Glossina Pallidipes* (Tsetse fly)

The tsetse fly pupae were obtained from laboratory cultures reared at International Atomic Energy Agency, (IAEA) laboratory at Vienna, Austria and maintained at 30°C, 80% RH till eclosion. 3-4 day adults were used for the experiments.

6. 5. Electroantennogramme (EAG) recording from the *A. aegypti* antenna

The 3-4 day adult female *A. aegypti* mosquitoes were anesthetized with CO_2, mounted on an electrode, and placed under a microscope. EAGs were recorded with electrolyte-filled (a mixture of 0.1 M Nacl, 0.005 M KCl and 0.002 M $CaCl_2.2H_2O$) glass capillary electrodes. The reference electrode was inserted into the haemolymph space through the head of the insect to the bottom of the antenna and connected to ground. A similar recording electrode touched the cuticle at other end of the antenna. Ag/AgCl electrodes connected the glass electrodes to a high-impedance pre amplifier ($\sim 10^{12}$ Ω) and an AC/DC amplifier (AM-92, Syntech, Netherland) and to the hard disk of PC via a 16-bit analog-digital IDAC card (Syntech) using the EAG software package (Syntech).

The humid air and odor delivery system was essentially the same as that described by Guerin and Visser.[117]

Charcoal filtered air from a central supply passed continuously over the preparation at a flow of 1 m/sec from a glass tube, which terminated within 1 cm of the fixed preparation. Each of the essential oil (10 mg) was dissolved in CH_2Cl_2 (1 ml) and 100 μl aliquots were pipetted onto a piece of filter paper and solvent allowed to evaporate. The filter paper was then placed in a syring. The needle of the syring was inserted through a hole in the glass tube and 1 ml of the volume was evacuated in 1 s using a solenoid valve into the main air stream.[117a] Only CH_2Cl_2 was used as control. Relative humidity was maintained at 95-98% at 20-22°C for the preparation.

6. 5. 1. Electroantennogramme (EAG) recordings from *A. aegypti* antenne for essential oils

15 essential oils were used for the EAG recordings from *A. aegypti* antenne. Four different antennae were used for each oil. Since responses of the antennae for each oil were not significantly different from each other the average was taken as a response (Table 9 and Figure 1).

6. 6. GC-Electroantennographic Detection (GC-EAD) with tsetse fly antennae

Essential oils were injected on to the GC (Carlo Erba, 5160 series, Italy) with the flame ionization detector (FID at 240 °C). Split/split less injector temperature at 220 °C, split ratio (1: 40), splitless time 1 min, carrier gas H_2, 30 m column ZB-5 Phenomenex (stationary phase 5%diphenyl-95%dimethyl polysiloxane), 30 m, 0.25 mm ID, film thickness 0.25 µm, GC oven: isotherm 5 min at 60°C, 4°C/min gradient to 220°C and 20 min isotherm at 220°C, transfer line to the EAG preparation at 240°C. The column effluent was split in to two, so that one half was directed to the FID and the other half to the electrophysiological preparation. The latter half was swept by a humid air stream to the electrophysiological preparation from the heated transfer line in the wall of the chromatograph in such a way that the column effluent was simultaneously monitored by the FID and fly antenna.

The preparation of an insect was done according to the method described by Guerin and Visser[117] as follows. EAGs were recorded with electrolyte-filled (0.1 M KCl) glass capillary electrodes (internal diameter 2 mm). The 3-4 day adult male *G. pallidipes* was anaesthetized with CO_2 and its head was removed. The tip of the reference electrode was painted red to observe insertion of the electrode. It was inserted into the pedicellus at the base of the funiculus and put immediately under the microscope in the air stream which was described earlier (air flow 1 m/sec) with the moisture level of 95-98% RH at 20-22°C and then connected to ground. The distance between the preparation and end of the air stream source was maintained to approximately 1 cm. The unbroken tip of the recording electrode was brought into contact with the funiculus surface upon which the tip broke. Micro manipulators (Leitz) were adjusted to get accurate positioning of the preparation and recording electrode. The EAG signal was captured via a silver wire in the electrolyte-filled (0.1 M KCl) glass electrode connected to a high-impedance amplifier (~10^{12} Ω) and an AC/DC amplifier (4-USB, Syntech) and recorded on the hard disk of a PC with the FID signal via a 16-bit analog-digital IDAC card (Syntech, Netherland) by using GC-EAD software package (Syntech, Netherland).

The air delivery system was setup as same as that of described earlier, Guerin and Visser.[117]

6. 6. 1. GC-electroantennographic detection (GC-EAD) with tsetse fly antennae for essential oils

11 essential oils, *C. citrinus*, *P. betle* leaf, *E. globules*, *H. indicus* L., *T. erecta* L., *E. odoratum* L., *C. flexuosus* (lemon grass), *P. nigrum* leaf (pepper), *P. zeylanicus*, *M. fragrans* (mace) and *C. nardus* (Ceylon citronella) were used for the GC-electroantennographic detection using *G. pallidipes.* Three different antennae were used for each oil.

6. 7. GC-MS analysis of essential oils

The same GC-MS instruments and experimental conditions were used for essential oil analysis as in part 1 of this thesis except for *L. camara* and *P. cembra* analysis. The same instruments but with a DB wax (FFAP) (stationary phase-nitroterephthalic acid modified polyethylene glycol) capillary column (30 m, 0.25 mm ID, film thickness 0.25 µm) were used for analysis of *L. camara* and *P. cembra* oils. Temperatures- injector 220°C, transfer line 240°C, oven isotherm 5 min at 40°C, 5°C/min gradient to 230°C.

Purity of compounds was assessed by scaning the mass spectrum and establishing that each ion in the total ion chromatogramme was associated with the product in question.

6. 7. 1. Chiral GC column chromatography

A GC-chiral column was used for the enantiomeric analysis of *L. camara* head-space extract. The column was a 50% CD 6-O-TBDMS-2,3 di-O-n-But, ID 0.25 mm, 40 m long at 180 °C (isothermal), injector 180 °C, carrier gas He, flow 1.5 ml/min.

6. 8. Isolation of the most active chemostimuli from *C. citrinus* oil for *G. pallidipes* antennae

Since most GC-EAD active compounds present in the oil for tsetse antennae could not be identified from the available GC-MS libraries and mass spectral data records, the oil was subjected to column chromatography to isolate the active compounds.

6. 8. 1. Chromatography of the essential oil of *C. citrinus*

The essential oil of *C. citrinus* (2.14 g) was subjected to medium pressure liquid chromatography on silica gel using mixtures of toluene, EtOAc and MeOH and 8 fractions were obtained named as CC/1 to CC/8.

6. 8. 1. 1. Isolation of 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4. 3. 0]-non-1(6) -ene-2, 4-dione (154)

Fraction CC/6 (144 mg) obtained above from HPLC (column: Nucleosil SNG, C18, semi preparative, diameter 250mm. Solvent: Starting MeOH:H_2O 7:3 finishing MeOH during 20 min. Flow rate: 1.8 ml/min. Detector: UV λ_{max} 254 nm) gave 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4.3.0]-non-1(6)-ene-2,4-dione (154) as a white solid (14 mg), m.p 78-79°C. ^{1}H NMR (400 MHz): δ 2.76 (2H, s, 9-H_2), 1.49 (6H, s, 2xCH_3 at C-8), 1.40 (6H, s, 2xCH_3 at C-5), 1.36 (6H, s, 2xCH_3 at C-3). ^{13}C NMR: δ 214.44 (C-4), 195.33 (C-2), 176.95 (C-6), 109.82 (C-1), 91.24 (C-8), 55.48 (C-3), 45.73 (C-5), 39.96 (C-9), 28.50 (2x$\underline{C}H_3$ at C-8), 25.05 (2x$\underline{C}H_3$ at C-3), 24.40 (2x$\underline{C}H_3$ at C-5). IR: ν (cm^{-1}) 2974.6, 2936.4, 2872.5, 1713.5, 1636.4, 1628.1, 1470.4, 1413.0, 1287.3, 1121.4, 835.9 ; λ_{max} (nm, log ε): 274 (4.76) and 236 (4.07); m/z (rel. Int., %) : 236 (M^+) (48), 221 (4), 208 (4), 193

(6), 166 (100), 151 (20), 138 (8), 131 (10), 123 (24), 95 (8), 79(8), 70 (8), 41 (48). $C_{14}H_{20}O_3$, HRMS calculated 236.1413, found 236.1448.

6. 8. 1. 2. Isolation of flavesone (155)

The late eluting fraction was collected from fraction CC/6, as flavesone (155) (2mg), ^{1}H NMR (400 MHz) δ: 18.48 (s, 2'-O$\underline{H}$), 3.82 (1H, m, 2-H), 1.45 (6H, s, 2xC$\underline{H}_3$ at C-3'), 1.37 (6H, s, 2xC$\underline{H}_3$ at C-5'), 1.18 (6H, d, *J*=6.72 Hz, 2xC$\underline{H}_3$ at C-2). m/z (rel. Int., %) : 252 (M^+) (46), 237 (40), 224 (8), 219 (4), 209 (12), 191 (14), 182 (22), 181 (18), 164 (36), 153 (8), 149 (8), 139(22), 122 (12), 112 (6), 96 (10), 94 (6), 81 (20), 69 (23), 43 (100).

7. RESULTS

7. 1. Distillation of essential oils

Collected plant materials were subjected to laboratory hydro distillation for 6 hr using Clevenger arm distillation apparatus. The oil yields and countries and cities from which plant materials were collected, are given in the Table 8.

Table 8. Yields of essential oils from laboratory distillation

No.	Name of the plant	Family	Yield %,v/w	Collected at (city-country)
1.	*Piper betle* L.leaf	Piperaceae	0.37	Kottawa-SL*
2.	*Piper nigrum* leaf (Pepper)	Piperaceae	1.54	Aranayake-SL
3.	*Cymbopogon flexuosus* D. C. (Lemon grass) leaf	Gramineae	0.50	Hambantota-SL
4.	*Cymbopogon nardus* (Ceylon citronella)	Gramineae	0.90	Hambantota-SL
5.	*Callistemon citrinus* leaf	Myrtaceae	1.10	Kottawa-SL
6.	*Myristica fragrans* (Mace)	Myristicaceae	17.60	Rathnapura-SL
7.	*Myristica fragrans* (Nutmeg)	Myristicaceae	3.70	Rathnapura-SL
8.	*Eucalyptus globules* leaf	Myristicaceae	0.90	Colombo-SL
9.	*Pinus cembra* leaf	Pinaceae	0.79	Engedin-Switzerland
10.	*Tagetes erecta* flower	Asteraceae	0.42	Kottawa-SL
11.	*Eupotorium odoratum* L. leaf	Compositae	0.22	Kottawa-SL
12.	*Plectranthus zelanicus* leaf	Lamiaceae	0.34	Kottawa-SL
13.	*Ocimum gratissimum* L. leaf	Lamiaceae	0.92	Kottawa-SL
14.	*Hemidesmas indicus* L. leaf	Asclepiadaceae	0.30	Aranayake-SL
15.	*Lantana camara* leaf	Verbenaceae	0.35	Kottawa-SL
16.	*Lantana camara* leaf	Verbenaceae	0.31	Neuchatel (Papilior-ama) Switzerland
17.	*Lantana camara* leaf	Verbenaceae	0.30	Neuchatel (Botanical garden), Switzerland
18.	*Lantana camara* leaf	Verbenaceae	0.28	Papignon-France
19.	*Lantana camara* leaf	Verbenaceae	0.32	Niroby, Kenya

*SL – Sri Lanka

7. 2. Electroantennogramme responses of *A. aegypti*

Average EAG responses of 0.477 and 0.475 mV were recorded with oils of *C. flexuosus* and *L. camara* (Papiliorama, Neuchatel, Switzerland) respectively, from *A. aegypti* antennae. An average response of 0.264 mV was recorded from the oil of *H. indicus.* Significantly lower responses were recorded for the blanks (Table 9 and Figure 1).

Table 9. Electroantennographic responses (mV) on *A. aegypti* (Mosquito) antenna for 1 mg doses of each essential oil

Essential oil	Ante. 1 (mV)	Ante. 2 (mV)	Ante. 3 (mV)	Ante. 4 (mV)	Avarage (mV)	STD (mV)
Cymbopogon flexuosus D. C. (Lemon grass)	0.370	0.4	0.572	0.564	0.477	0.092
Lantana camara (Papiliorama, Switzerland)	0.461	0.391	0.606	0.443	0.475	0.080
Myristica fragrans (Mace)	0.370	0.355	0.505	0.632	0.466	0.112
Cymbopogon nardus	0.490	0.418	0.423	0.528	0.465	0.046
Pinus cembra (Engadin, Swistzerland)	0.415	0.466	0.499	0.457	0.459	0.030
Callistemon citrinus	0.336	0.366	0.629	0.463	0.449	0.114
Eucalyptus globules	0.420	0.501	0.387	0.472	0.445	0.044
Myristica fragrans (Nutmeg)	0.300	0.362	0.661	0.391	0.429	0.138
Lantana camara (Sri Lanka)	0.302	0.519	0.507	0.331	0.415	0.099
Tagetes erecta (flowers)	0.266	0.368	0.345	0.594	0.393	0.122
Plectranthus zeylanicus	0.424	0.434	0.235	0.392	0.371	0.080
Ocimon graticimom L.	0.460	0.267	0.254	0.353	0.334	0.082
Piper betle L.	0.239	0.222	0.423	0.437	0.33	0.100
Eupatorium odoratum L.	0.270	0.273	0.361	0.333	0.309	0.039
Hemidesmus indicus L.	0.165	0.269	0.275	0.348	0.264	0.065
Blank	0.073	0.118	0.095	0.117	0.101	0.018

Figure 2 illustrates electroantennogram responses recordes from *A. aegypti* antennae to the *P. zelanicus* oil and to the blank. Average

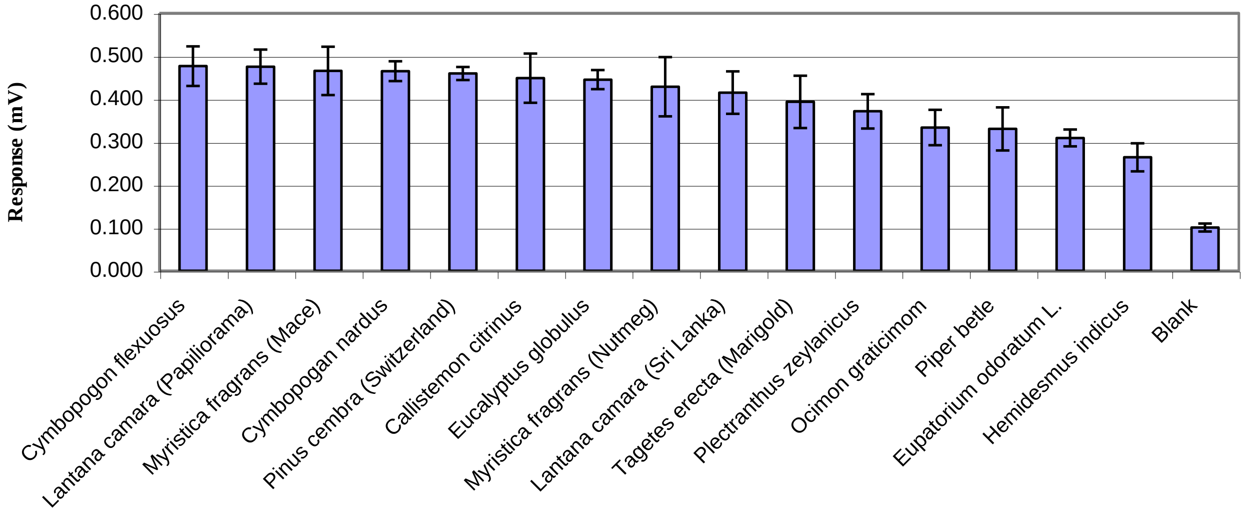


Figure 1. EAG responses of *A. aegypti to* essential oils (1 mg dose, average of four antennae)

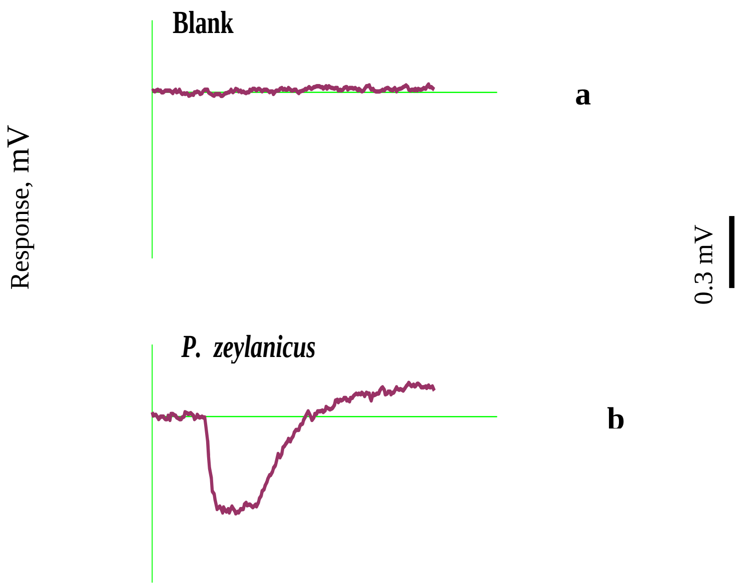


Figure 2. Electroantennogramme response of *A. aegypti* to *P. zeylanicus* oil (a-blank and b-oil)

7. 3. GC-EAD of active fractions in essential oils for *G. pallidipes* antennae

7. 3. 1. *C. citrinus* oil

36 compounds were identified from the oil out of which 1,8-cineole (28) was found to be the major (53.70 %) compound present. 12 compounds were found to activate *G. pallidipes* antennae. The major GC-EAG response was recorded to the β-diketone 154 followed by limonene (30), α-terpineol (1), spatulenol (121), α-humulene (101) and to the β-Caryophyllene oxide (122) (Table 10 & Figure3).

Table 10. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *C. citrinus* oil (simple letters in the last column are for the EAD responses recorded in figure 3)

Peak No.	Name	Percentage (%, w/w)	RI	R.I. Reference[42]	Active peaks in fig. 3
1	α-Thujene (55)	0.04	926	931	
2	α-Pinene (32)	3.12	932	939	
3	β-Pinene (33)	0.13	974	980	
4	α-Phellandrene (57)	0.31	998	1005	
5	Δ-3-Carene (142)	trace	1004	1011	
6	*p*-cymene (29)	2.7	1024	1026	a
7	Limonene (30)	4.32	1028	1031	b
8	1,8-Cineole (28)	53.70	1032	1033	c
9	γ-Terpinene (62)	0.13	1065	1062	
10	Terpinolene (65)	0.06	1097	1088	
11	Linalool (68)	0.22	1111	1098	
12	*endo*-Fenchol (157)	0.05	1124	1112	
13	*cis*-Pinene hydrate (158)	0.02	1133	1121	
14	α-Campholenal (159)	0.03	1138	1125	
15	*trans*-Pinocarveol (160)	0.75	1147	1139	d
16	Camphene hydrate (161)	0.03	1156	1148	
17	Terpin-4-ol (73)	1.14	1179	1177	e

Table 10 (Contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae for *C. citrinus* oil (simple letters in the last column are for the EAD responses recorded in figure 3)

Peak No.	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 3
18	*m*-Cymen-8-ol (74)	0.19	1187	1180	
19	α-Terpineol (1)	6.19	1189	1189	f
20	*trans*-Carveol (162)	0.09	1219	1217	
21	*cis*-Carveol (163)	0.05	1230	1229	
22	Unknown (MW 157)	2.55	1277	-	g
23	*trans*-Pinocarvyl acetate (164)	0.04	1306	1297	
24	Carvacrol (165)	0.11	1310	1298	
25	Unknown	0.05	1326	1316	
26	Eugenol (21)	0.13	1362	1356	
27	β-Caryophyllene (27)	0.32	1413	1418	h
28	Aromadendrene (100)	0.31	1436	1439	
29	α-Humulene (101)	0.08	1453	1454	i
30	α-Selinene (108)	trace	1497	1494	
31	β-Diketone 154	11.11	1524	-	j
32	Flavesone (155)	1.03	1546	1547[42a]	
33	Spathulenol (121)	2.91	1577	1576	k
34	β-Caryophyllene oxide (122)	0.72	1583	1581	l
35	Globulol (123)	0.11	1583	1583	
36	Humulene epoxide II (124)	0.15	1608	1606	
37	Leptospermone (156)	0.08	1616	1623[42a]	
38	Unknown (MW 250)	0.72	1626	-	

7. 3. 2. *P. betle* L. leaf oil (Laboratory distillation)

53 compounds were identified from the oil of which 24 compounds were active to the *G. pallidipes* antennae. Safrole (25) was found to be the major active compound followed by terpin-4-ol (73), β-caryophyllene (27), eugenol (21), germacrene D (105) and α-humulene (101) (Table 11 & Figure 4).

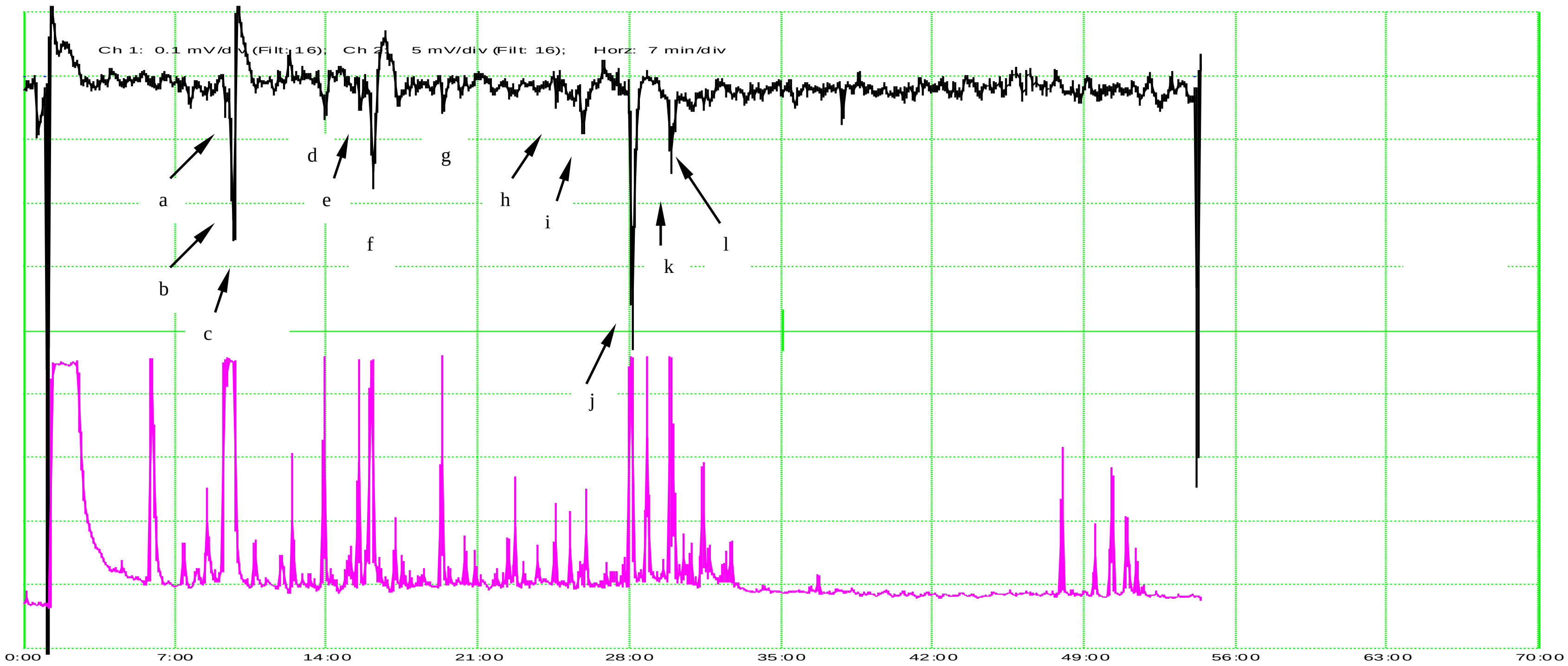


Figure 3. GC-EAD analysis of *C. citrinus* oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 10)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C.

Table 11. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from laboratory distilled *P. betle* leaf (simple letters in the last column are for the EAD responses recorded in to figure 4)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig.4
1	α- Thujene (55)	0.36	926	931	
2	α-Pinene (32)	0.67	933	939	a
3	Camphene (26)	0.27	948	953	
4	Sabinene (56)	3.66	971	976	b
5	Myrcene (31)	0.84	987	991	c
6	α-Phellandrene (57)	0.09	1008	1005	
7	α-Terpinene (34)	1.00	1013	1018	c^1
8	*p*-cymene (29)	0.72	1025	1026	d
9	β-Phellandrene (59)	1.22	1029	1031	e
10	1,8-Cineole (28)	0.32	1034	1033	
11	(*Z*)-β-Ocimene (61)	trace	1041	1041	
12	(*E*)-β-Ocimene (38)	0.05	1054	1050	f
13	γ-Terpinene (62)	1.41	1065	1062	g
14	Terpinolene (65)	0.33	1097	1088	h
15	Linalool (68)	0.38	1111	1098	i
16	Unknown	trace	1120	1099	j
17	*Cis*-*p*-Menth-2-en-1-ol (70)	0.04	1132	1121	k
18	Terpin-4-ol (73)	6.29	1178	1177	l
19	α-Terpineol (1)	0.19	1190	1189	l^1
20	Safrole (25)	43.50	1296	1285	m
21	2-Undecanone	trace	1291	1291	
22	δ-Elemene (87)	0.05	1339	1339	
23	α-Cubebene (88)	0.06	1349	1351	
24	Eugenol (21)	14.4	1361	1356	n
25	α-Ylangene (89)	0.08	1369	1372	
26	α-Copaene (92)	0.32	1372	1376	n^1
27	β-Bourbonene (93)	0.09	1381	1384	

Table 11 (Contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from laboratory distilled *P. betle* leaf (simple letters in the last column are for the EAD responses recorded in figure 4)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 4
28	β-elemene (94)	0.29	1386	1391	
29	Methyl eugenol (24)	0.63	1401	1401	
30	β-Caryophyllene (27)	1.43	1413	1418	o
31	β-Gurjunene (97)	0.10	1424	1432	
32	Aromadendrene (100)	0.04	1436	1439	
33	α-Humulene (101)	1.05	1452	1454	p
34	γ-Muurolene (104)	1.20	1477	1477	q
35	Germacrene D (105)	1.79	1482	1480	r
36	β-Selinene (106)	1.26	1487	1485	
37	α-Selinene (108)	1.60	1497	1494	
38	*trans*-β-Guaiene (109)	0.23	1501	1500	
39	(*E*, *E*)-α-Farnesene (167)	0.25	1507	1508	
40	γ-Cadinene (112)	0.09	1516	1513	
41	7-*epi*-α-Selinene (114)	0.24	1520	1517	
42	δ-Cadinene (115)	0.62	1524	1524	
43	Eugenyl acetate (24a)	3.94	1531	1524	s
44	α-Cadinene (117)	0.05	1538	1538	
45	Selina-3, 7(11)-diene (118)	0.03	1542	1542	
46	Germacrene B (119)	0.38	1557	1556	
47	Spathulenol (121)	0.19	1577	1576	t
48	Globulol (123)	0.18	1583	1583	
49	Humulene epoxide II (124)	trace	1605	1606	
50	1-*epi*-Cubenol (125)	0.15	1626	1627	u
51	Cubenol (126)	0.32	1642	1642	
52	Allylpyrocatechol diacetate (16)	6.08	1647	-	
53	α-Cadinol (128)	0.50	1660	1653	

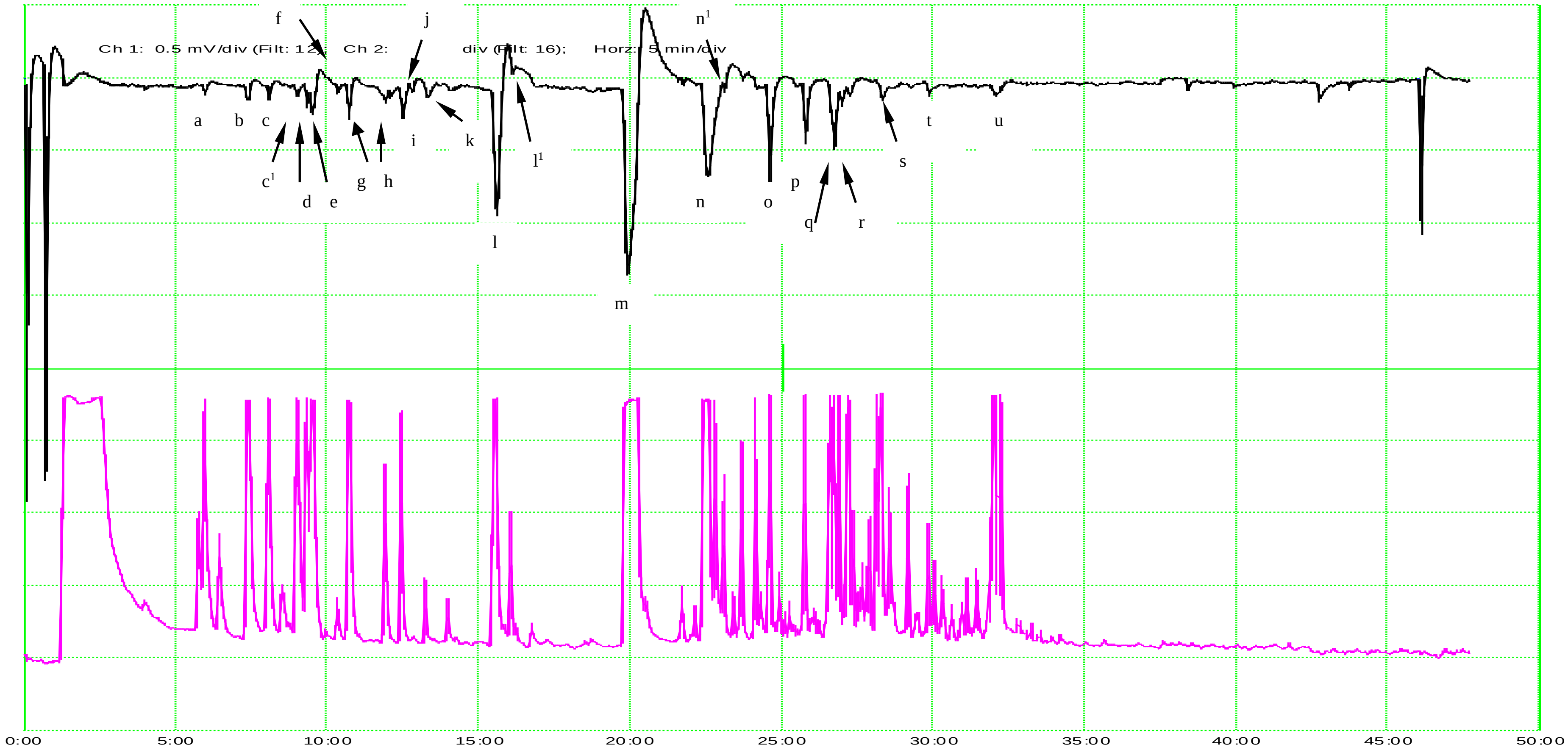


Figure 4. GC-EAD analysis of *P. betle* oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 11)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

7. 3. 3. *P. nigrum* leaf oil

52 compounds were identified from this oil out of which 21 were recorded as GC-EAD active stimulants. GC-EAD major responses were recorded to β-caryophyllene (27) followed by germacrene D (105), spatulenol (121), δ-elemene (87), α-humulene (101), methyl geranate (170), bicyclogermacrene (149) and (*E*)-nerolidol (120). (*E*)-nerolidol (120) (17.90 %) and bicyclogermacrene (149) (16.32) were found to be the major components of the oil (Table 12 and Figure 5).

Table 12. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *P. nigrum* leaf oil (simple letters in the last column are for the EAD responses recorded in figure 5)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 5
1	α-Pinene (32)	1.61	934	945	a[1]
2	Camphene (26)	0.07	950	953	
3	Sabinene (56)	0.05	973	976	
4	β-Pinene (33)	0.57	975	980	
5	3-Octanon	0.12	984	986	
6	Myrcene (31)	0.13	988	991	
7	Δ-3 Carene (142)	0.02	1006	1011	
8	Limonene (30)	0.17	1030	1031	a
9	1,8 Cineole (28)	trace	1035	1033	
10	Linalool (68)	0.31	1112	1098	b
11	Camphor (41)	0.02	1156	1143	
12	Terpin-4-ol (73)	0.03	1181	1177	c
13	α-Terpineol (1)	0.26	1191	1189	d
14	Methyl citronellate (168)	0.34	1265	1261	e
15	Bornyl acetate (169)	0.06	1293	1285	f
16	Methyl geranate (170)	0.03	1333	1323	g
17	δ-Elemene (87)	5.16	1340	1339	h
18	α-Cubebene (88)	1.71	1351	1351	
19	Citronellyl acetate (171)	0.11	1356	1354	i

Table 12 (Contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *P. nigrum* leaf oil (simple letters in the last column are for the EAD responses recorded in figure 5)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 5
20	Cyclosativene (172)	0.11	1365	1368	
21	α-Ylangene (89)	0.26	1371	1372	
22	α-Copaene (92)	3.31	1374	1376	j
23	β-Bourbonene (93)	0.09	1382	1384	
24	β-Cubebene (173)	Trace	1386	1390	
25	β-elemene (94)	1.00	1388	1391	k
26	α-Gurgunene (174)	2.29	1404	1409	k^1
27	Aristolene (175)	Trace	1427		
28	β-Caryophyllene (27)	3.07	1416	1418	l
29	β-Gurjunene (97)	0.34	1427	1432	
30	Aromadendrene (100)	0.67	1438	1439	
31	α-Guaiene (176)	0.12	1443	1439	
32	α-Humulene (101)	1.30	1455	1454	m
33	β-Santalene (177)	0.11	1460	1462	
34	*allo*-Aromadendrene (178)	0.19	1463	1461	n
35	γ-Gurjunene (179)	0.46	1474	1473	n^1
36	γ-Muurolene (104)	0.65	1478	1477	
37	Germacrene D (105)	5.55	1484	1480	o
37	β-Selinene (106)	2.22	1490	1485	
38	Bicyclogermacrene (149)	16.32	1494	1494	p
39	α-Muurolene (180)	0.98	1503	1499	
40	Germacrene A (111)	0.38	1510	1503	
41	γ-Cadinene (112)	0.14	1517	1513	
42	*cis*-Calamenene (181)	0.37	1520	1521	
43	δ-Cadinene (115)	2.81	1526	1524	
44	Cadina-1, 4-diene (116)	0.13	1535	1532	
45	α-Cadinene (117)	0.08	1540	1538	

Table 12 (Contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *P. nigrum* leaf oil (simple letters in the last column are for the EAD responses recorded in figure 5)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 4
46	(*E*)-Nerolidol (120)	17.90	1562	1564	q
47	Spathulenol (121)	6.72	1579	1576	r
48	β-Caryophyllene oxide (122)	2.01	1584	1581	r^1
49	Glogulol (123)	1.34	1592	1583	r^{11}
50	1-*epi*-Cubenol (125)	2.18	1630	1629	s
51	*epoxy-allo*, Alloaromadendrene (182)	0.67	1641	1641[42a]	
52	*epi*-α-Muurolol (183)	1.74	1644	1641	
53	α-Muurolol (127)	4.38	1649	1645	
54	α-Cadinol (128)	1.21	1658	1653	
55	(*E*)-14-hydroxy-9-*epi*-Caryophyllene (184)	1.52	1683	1670	
56	Unknown	trace	1738	-	t
57	Unknown	trace	1862	-	u

7. 3. 4. *C. flexuosus* (Lemon grass)

19 active peaks were recorded with *G. pallidipes* antenna by GC-EAD analysis of *C. flexuosus*. The major active compound was identified as citral b (neral) (139) followed by citral a (geranial) (138), geranyl acetate (141), β-caryophyllene (27), α-humulene (101) and isopulegol (185). A total of 51 compounds were identified from this oil by GC-MS. Citral a (138) (37.93 %) and citral b (139) (27.95 %) were found to be the major compounds present in the oil (Table 13 & Figure 6).

7. 3. 5. *C. nardus* (Citronella)

29 active peaks were recorded for the *G. pallidipes* antenna by GC-EAD analysis of *C. nardus*. Borneol (42) followed by geranyl acetate (141), geraniol (37), limonene (30) and γ-muurolene (104) were recorded as major active compounds. 64 compounds were identified from this oil by GC-MS analysis of which geraniol (37) (22.60 %) and (*E*)-methyl isoeugenol (186) (15.50 %) were the major compounds present in the oil (Table 14 & Figure 7).

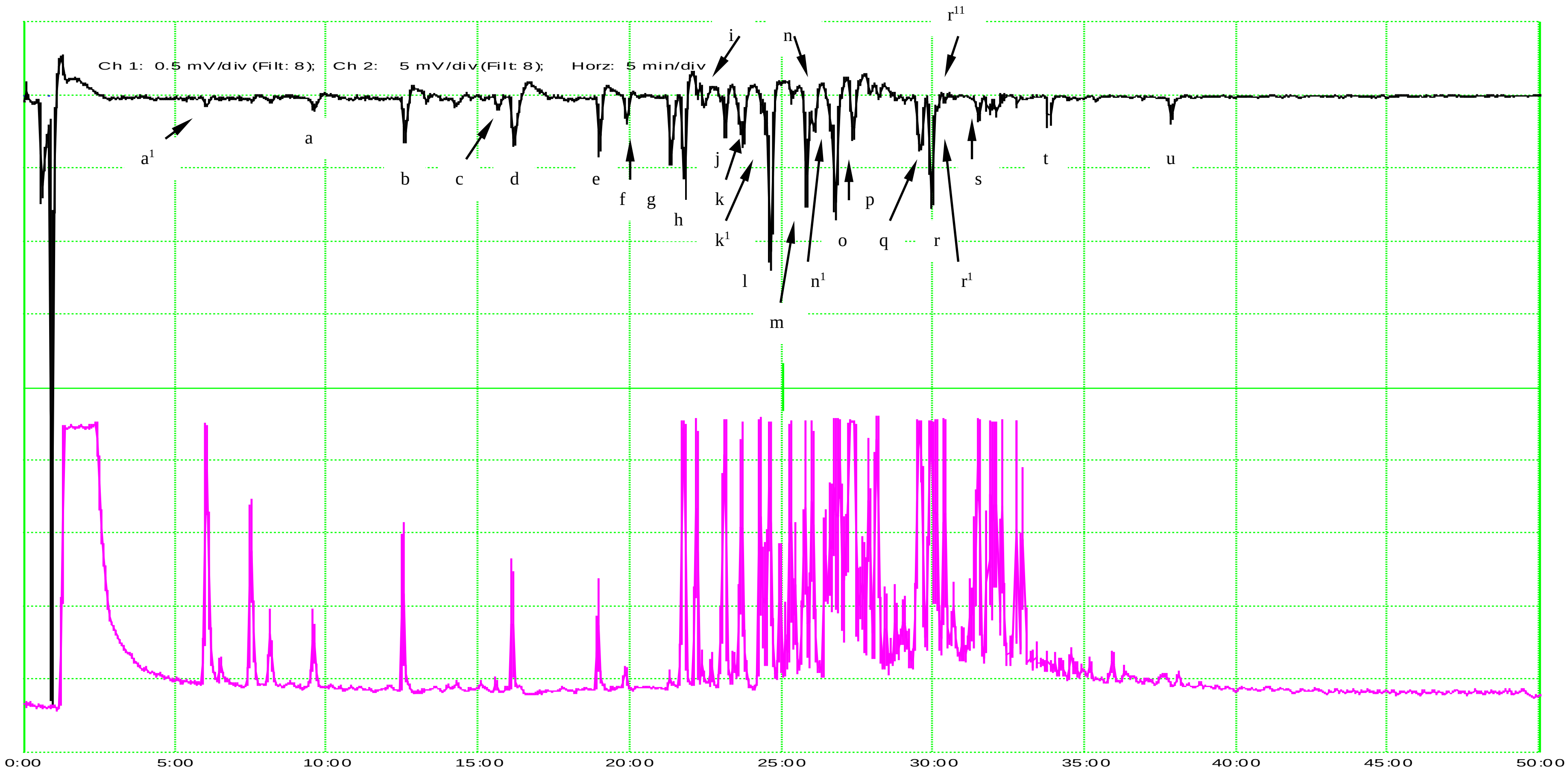


Figure 5. GC-EAD analysis of *P. nigrum* leaf oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 12)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C.

Table 13. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *C. flexuosus* (lemon grass) oil (simple letters in the last column are for the EAD responses recorded in figure 6)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference [42]	Active peaks In fig. 6
1	Tricyclene (187)	0.05	916	926	
2	α-Pinene (32)	0.15	928	939	
3	Camphene (26)	0.30	944	953	
4	β-Pinene (33)	0.09	970	980	
5	1-Octen-3-ol (133a)	trace	978	978	a
6	Dehydro 1, 8-Cineole (188)	0.06	984	991	
7	Δ-3-Carene (142)	0.03	999	1011	
8	*p*-Cymene (29)	trace	1020	1026	
9	Limonene (30)	1.29	1023	1031	b
10	1, 8-cineole (28)	0.13	1024	1033	
11	(*Z*)-β-Ocimene (61)	0.19	1037	1040	
12	(*E*)-β-Ocimene (38)	0.11	1050	1050	c
13	Linalool (68)	0.23	1107	1098	
14	*cis*-Thujone (189)	0.05	1113	1102	
15	*cis*-Verbenol (190)	0.08	1145	1140	
16	Isopulegol (185)	1.15	1149	1146	d
17	Citronellal (54)	9.71	1156	1153	e
18	Isopulegole Iso (191)	2.31	1156	1158	
19	Borneol (42)	0.33	1167	1165	e^1
20	Unknown	0.31	1182	1193	
21	α-Terpineol (1)	0.08	1187	1189	f
22	Methyl salicylate (193)	0.07	1193	1190	
23	n-Decanal	0.09	1198	1192	g
24	Citronellol (140)	1.10	1223	1228	h
25	Citral b (neral) (139)	27.95	1239	1240	i
26	Geraniol (37)	1.85	1253	1255	

Table 13 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *C. flexuosus* (lemon grass) oil (simple letters in the last column are for the responses recorded in figure 6)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference [42]	Active Peaks In fig. 6
27	Citral a (geranial) (138)	37.93	1272	1270	j
28	Bornyl acetate (169)	0.04	1287	1285	
29	Citronellyl acetate (171)	0.16	1351	1354	
30	Eugenol (21)	0.20	1358	1356	j^1
31	Neryl acetate (194)	0.13	1361	1365	
32	Cyclosativene (172)	0.16	1369	1368	k
33	Geranyl acetate (141)	3.48	1377	1383	l
34	β-Elemene (94)	0.19	1383	1391	
35	β-Longipinene (195)	0.03	1395	1398	
36	Methyl eugenol (24)	0.13	1398	1401	
37	β-Caryophellene (27)	2.00	1408	1418	m
38	*cis*-α-Bergamotene (196)	0.04	1425	1415	
39	α-Santalene (197)	0.03	1436	1420	
40	α-Humulene (101)	0.20	1447	1454	n
41	β-Chamigrene (198)	0.04	1474	1475	
42	*epi*-Cubebol (107)	trace	1490	1493	o
43	Cuparene (199)	0.08	1504	1502	
44	γ-Cadinene (112)	0.21	1511	1513	p
45	δ-Cadinene (115)	0.17	1520	1524	
46	(*E*)-γ-Bisabolene (200)	trace	1528	1533	
47	Elemol (135)	0.15	1546	1549	
48	Elemicin (43)	0.14	1555	1554	
49	β-Caryophyllene oxide (122)	0.31	1578	1581	q
50	Humulene epoxide II (124)	0.05	1602	1606	
51	(*E*)-Isoelemicin (201)	0.04	1650	1649	

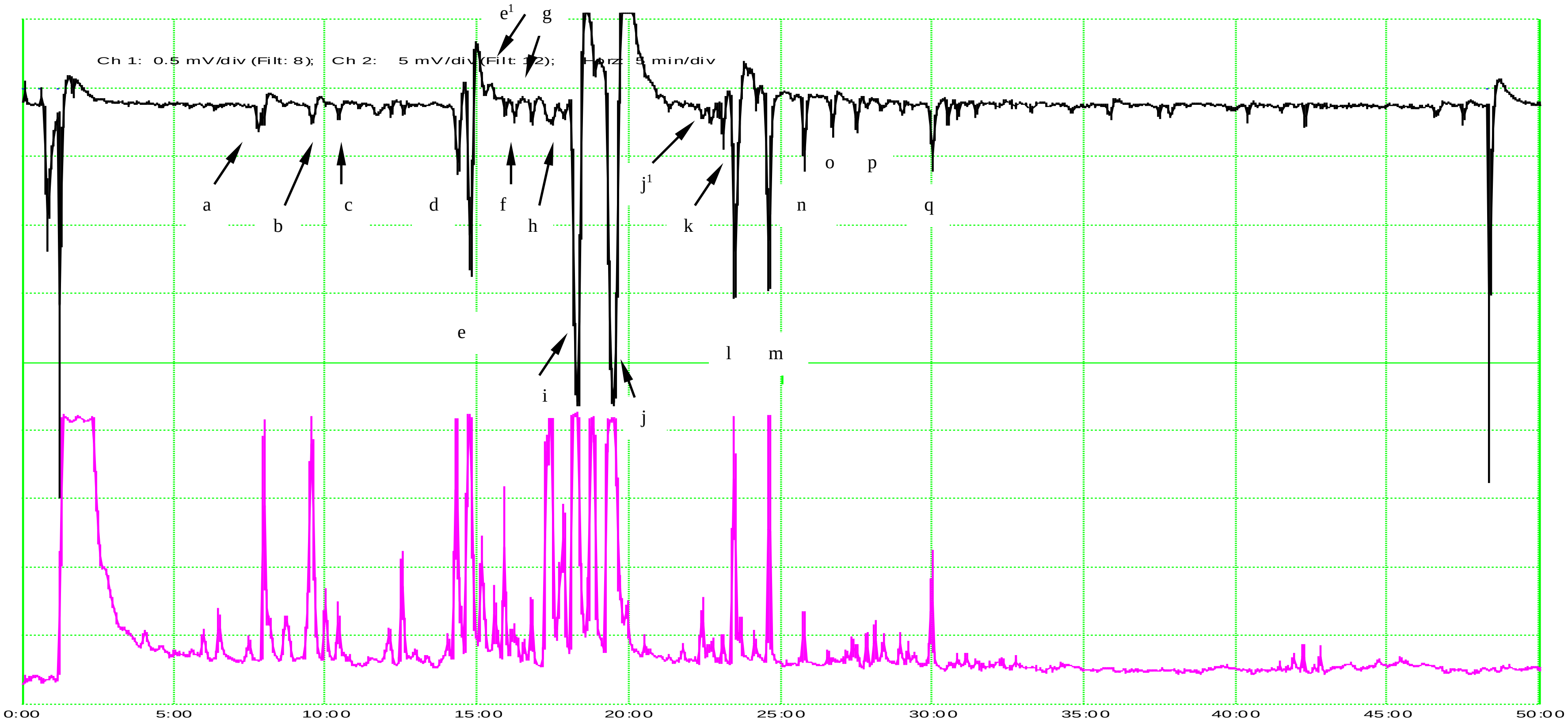


Figure 6. GC-EAD analysis of *C. flexuosus* (lemon grass) oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 13)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 14. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *C. nardus* (citronella) oil (simple letters in the last column are for the EAD responses recorded in figure 7)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 7
1	Tricyclene (187)	1.24	922	926	
2	α-Pinene (32)	2.27	934	939	a
3	Camphene (26)	8.46	949	953	b
4	Verbenene (202)	trace	955	967	
5	Sabinene (56)	0.18	973	976	
6	β-Pinene (33)	0.05	976	980	
7	1-Octen-3-ol (133a)	trace	980	978	c
8	Myrcene (31)	0.54	988	991	
9	Δ-2-Carene (203)	0.03	996	1001	
10	α-Phellendrene (57)	trace	999	1005	
11	*p*-cymene (29)	0.16	1027	1026	
12	Limonene (30)	8.90	1030	1031	d
13	1, 8-Cineole (28)	0.06	1036	1033	
14	(*Z*)-β-Ocimene (61)	0.44	1043	1040	
15	(*E*)-β-Ocimene (38)	0.15	1056	1050	e
16	Octanol	trace	1080	1070	f
17	Terpinolene (65)	0.29	1099	1088	
18	Linalool (68)	0.45	1112	1098	g
19	Nonanal	trace	1116	1098	
20	*trans*-*para*-Menth-2-en-1-ol (204)	0.03	1134	1140	
21	Camphene hydrate (161)	0.68	1157	1148	h
22	Citronellal (54)	1.55	1161	1153	i
23	Isopulegol-*iso* (191)	0.62	1164	1156	j
24	Borneol (42)	8.36	1171	1165	k
25	Isopulegol-(*neo*-*iso*) (205)	0.83	1174	1168	
26	Terpin-4-ol (73)	0.92	1181	1177	k^1

Table 14 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *C. nardus* (citronella) oil (simple letters in the last column are for the EAD responses recorded in figure 7)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active Peaks In fig. 7
27	*P*-Cymen-8-ol (206)	0.09	1188	1183	
28	α-Terpineol (1)	1.46	1191	1189	l
29	Myrtenol (206a)	0.14	1196	1194	m
30	n-Decanal	trace	1208	1204	
31	*trans*-Carveol (162)	trace	1223	1217	
32	Citronellol (140)	1.59	1230	1228	n
33	Citral b (neral) (139)	0.16	1249	1240	o
34	Geraniol (37)	22.60	1260	1255	p
35	Citral a (geranial) (138)	0.55	1281	1270	
36	Bornyl acetate (169)	0.24	1294	1285	p^1
37	Geranyl formate (207)	trace	1312	1300	
38	α-Terpinyl acetate (35)	0.37	1354	1350	q
39	Citronellyl acetate (171)	0.15	1357	1354	q^1
40	Neryl acetate (194)	trace	1368	1365	
41	Geranyl acetate (141)	1.33	1383	1383	r
42	β-elemene (94)	0.70	1388	1391	
43	Methyl eugenol (24)	0.78	1404	1401	r^1
44	β-Caryophyllene (27)	0.44	1416	1418	s
45	*trans*-α-Bergamotene (208)	0.07	1433	1436	
46	Aromadendrene (100)	trace	1444	1439	
47	α-Humulene (101)	0.12	1455	1454	t
48	(*Z*)-Methyl iso eugenol (102)	0.35	1464	1456	
49	γ-Muurolene (104)	1.06	1485	1477	u
50	β-Selinene (106)	trace	1490	1485	
51	(*Z, E*)-α-Farnesene (209)	5.85	1496	-	v
52	(*E)*-Methyl isoeugenol (186)	15.50	1505	1495	v^1

Table 14 (conand.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *C. nardus* (citronella) oil (simple letters in the last column are for the EAD responses recorded in figure 7)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active Peaks in fig 7
53	β-Bisabolene (210)	0.34	1510	1509	
54	γ-Cadinene (112)	trace	1519	1513	
55	δ-Cadinene (115)	0.07	1527	1524	
56	Citronyl n-butyrate (211)	0.24	1529	1529	
57	Unknown	0.08	1536	-	
58	Elemol (135)	2.67	1552	1549	w
59	Geranyl n-butyrate (213)	4.38	1560	1562	x
60	β-Caryophyllene oxide (122)	0.04	1585	1581	
61	Unknown	0.06	1597	-	
62	γ-Eudesmol (215)	0.32	1634	1630	
63	*epi*-α-Cadinol (216)	Trace	1644	1640	
64	α-Eudesmol (217)	0.35	1656	1652	
65	Valerianol (218)	0.36	1658	1655	
66	Bulnesol (219)	0.04	1675	1666	
67	Unknown	0.40	1752	-	

7. 3. 6. *M. fragrans* (Mace)

42 compounds were identified from the oil out of which 19 compounds were active on the *G. pallidipes* antennae. Elemicin (43) followed by myristicin (44), terpin-4-ol (73), safrole (25), β-caryophyllene (27) and methyl eugenol (24) were found to be the major active compounds for the antenna. Sabinene (56) (22.70 %) followed by terpin-4-ol (73) (18.50 %) to be the first and second major components of the oil (Table 15 & Figure 8).

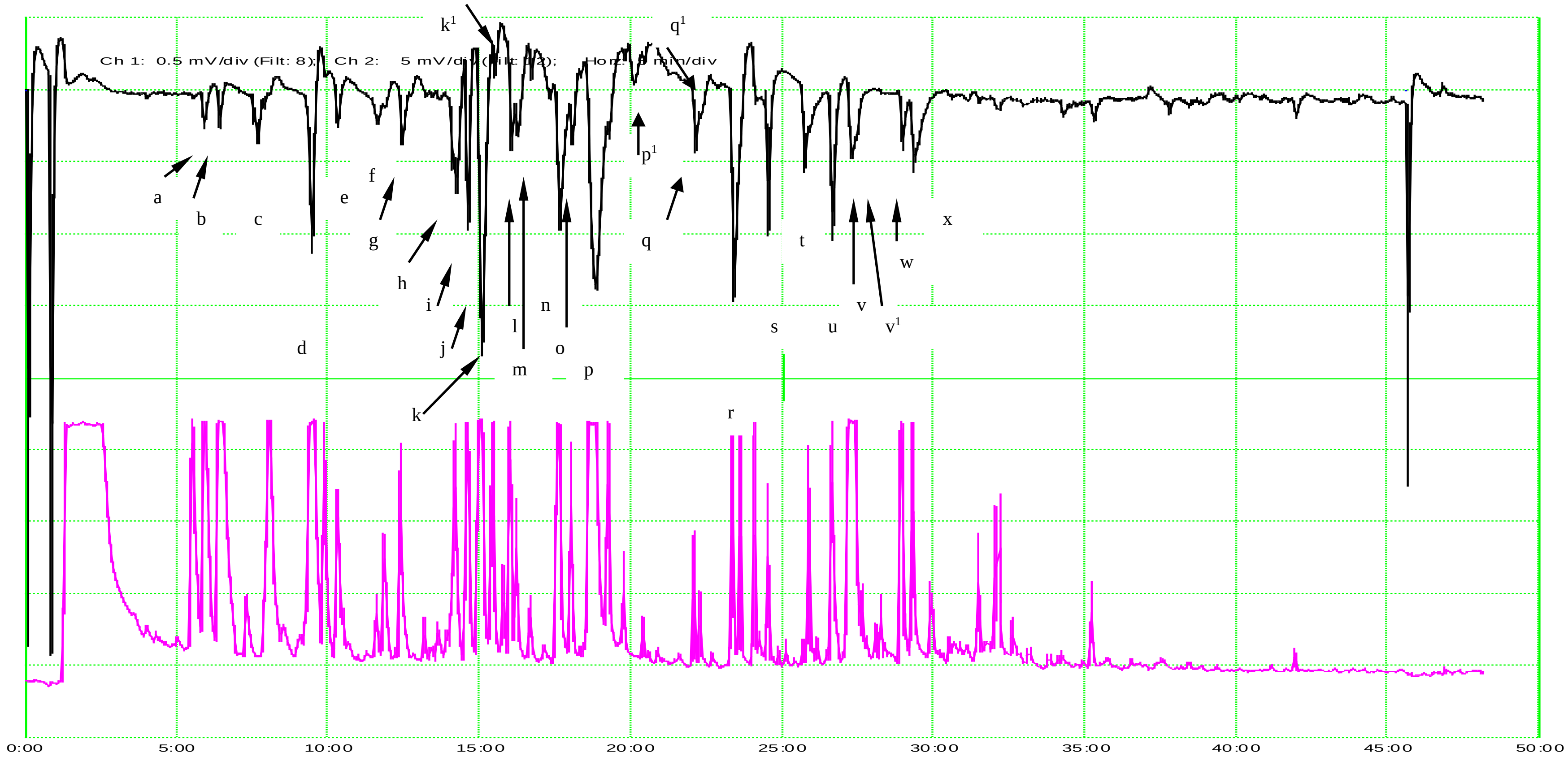


Figure 7. GC-EAD analysis of *C. nardus* (citronella) oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 14)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 15. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *M. fragrans* (mace) oil (simple letters in the last column are for the EAD responses recorded in figure 8)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig 8
1	Tricyclene (187)	1.55	926	926	
2	α-Pinene (32)	10.10	933	939	a
3	Camphene (26)	0.15	949	953	
4	Sabinene (56)	22.71	971	976	b
5	β-Pinene (33)	9.57	974	980	
6	Myrcene (31)	1.54	987	991	
7	α-Phellendrene (57)	0.74	998	1005	
8	Δ-3-Carene (142)	1.71	1004	1011	
9	α-Terpinene (34)	2.55	1013	1018	b^1
10	*p*-Cymene (29)	1.6	1025	1026	b^{11}
11	β-Phellendrene (59)	4.37	1029	1031	c
12	1,8-Cineole (28)	0.25	1035	1033	
13	γ-Terpinene (62)	4.26	1066	1062	d
14	*cis*-Sabinene hydrade (63)	0.22	1078	1068	
15	n-Octanol	0.06	1086	1070	e
16	Terpinolene (65)	1.95	1098	1088	e^1
17	*p*-Cymenene (220)	0.03	1102	1089	
18	Linalool (68)	0.12	1112	1098	
19	*exo*-Fenchol (221)	trace	1126	1117	
20	*cis*-*para*-Menth-2-en-1-ol (70)	0.3	1132	1121	f
21	*trans*-*para*-Menth-2-en-1-ol (204)	0.08	1149	1140	
22	Terpin-4-ol (73)	18.50	1179	1177	g
23	*para*-Cymen-8-ol (206)	0.07	1188	1183	
24	α-Terpineol (1)	1.02	1190	1189	h
25	*cis*-Piperitol (75)	0.09	1193	1193	
26	*trans*-Piperitol (222)	0.02	1205	1205	

Table 15 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *M. fragrans* (mace) oil (simple letters in the last column are for the EAD responses recorded in figure 8)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 8
27	Bornyl acetate (169)	0.02	1292	1285	
28	Safrole (25)	2.45	1297	1285	i
29	Iso-3-thujyl acetate (223)	0.04	1306	1301	
30	α-Terpinyl acetate (35)	0.11	1353	1350	
31	Eugenol (21)	0.02	1364	1356	
32	α-Ylangene (89)	0.03	1374	1372	
33	Geranyl acetate (141)	0.01	1384	1383	j
34	Methyl eugenol (24)	0.74	1403	1401	k
35	β-Caryophyllene (27)	0.07	1414	1418	l
36	α-Humulene (101)	0.01	1454	1454	m
37	Germacrene D (105)	0.01	1483	1480	n
38	(*E*)-Methyl isoeugenol (186)	0.03	1507	1495	o
39	γ-Cadinene (112)	0.02	1525	1513	
40	Myristicin (44)	7.73	1529	1520	p
41	Elemicin (43)	3.93	1560	1554	q
42	(*E*)-Isoelemicin (201)	0.03	1656	1649	

7. 3. 7. *E. globules*

43 compounds were identified from the oil out of which 25 compounds were active on the *G. pallidipes* antenna. Piperitone (147) followed by terpin-4-ol (73), *cis*-pinocarveol (224), an unknown compound (peak f in Table 16 & Figure 9), α-terpineol (1) and α-gurjunene (174) were recorded as the most active constituents for *G. pallidipes* antenna. α-phellendrene (57) (28.40 %) and 1,8-cineol (28) (22.10 %) were the major compounds present in the oil (Table 16 & Figure 9).

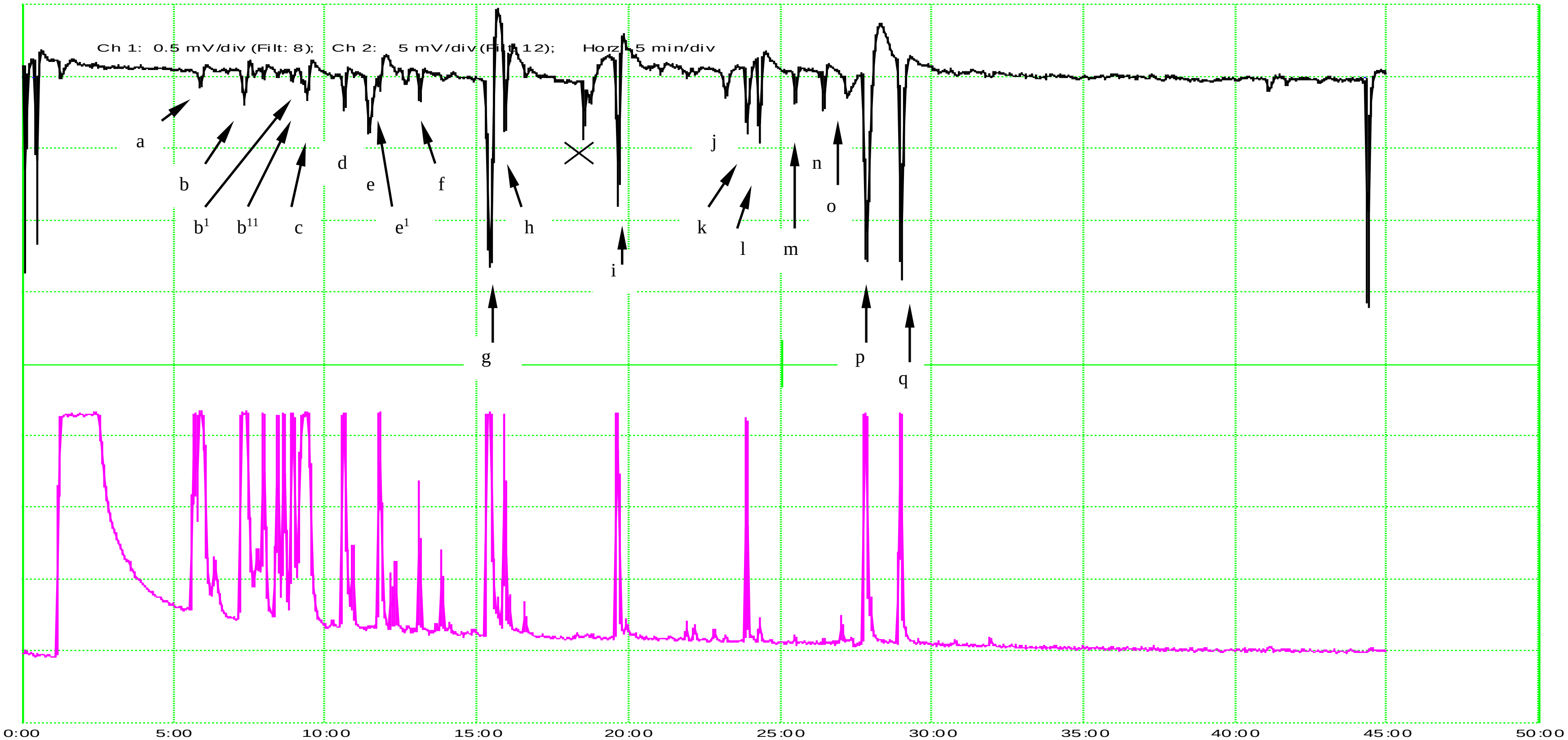


Figure 8. GC-EAD analysis of *M. fragrans* (mace) oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 15)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 16. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *E. globules* oil (simple letters in the last column are for the EAD responses recorded in figure 9)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 9
1	Tricyclene (187)	0.42	927	926	
2	α-Pinene (32)	11.80	934	939	a
3	Camphene (26)	0.02	949	953	
4	β-Pinene (33)	0.17	975	980	
5	3-Octanon	0.06	984	986	
6	Myrcene (31)	0.54	988	991	
7	α-Phellandrene (57)	28.40	998	1005	b
8	α-Terpinene (34)	trace	1014	1018	
9	*p*-Cymene (29)	12.90	1025	1026	c
10	Limonene (30)	4.98	1030	1031	c^1
11	1,8-Cineole (28)	22.10	1033	1033	d
12	γ-Terpinene (62)	1.90	1066	1062	e
13	Unknown	trace	1084	1080	f
14	Terpinolene (65)	0.91	1098	1088	g
15	Isopentylisovalerate (225)	0.41	1117	1103	h
16	*cis-para*-Menth-2-en-1-ol (70)	0.03	1134	1121	i
17	Isoborneol (226)	0.08	1175	1156	
18	Borneol (42)	0.24	1177	1165	j
19	Terpin-4-ol (73)	2.35	1180	1177	k
20	*P*-Cymen-8-ol (206)	0.06	1183	1183	
21	α-Terpineol (1)	0.83	1191	1189	l
22	*cis*-pinocarveol (224)	0.56	1199	1183	m
23	*trans*-Piperitol (222)	0.03	1207	1205	
24	Unknown	0.07	1227	-	
25	Hexyl-3-methyl Butanoate	0.28	1243	1243	n
26	Carvotanacetone (228)	trace	1247	1246	

Table 16 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *E. globules* oil (simple letters in the last column are for the EAD responses recorded in figure 9)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 9
27	Piperitone (147)	2.02	1262	1252	o
28	Thymol (82)	0.01	1320	1290	p
29	Carvacrol (165)	0.10	1311	1298	q
30	*cis*-2,3-Pinanediol (166)	1.14	1326	1316	r
31	α-Copaene (92)	0.41	1371	1376	s
32	α-Gurjunene (174)	0.21	1403	1409	t
33	Aromadendrene (100)	0.72	1438	1439	
34	*allo*-Aromadendrene (178)	0.15	1462	1461	u
35	Viridiflorene (229)	0.06	1492	1493	
36	*trans*-β-Guaiene (109)	0.98	1498	1500	v
37	δ-Cadinene (115)	0.06	1526	1524	
38	Germacrene B (119)	0.81	1562	1556	w
39	Germacrene D-4-ol (230)	0.01	1569	1574	
40	Globulol (123)	0.70	1584	1583	x
41	10-*epi*-γ-Eudesmol (231)	trace	1628	1619	
42	γ-Eudesmol (215)	0.40	1633	1630	
43	Hinesol (232)	0.06	1642	1638	
44	β-Eudesmol (233)	0.48	1654	1649	y
45	α-Eudesmol (217)	0.61	1657	1652	

7. 3. 8. *T. erecta* (Marigold) oil

Piperitone (147) (63.60 %) was identified as the major compound with other 40 compounds by GC-MS. 19 compounds were identified as GC-EAD active for the *G. pallidipes* antenna. An unknown compound (peak c in Table 17 & Figure 10) followed by piperitone (147), 1-octen-3-ol (133a), spatulenol (121), *para*-cymen-8-ol (206) and terpin-4-ol (73) were recorded as the major active compounds for the *G. pallidipes* antenna (Table 17 and Figure 10).

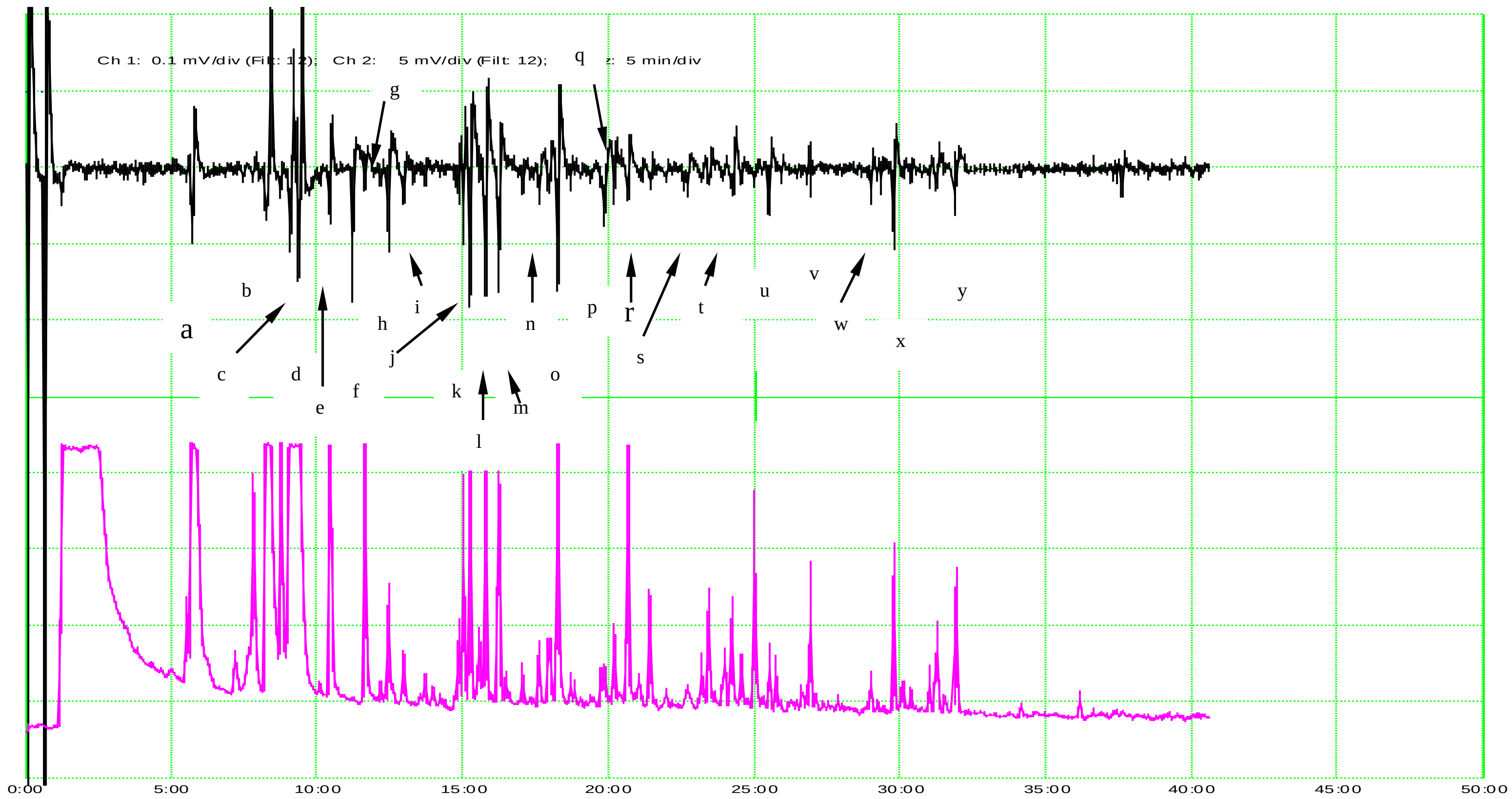


Figure 9. GC-EAD analysis of *E. globules* oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 16)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 17. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *T. erecta* (marigold) oil (simple letters in the last column are for the EAD responses recorded in figure 10)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 10
1	Tricyclene (187)	0.11	929	926	
2	Sabinene (56)	0.32	968	976	
3	β-Pinene (33)	0.05	971	980	
4	1-Octen-3-ol (133a)	trace	974	978	a
5	Myrcene (31)	0.08	985	991	
6	*p*-cymene (29)	0.39	1020	1026	
7	Limonene (30)	13.60	1024	1031	b
8	Unknown (224a)	trace	1070	1080	c
9	Fenchone (64)	trace	1078	1087	
10	*p*-cymenene (220)	0.39	1097	1089	
11	Linalool (68)	0.68	1108	1098	d
12	*trans*-Thujone (234)	trace	1110	1114	
13	(*E*)-Myroxide (148)	trace	1141	1142	
14	Borneol (42)	0.06	1168	1165	
15	Terpin-4-ol (73)	0.77	1176	1177	e
16	*p*-Cymen-8-ol (206)	2.61	1184	1183	f
17	α-Terpineol (1)	0.27	1188	1189	f^1
18	*trans*-Carveol (162)	0.10	1215	1217	
19	Methyl thymol (235)	0.27	1231	1235	
20	Carvone (4)	0.10	1247	1242	
21	Piperitone (147)	63.60	1255	1252	g
22	2-Phenyl ethyl acetate (236)	0.66	1261	1256	
23	Bornyl acetate (169)	0.48	1288	1285	g^1
24	Isobornyl acetate (237)	0.16	1294	1285	
25	6-hydroxy Carvotanacetone, (238)	0.16	1310	1304	
26	*trans*-Carvyl acetate (239)	trace	1340	1337	

Table 17 (Contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *T. erecta* (marigold) oil (simple letters in the last column are for the EAD responses recorded in figure 10)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig. 10
27	Eucarvone (240)	4.40	1344	-	h
28	Piperitenon oxide (241)	0.22	1368	1363	i
29	Geranyl acetate (141)	0.02	1380	1383	j
30	β-Elimene (94)	trace	1386	1391	
31	1,7-di-*epi*-α-Cedrene (242)	0.06	1388	1397	
32	Cyperene (243)	0.08	1390	1398	k
33	β-Caryophyllene (27)	0.45	1409	1418	l
34	*Para*-Menth-1-ene-9-ol-acetate (244)	0.16	1414	1420	
35	(*Z*)-β-Farnesene (245)	0.34	1448	1443	m
36	Caryophyllene oxide isomer	0.29	1551	-	n
37	(*E*)-Nerolidol (120)	0.65	1557	1564	o
38	Spathulenol (121)	1.82	1573	1576	p
39	β-Caryophyllene oxide (122)	2.11	1579	1581	q
40	Unknown	0.22	1633	-	r
41	*epi*-α-Muurolol (183)	0.07	1651	1641	
42	Bisabolone (246)	0.75	1743	-	s

7. 3. 9. *E. odoratum* L. oil

53 compounds were identified from GC-MS and by comparison of retention indices. 16 compounds were identified as GC-EAD active compounds for the *G. pallidipes* antenna of which was cyperene (243) found to be major active compound followed by β-caryophyllene (27), α-humulene (101), germacrene D (105) and 2-*epi*-caryophyllene (247) (Table 18 and Figure 11). α-pinene (32) (19.70 %), β-caryophyllene (27) (12.10 %) and 2-*epi*-caryophyllene (247) (8.23 %) were the major compounds present in the oil.

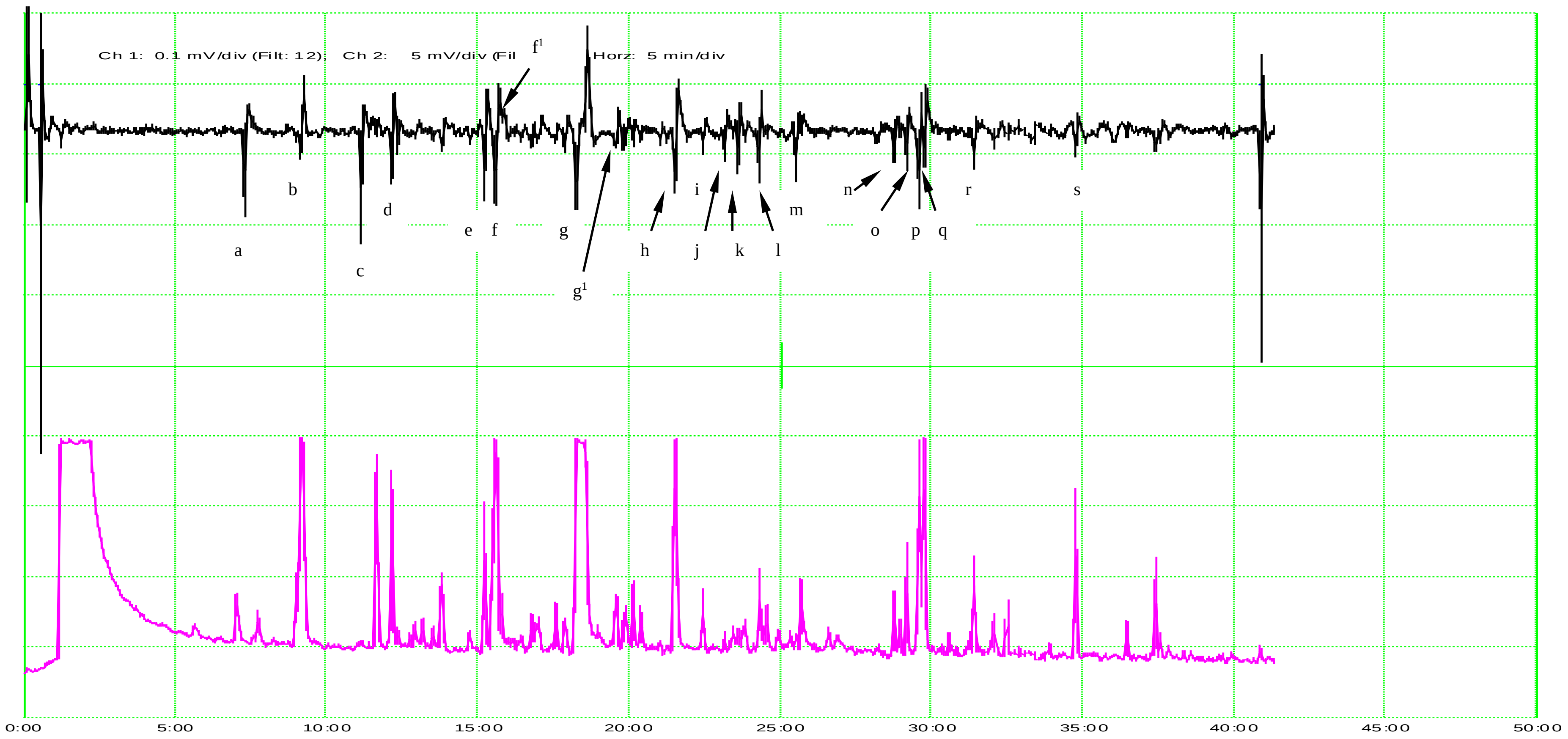


Figure 10. GC-EAD analysis of *T. erecta* oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 17)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 18. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *E. odoratum* oil (simple letters in the last column are for the EAD responses recorded in figure 11)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 11
1	Tricyclene (187)	0.08	925	926	
2	α-Pinene (32)	19.70	931	939	a
3	Camphene (26)	trace	948	953	
4	Sabinene (56)	1.01	971	976	
5	β-Pinene (33)	6.95	973	980	
6	1-Octen-3-ol (133a)	trace	980	978	b
7	Myrcene (31)	0.16	986	991	
8	*p*-Cymene (29)	0.16	1024	1026	
9	Limonene (30)	0.6	1027	1031	
10	(*E*)-β-Ocimene (38)	0.03	1053	1050	
11	Linalool (68)	0.07	1112	1098	
12	Geijerene (249)	2.94	1146	1144	c
13	Terpin-4-ol (73)	0.02	1179	1177	
14	α-Terpineol (1)	0.05	1190	1189	
15	Myrtenol (206a)	trace	1194	1193	
16	IsoGeijerene C	0.25	1250	1250	
17	Unknown	0.56	1288	-	d
18	Pregeijerene (250)	0.41	1294	1288	
19	δ-Elemene (87)	0.23	1338	1339	
20	α-Cubebene (88)	0.14	1348	1351	
21	α-Copaene (92)	2.96	1372	1376	d^1
22	β-Bourbonene (93)	1.23	1380	1384	
23	β-Elemene (94)	0.50	1385	1391	
24	Cyperene (243)	0.23	1393	1398	e
25	γ-Caryophyllene (251)	trace	1402	1404	f
26	α-Gurjunene (174)	trace	1406	1409	

Table 18 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *E. odoratum* oil (simple letters in the last column are for the EAD responses recorded in figure 11)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 11
27	β-Caryophyllene (27)	12.1	1413	1418	g
28	β-Gurjunene (97)	0.37	1424	1432	
29	2-*epi*-Caryophyllene (247)	8.23	1428		h
30	α-Guaiene (176)	0.17	1440	1439	
31	α-Humulene (101)	4.32	1451	1454	i
32	*allo*-Aromadendrene (178)	0.08	1460	1461	
33	γ-Muurolene (104)	0.96	1473	1477	i^1
34	Germacrene D (105)	6.08	1481	1480	j
35	*epi*-Cubebol (107)	trace	1495	1493	
36	Bicyclogermacrene (149)	0.40	1496	1494	j^1
37	α-Muurolene (180)	0.88	1501	1499	
38	Germacrene A (111)	0.62	1507	1503	
39	γ-Cadinene (112)	0.16	1515	1513	
40	δ-Cadinene (115)	5.58	1524	1524	
41	α-Cadinene (117)	0.06	1537	1538	
42	α-Calacorene (252)	0.29	1545	1542	
43	Elemol (135)	0.47	1549	1549	
44	Germacrene B (119)	0.52	1557	1556	
45	(*E*)-Neralidol (120)	trace	1560	1564	
46	Spathulenol (121)	1.66	1577	1576	k
47	β-Caryophyllene oxide (122)	2.84	1582	1581	l
48	Sesquiterpene alcohol (MW 220)	0.06	1596	1598	m
49	Humulene epoxide II (124)	1.26	1607	1606	n
50	1-*epi*-Cubenol (125)	1.02	1626	1627	o
51	γ-Eudesmol (215)	0.54	1630	1630	
52	Cubenol (126)	1.13	1641	1642	

Table 18 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *E. odoratum* oil (simple letters in the last column are for the EAD responses recorded in figure 11)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig 11
53	α-Muurolol (127)	0.37	1647	1645	
54	β-Eudesmol (233)	0.20	1652	1649	
55	α-Cadinol (128)	2.11	1655	1653	
56	Sesquiterpene alcohol (MW220)	0.16	1690	-	p

7. 3. 10. *P. zeylanicus* oil

65 compounds were identified by GC-MS and by comparison with retention indices. 29 compounds were identified as GC-EAD active compounds for the *G. pallidipes* antenna. The major active response was recorded from 1-octen-3-ol (133a) followed by geranyl acetate (141), β-caryophyllene (27), γ-caryophyllene (251), α-humulene (101) and 1-octen-3-yl-acetate (253). Valerianol (218) (12.20 %), γ-caryophyllene (251) (10.30 %), geranyl acetate (141) (4.71 %) were to be major compounds present in the oil. 1-octen-3-ol (133a) (2.29 %) which evoked a high response from the *G. pallidipes* was found to be present in this oil in higher proportions relative to the other analyzed oils (Table 19 and Figure 12).

7. 3. 11. *H. indicus* L. oil

78 compounds were identified by GC-MS and by the comparison of retention indices. 23 compounds were identified as GC-EAD active compounds on the *G. pallidipes* antenna. The major active response was recorded from piperitone (147) followed by β-caryophyllene (27), α-humulene (101), neryl isovalerate (254), (*E*)-nerolidol (120) and cyperene (243). GC-MS also revealed that 2-hydroxy-4-methoxy benzaldehyde (22.51 %) to be the major compound followed by β-caryophyllene (27) (9.71 %) and α-Humulene (101) (4.48 %) (Table 20 and Figure 13).

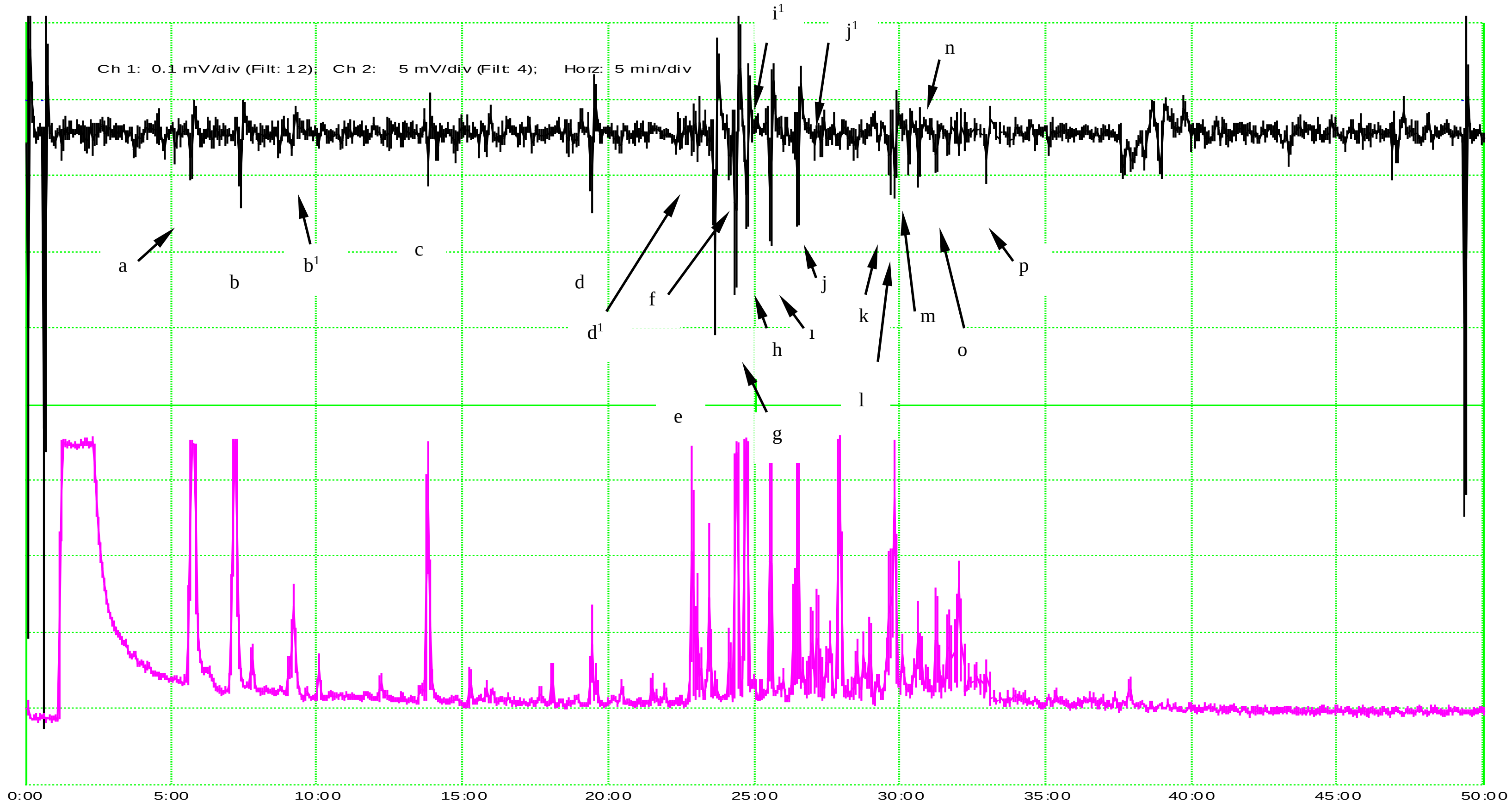


Figure 11. GC-EAD analysis of *E. odoratum* oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 18)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 19. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *P. zeylanicus* oil (simple letters in the last column are for the EAD responses recorded in figure 12)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 12
1	α-Pinene (32)	0.54	935	939	
2	Camphene (26)	0.12	950	953	
3	Sabinene (56)	0.05	976	976	
4	1-Octen-3-ol (133a)	2.29	981	978	a
5	3-Octanol	0.13	996	993	b
6	α-Phellandrene (57)	0.13	1008	1005	
7	*p*-cymene (29)	0.62	1028	1026	c
8	Limonene (30)	0.19	1032	1031	d
9	α-Pinene oxide (255)	0.06	1091	1095	
10	Linalool (68)	0.99	1113	1098	e
11	1-Octen-3-yl acetate (253)	4.00	1125	1110	f
12	3-Octanol acetate	trace	1138	1124	
13	Borneol (42)	0.26	1173	1165	g
14	Terpin-4-ol (73)	0.09	1182	1177	h
15	α-Terpineol (1)	0.10	1193	1189	i
16	1-Dodecene	trace	1199	1192	
17	*endo*-Fenchyl acetate (256)	0.07	1220	1220	
18	Isobornyl formate (257)	0.07	1232	1233	
19	Geraniol (37)	0.20	1263	1255	j
20	Bornyl acetate (169)	0.53	1294	1285	k
21	Dehydro elsholtzia ketone (258)	trace	1310	1298	
22	δ-Elemene (87)	0.17	1341	1339	
23	Cyclosativene (172)	0.06	1368	1368	
24	α-Ylangene (89)	0.16	1371	1372	
25	α-Copaene (92)	1.69	1375	1376	k^1
26	Geranyl acetate (141)	4.71	1383	1383	l

Table 19 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *P. zeylanicus* oil (simple letters in the last column are for the EAD responses recorded in figure 12)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks in fig 12
27	β-Elemene (94)	2.09	1389	1391	l[1]
28	1,7-di-*epi*-α-Cedrene (242)	trace	1397	1397	
29	γ-Caryophyllene (251)	10.30	1402	1404	m
30	α-Cedrene (259)	trace	1409	1409	
31	β-Caryophyllene (27)	4.14	1418	1418	n
32	β-Gurjunene (97)	0.24	1428	1432	
33	*trans*-α-Bergamotene (208)	0.67	1433	1436	
34	α-Guaiene (176)	0.36	1438	1439	
35	Aromadendrene (100)	0.53	1444	1439	
36	α-Humulene (101)	3.69	1456	1454	o
37	γ-Muurolene (104)	0.82	1480	1477	p
38	*cis*-β-Guaiene (260)	0.58	1484	1490	
39	Seline-4, 6-diene (261)	3.70	1489	-	
40	β-Selinene (106)	2.17	1481	1485	
41	Epizonarene (262)	1.85	1500	1497	
42	*trans*-β-Guaiene (109)	0.90	1505	1500	
43	β-Bisabolene (210)	0.87	1511	1509	
44	β-Curcumene (153)	0.23	1515	1512	
45	γ-Cadinene (112)	2.20	1519	1513	q
46	7-*epi*-α-Selinene (114)	0.41	1523	1517	
47	δ-Cadinene (115)	3.33	1528	1524	
48	Cadina-1,4-diene (116)	0.12	1536	1532	
49	α-Cadinene (117)	0.22	1542	1538	
50	α-Calacorene (252)	0.30	1549	1542	
51	Elemol (135)	0.09	1554	1549	
52	Caryophyllene oxide isomer	0.22	1558	-	r

Table 19 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *P. zeylanicus* oil (simple letters in the last column are for the EAD responses recorded in figure 12)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peak in fig 12
53	(*E*)-Nerolidol (120)	0.11	1564	1564	s
54	Spathulenol (121)	0.54	1581	1576	t
55	β-Caryophyllene oxide (122)	1.58	1586	1581	u
56	Globulol (123)	1.72	1593	1583	v
57	Alloaromadendrene oxide isomer	0.13	1600	-	w
58	Humulene epoxide II (124)	1.20	1612	1606	x
59	1, 10-di-*epi*-Cubenol (263)	0.91	1616	1614	
60	1-*epi*-Cubenol (125)	1.10	1631	1627	y
61	3-Isothujopsanone (264)	0.59	1638	1637	
62	*epi*-α-Cadinol (216)	2.99	1645	1640	z
63	α-Muurolol (127)	0.89	1651	1645	
64	α-Cadinol (128)	5.89	1660	1653	z^1
65	Valerianol (218)	12.20	1666	1655	
66	β-Bisabolol (265)	0.90	1675	1671	
67	α-Bisabolol (266)	1.14	1690	1683	
68	Unknown	0.06	1743	-	z^2
69	14-hydroxy-α-Muurolene (267)	0.17	1752	1775	z^3
70	Unknown	0.06	2081	-	z^4
71	Unknown (MW 286)	0.02	2134	-	z^5

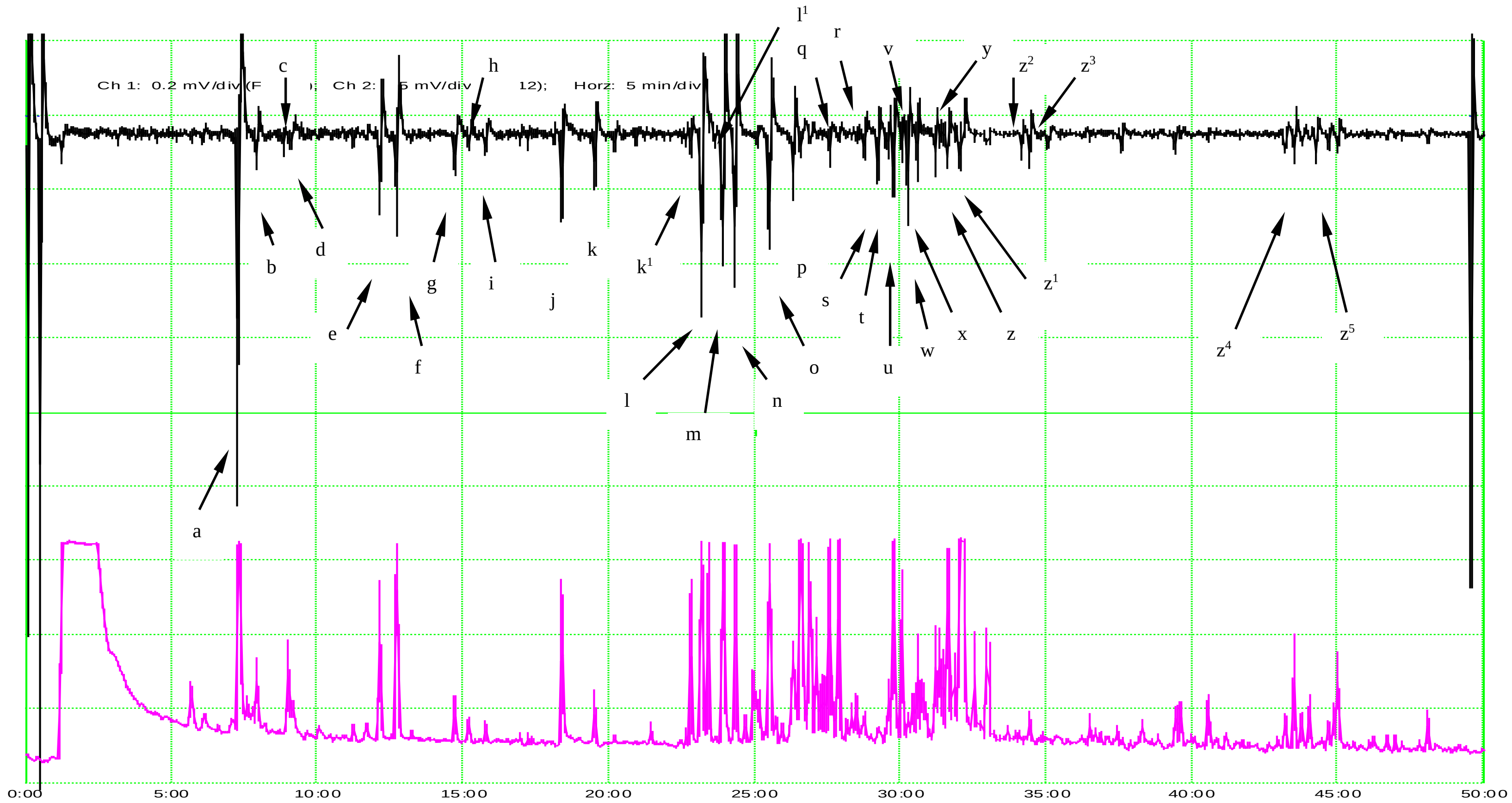


Figure 12. GC-EAD analysis of *P. zeylanicus* oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 19)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 20. Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *H. indicus* oil (simple letters in the last column are for the EAD responses recorded in figure 13)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 13
1	(*Z*)-3-Hexenol	1.27	844	857	
2	n-Hexanol	0.25	858	867	
3	α-Pinene (32)	0.03	935	939	
4	Benzaldehyde	0.04	961	961	
5	Sabinene (56)	0.01	968	976	
6	1-Octen-3-ol (133a)	trace	972	978	a
7	β-Pinene (33)	0.01	974	980	
8	Myrcene (31)	0.01	983	991	
9	n-Octanal	trace	997	1001	
10	*p*-cymene (29)	0.04	1019	1026	
11	Limonene (30)	0.12	1023	1031	
12	1,8-Cineole (28)	0.01	1029	1033	
13	Salicyldehyde (268)	0.14	1048	1041	
14	Benzene acetaldehyde	0.11	1049	1043	
15	γ-Terpinene (62)	0.02	1061	1062	
16	n-Octanol	0.09	1078	1076	
17	Terpinolene (65)	0.03	1093	1088	
18	*p*-Cymenene (220)	0.04	1096	1089	
19	Linalool (68)	0.20	1106	1098	
20	n-Nonanal	0.32	1111	1098	
21	*cis*-Pinene hydrate (158)	0.01	1128	1121	
22	Menthone (269)	0.09	1157	1154	
23	n-Pentyl benzene (270)	0.05	1167	1158	
24	Terpin-4-ol (73)	0.02	1175	1177	
25	*m*-Cymen-8-ol (74)	0.12	1183	1180	
26	α-Terpineol (1)	0.06	1187	1189	

Table 20 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *H. indicus* oil (simple letters in the last column are for the EAD responses recorded in figure 13)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 13
27	Methyl salicylate (193)	0.72	1190	1190	
28	(*E*)-4-Decenal	0.09	1197	1195	
29	Piperitone (147)	3.71	1256	1252	b
30	Phenyl ethyl acetate	trace	1257	1256	
31	(*E*)-2-Decenal	0.17	1261	1261	c
32	Ethyl salicylate (271)	0.35	1272	1267	
33	Thymol (82)	trace	1296	1290	
34	Methyl geranate (170)	0.02	1327	1323	
35	2-Hydroxy-4-methoxy Benzaldehyde	22.51	1337	1341	d
36	Eucarvone (240)	0.63	1346	-	e
37	Eugenol (21)	0.10	1359	1356	
38	Cyclosativene (172)	0.13	1361	1368	
39	α-Copaene (92)	1.23	1370	1376	e^1
40	Geranyl acetate (141)	0.16	1377	1383	f
41	β-elemene (94)	0.62	1382	1391	
42	Cyperene (243)	0.05	1390	1398	g
43	Italicene (272)	0.12	1393	1401	
44	α-Cedrene (259)	0.83	1400	1409	
45	*cis*-α-Bergamotene (196)	0.27	1403	1415	
46	β-Caryophyllene (27)	9.71	1411	1418	h
47	(*E*)-α-Ionone (96)	0.12	1420	1426	
48	*trans*-α-Bergamorene (208)	0.73	1426	1436	
49	(*Z*)-β-Farnesene (244)	0.13	1434	1443	
50	Unknown	0.08	1443	-	
51	α-Humulene (101)	4.88	1448	1454	i
52	Germacrene D (105)	0.55	1478	1480	j

Table 20 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *H. indicus* oil (simple letters in the last column are for the EAD responses recorded in figure 13)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peak In fig 13
53	(*E*)-β-Ionone (274)	0.42	1484	1485	
54	(*Z, E*)-α-Farnesene (209)	1.77	1489	-	
55	Bicyclogermacrene (149)	0.18	1494	1494	
56	*trans*-(β)-Guaiene (109)	0.18	1496	1500	
57	(*E, E*)-α-Farnesene (167)	0.90	1503	1508	
58	Butylated hydroxy toluene (275) (artifact)	3.24	1510	1512	k
59	δ-Cadinene (115)	0.53	1520	1524	
60	α-Calacorene (252)	0.12	1541	1542	
61	Germacrene B (119)	0.26	1553	1556	
62	(*E*)-Nerolidol (120)	5.22	1557	1564	l
63	Dodecanoic acid	0.75	1568	1567	
64	Spathulenol (121)	0.40	1576	1576	l^1
65	β-Caryophyllene oxide (122)	2.24	1579	1581	m
66	Neryl isovalarate (254)	0.58	1595	-	n
67	Humulene epoxide II (124)	0.24	1602	1606	
68	γ-Eudesmole (215)	0.44	1626	1630	
69	*epi*-α-Cadinol (216)	0.38	1636	1640	
70	α-Cadinol (128)	0.52	1651	1653	
71	(*E*)-Citronyl tiglate (276)	0.39	1667	1667	o
72	14-Hydroxy-9-*epi* Caryophyllene (184)	0.32	1664	1670	p
73	Myristaldehyde (277)	3.14	1705	-	q
74	(*E, Z*)-Farnesol (137)	0.18	1737	1742	r
75	(6*S*, 7*R*)-Bisabolone (246)	0.37	1743	1744	
76	Unknown	0.74	1819	-	s
77	Hexahydrofarnesyl acetone (278)	2.34	1828	-	t
78	Benzyl salicylate (279)	0.15	1866	1863	u

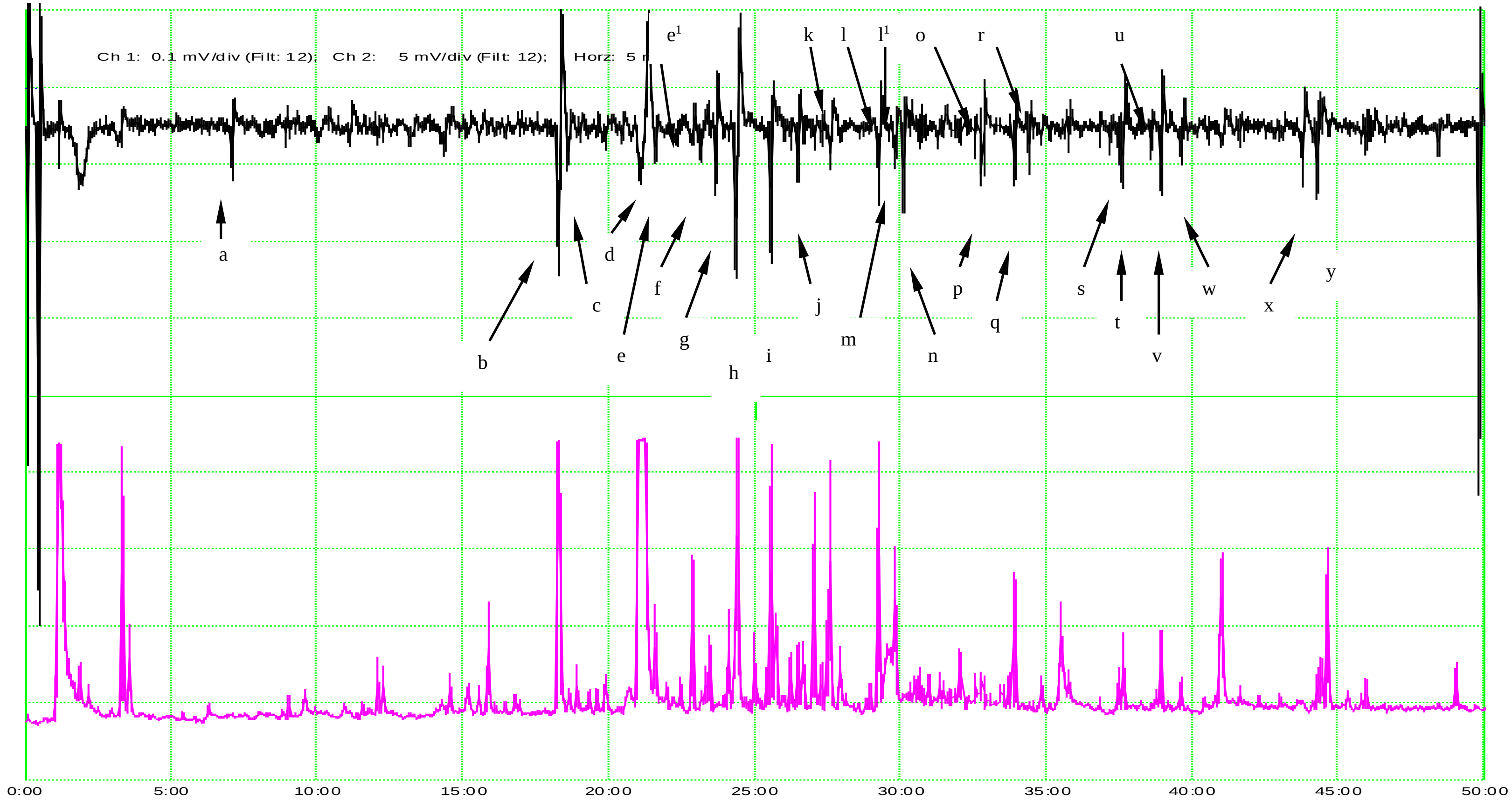


Figure 13. GC-EAD analysis of *H. indicus* oil using *G. Pallidipes* antenna (simple letters in the spectrum are stimuli identified in table 20)(Upper trace-Electroantennogram, Lower trace-Flame ionization detection)

GC column: ZB-5 (5%Diphenyl:95%Dimethyl polysiloxane), Oven: isotherm 5 min at 60°C, 4 °C/min gradient to 220°C, 20 min isotherm at 220°C

Table 20 (contd.). Chemicals identified by GC-EAD as chemostimuli for *G. pallidipes* antennae from *H. indicus* oil (simple letters in the last column are for the EAD responses recorded in figure 13)

Peak No	Name	Percentage (%, w/w)	RI	RI Reference[42]	Active peaks In fig. 13
79	5, 8, 11-heptadecatriene-1-ol	2.09	1883	-	v
80	Unknown	0.46	1904	-	w
81	Unknown	0.19	2056	-	x
82	Heneicosane	0.92	2076	2100	y

Reproducibility of results was confirmed by comparing three different antennal responses to each oil. Responses recorded with the three different antennae were considered as active products of the oils (Figure 13a)

7. 4. EAG active compounds from 11 essential oils for *G. Pallidipes* antenna

The active compounds identified from these 11 essential oils were grouped according to its chemical structures. 12 monoterpene hydrocarbons, 31 oxygenated monoterpenes, 19 sesquiterpene hydrocarbons, 15 oxygenated sesquiterpenes, 11 phenylic compounds, 9 oxygenated long/short chain hydrocarbons and 1 bicyclic β-diketone were found to be active to the tsetse antenna (Table 21).

To list the major GC-EAD response compounds from the 11 essential oils, the six most active peakes recorded for each oil were considered. Over the 11 oils 35 compounds were found to evoke major response from *G. Pallidipes* antennae.

They are identified as limonene (30) as a monoterpene hydrocarbon, *cis* pinocarveol (224), isopulegol (185), citronellal (54), borneol (42), terpin-4-ol (73), α-terpineol (1), citral b (neral) (139), piperitone (147), geraniol (37), citral a (geranial) (138), methyl geranate (170), geranyl acetate (141), neryl isovalerate (254) as oxygenated monoterpenes, cyperene (243), γ-caryophyllene (251), β-caryophyllene (27), 2-*epi*-caryophyllene (247), α-gurjunene (174), δ-elemene (87), α-humulene (101), γ-muurolene (104), germacrene D (105) as sesquiterpenes, (*E*)-nerolidol (120), spathulenol (121) as oxygenated sesquiterpenes, safrole (25), eugenol (21), methyl eugenol (24), myristicin (44), elemicin (43), *p*-cymen-8-ol (206) as phenylic compounds, 1-octen-3-ol (133a), 1-

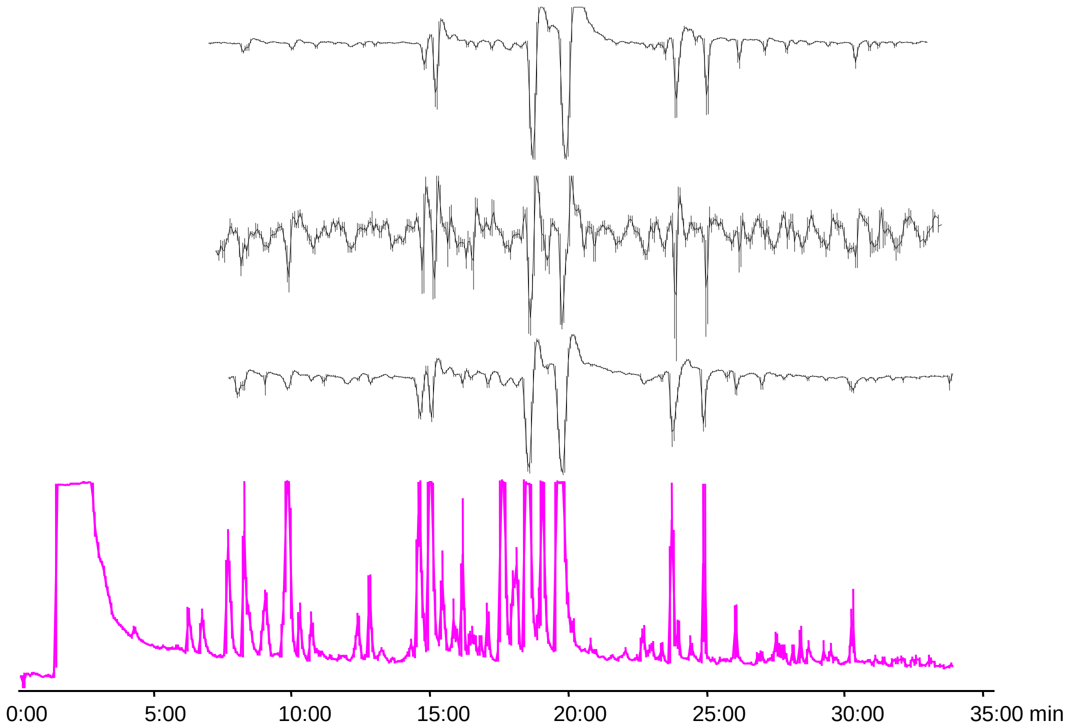


Figure 13a. For three antennae of differing sensitivity to show responses for the part of the chromatogramme where there were most distinct responses

octen-3-yl-acetate (253) as oxygenated short chain hydrocarbons and 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4. 3. 0]-non-1(6)-ene-2, 4-dione (154) as a bicyclic diketone.

7. 5. GC-EAD dose responses for active compounds

Dose response curves were obtained with the isolated compounds of 154 together with β-caryophyllene (27) and geranyl acetate (141). Allylhexanoate, which we suppose to be one of the active compounds present in essential oils of *T. erecta* and *E. citrodara* (reported as an unknown compound indicated as c in Figure 10 & Table 17 and f in Figure 9 & Table 16) was also tested on tsetse antenna to obtain the dose response curve. The GC-EAD responses of compound 154, geranyl acetate and allylhexanoate were compared with β-caryophyllene, which showed high GC-EAD responses throughout 10 of 11 essential oils. (Figures 14a, 14b, 14c).

Table 21. GC-EAG active compounds for the *G. pallidipes* antenna identified from the 11 essential oils by GC-MS analysis. (Active compounds are obtained from table 10-20)

Compound	Oil A	Oil B	Oil C	Oil D	Oil E	Oil F	Oil G	Oil H	Oil I	Oil J	Oil K
Monoterpen hydrocarbons											
α-Pinene (32)		+			+	+	+		+		
Camphene (26)					+						
Sabinene (56)		+				+					
Myrcene (31)		+									
α-Phellandrene (57)							+				
α-Terpenene (34)		+									
β-Phellendrene (59)		+				+					
Limonene (30)	+		+	+	+			+		+	
(*E*)-β-Ocimene (38)		+		+	+						
γ-Terpinene (62)		+				+	+				
Terpinolene (65)		+					+				
Geijerene (249)									+		
Oxygenated monoterpenes											
1, 8-Cineole (28)							+				
Linalool (68)		+	+		+			+		+	
cis-*p*-Menth-2-en-1-ol (70)		+				+	+				
trans-pinocarveol (160)	+										
cis-pinocarveol (224)							+				
Isopulegol (185)				+							
Camphene hydrate (161)					+						
Citronellal (54)				+	+						
Iso isopulegol (191)					+						
Borneol (42)					+		+			+	
Terpin-4-ol (73)		+	+			+	+	+		+	
p-cymen-8-ol (206)								+			

Table 21 (contd.). GC-EAG active compounds for the *G. pallidipes* antenna identified from the 11 essential oils by GC-MS analysis. (Active compounds are obtained from table 10-20)

Compound	Oil A	Oil B	Oil C	Oil D	Oil E	Oil F	Oil G	Oil H	Oil I	Oil J	Oil K
α-Terpineol (1)	+		+	+	+	+	+			+	
Myrtenol (206a)					+						
Citronellol (140)				+	+						
Neral (139)				+	+						
Piperitone (147)							+	+			+
Geraniol (37)					+					+	
Methyl citronellate (168)			+								
Geranial (138)				+							
Bornyl acetate (169)			+							+	
Methyl geranate (170)			+								
α-Terpinyl acetate (35)					+						
Citronellyl acetate (171)			+	+							
Piperitenone oxide (241)								+			
Geranyl acetate (141)					+	+		+		+	+
Geranyl n-butyrate (213)					+						
(*E*)-Citronellyl tiglate (276)											+
Eucarvone (240)								+			+
Neryl isovalerate (254)											+
Sesquiterpenes											
Cyclosativene (172)				+							
α-Copaene (92)			+				+				
β-Elemene (94)			+								
Cyperene (243)								+	+		+
γ-Caryophyllene (251)									+	+	

Table 21 (contd.). GC-EAG active compounds for the *G. pallidipes* antenna identified from the 11 essential oils by GC-MS analysis. (Active compounds are obtained from table 10-20)

Compound	Oil A	Oil B	Oil C	Oil D	Oil E	Oil F	Oil G	Oil H	Oil I	Oil J	Oil K
β-Caryophyllene (27)	+	+	+	+	+	+		+	+	+	+
2-*epi*-Caryophyllene (247)									+		
α-Gurjunene (174)							+				
δ-Elemene (87)			+								
(*Z*)-β-Farnesene (245)								+			
α-Humulene (101)	+	+	+	+	+	+			+	+	+
allo-Aromadendrene (178)			+				+				
γ-Muurolene (104)		+			+					+	
Germacrene D (105)		+	+			+			+		+
Bicyclogermacrene (149)			+								
trans-β-Guaiene (109)							+				
δ-Cadinene (115)				+							
(*Z, E*)-α-Farnesene (209)					+						
Germacrene B (119)							+				
Oxygenated sesquiterpenes											
epi-Cubebol (107)				+						+	
Elemol (135)					+						
(*E*)-Nerolidol (120)			+					+		+	+
Spatulenol (121)	+	+	+					+	+	+	
caryophyllene oxide isomer								+		+	
β-caryophyllene oxide (122)	+			+				+	+	+	+
Globulol (123)							+			+	
Humulene epoxide II (124)									+	+	
1-*epi*-Cubenol (125)		+	+						+	+	
epi-α-Cadinol (216)										+	

Table 21 (contd.). GC-EAG active compounds for the *G. pallidipes* antenna identified from the 11 essential oils by GC-MS analysis. (Active compounds are obtained from table 10-20)

Compound	Oil A	Oil B	Oil C	Oil D	Oil E	Oil F	Oil G	Oil H	Oil I	Oil J	Oil K
β-Eudesmol (233)							+				
α-Cadinol (128)										+	
14-Hydroxy-9-*epi*-Caryophyllene (184)											+
14-Hydroxy-α-Muurolene (267)										+	
Alloaromadendrene oxide (182)										+	
Bisabolone (246)								+			
Phenylic compounds											
p-Cymene (29)		+					+			+	
Safrole (25)		+				+					
Carvacrol (165)							+				
Eugenol (21)		+									
Eugenyl acetate (24a)		+									
Methyl eugenol (24)						+					
(*E*)-Methyl isoeugenol (186)						+					
Myristicin (44)						+					
Elemicin (43)						+					
Thymol (82)							+				
2-Hydroxy-4-methoxy Benzyldehyde											+
Oxygenated long/short chain hydrocarbons											
Unknown							+	+			
Isopentyl isovalerate (225)							+				
1-Octen-3-ol (133a)				+	+			+	+	+	+
n-decanal				+							
Octanol					+	+					

Table 21 (contd.). GC-EAG active compounds for the *G. pallidipes* antenna identified from the 11 essential oils by GC-MS analysis. (Active compounds are obtained from table 10-20)

Compound	Oil A	Oil B	Oil C	Oil D	Oil E	Oil F	Oil G	Oil H	Oil I	Oil J	Oil K
Hexyl-3-methyl Butanoate							+				
3-Octanol										+	
1-Octen-3-yl acetate (253)										+	
(*E*)-2-Decenal											+
Myristaldehyde (277)											+
β-diketone											
Compound 154	+										

+ - detected and identified from the oils

A – *C. citrinus* leaf oil

B – *P. betle* leaf oil (laboratory distillation)

C – *P. nigrum* (Pepper) leaf oil

D – *C. flexuosus* (Lemon grass) oil

E – *C. nardus* (Citronella) oil

F – *M. fragrans* (Mace) oil

G – *E. citrodara* oil

H – *T. erecta* (Marigold) oil

I – *E. odoratum* oil

J – *P. zeylanicus* oil

K – *H. indicus* oil

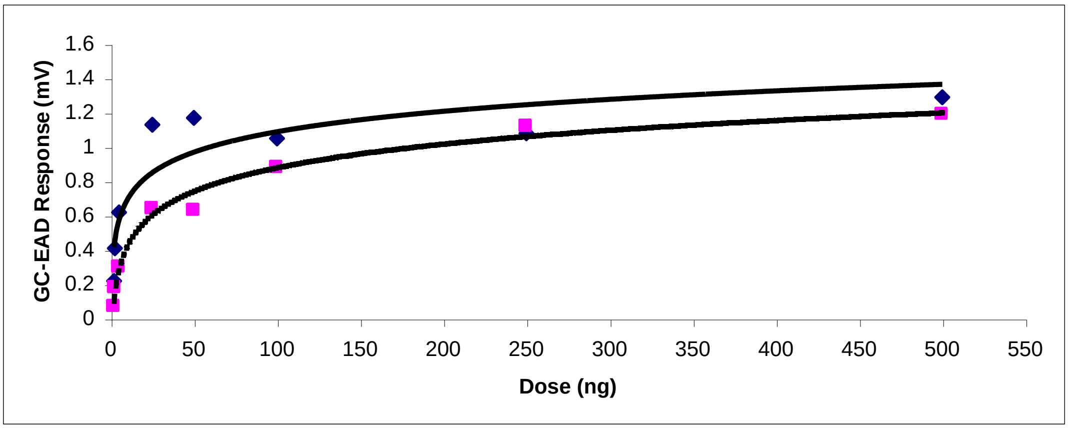


Figure 14a. Dose-response curves to β-Caryophyllene (——— , upper trace) and Geranyl acetate (■ , - - - - , lower trace) for *G. Pallidipes* antennae.

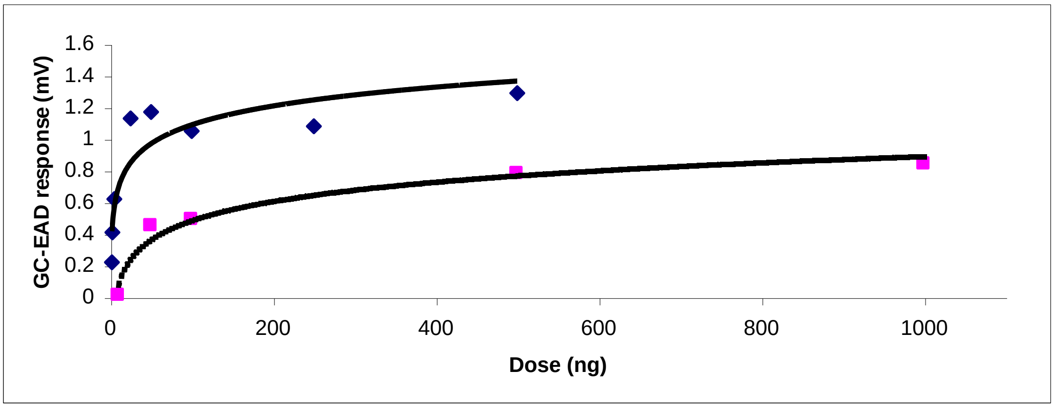


Figure 14b. Dose-response curves to β-Caryophyllene (——— , upper trace) and compound (154) (■ , - - - - , lower trace) for *G. Pallidipes* antennae.

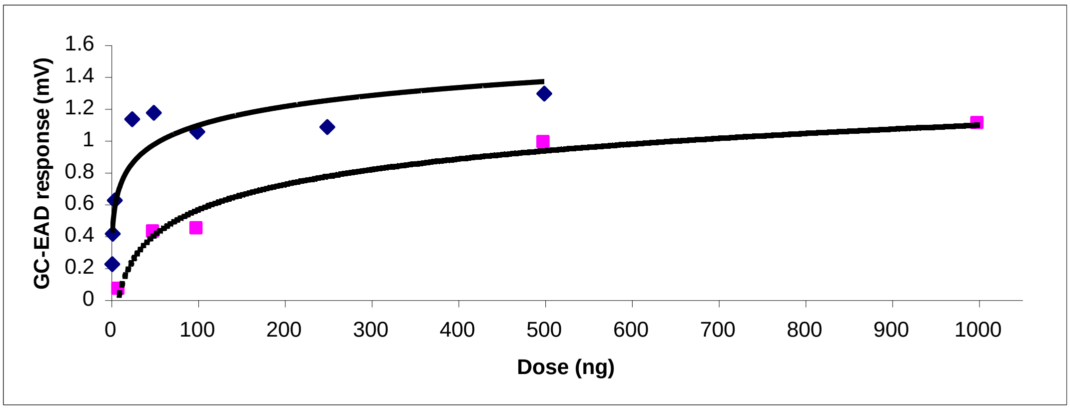


Figure 14c. Dose-response curves to β-Caryophyllene (——— , upper trace) and Allylhexanoate (■ - - - - , lower trace) for *G. Pallidipes* antennae.

7. 6. Chemical composition of the essential oils and head space of *L. camara*

Essential oils and head spaces of *L. camara* obtained from different continents were analyzed by GC-MS. Table 22 illustrates chemical compositions of analyzed samples.

The essential oil sample obtained from Sri Lanka (sample number A in Table 22) showed β-caryophyllene (27) (27.51 %) followed by α-humulene (101) (8.69 %), sabinene (56) (7.84 %) and germacrene D (105) (7.50 %) as the major compounds present in the oil. The essential oil obtained from distillation of plant material collected from Papiliorama, Neuchatel, Switzerland (sample number B in Table 22) also showed that the β-caryophyllene (27) (18.20 %) is the major followed by α-humulene (101) (15.60 %), γ-curcumene (150) (11.60 %), α-zingiberene (152) (10.27 %), whereas the essential oil sample distilled from the plant material obtained of the Botanical Garden, University of Neuchatel, Switzerland (sample number C in Table 22) showed (*E*)-nerolidol (120) (27.95 %) was the major component followed by β-caryophyllene (27) (10.21 %) and 1-octen-3-ol (133a) (6.22 %). The plant material obtained from France (sample number D in Table 22) showed the major compounds to be γ-curcumene (150) (13.50 %), α-zingiberene (152) (10.60 %), β-caryophyllene (27) (9.85 %) and α-humulene (101) (8.06 %). Kenya oil (sample number E in Table 22) showed the major component to be β-caryophyllene (27) (13.42 %) followed by bicyclogermacrene (149) (11.30 %) and sabinene (56) (9.17 %).

The head-space extract (adsorbent, Porapack Q) of plant material obtained from the Botanical Garden, University of Neuchatel, Switzerland (sample number F in Table 22) showed β-caryophyllene (27) (29.56 %) was the major followed by bicyclogermacrene (149) (11.30 %), α-pinene (32) (6.61 %) and α-humulene (101) (5.31 %). The head-space extract obtained from the plant material collected from Kenya (sample number H in Table 22) also showed β-caryophyllene (27) (30.67 %) as the major component followed by bicyclogermacrene (149) (23.37 %), germacrene D (105) (9.74 %) and α-humulene (101) (8.74 %).

Direct analysis of head-space vapor (without absorbent) of the *Lantana* sample from Botanical Garden, University of Neuchatel, Switzerland (sample number G in Table 22) revealed that β-caryophyllene (27) (22.81 %) as the major followed by bicyclogermacrene (149) (8.71 %), γ-curcumene (150) (8.52 %), α-zingiberene (152) (8.08 %) and α-pinene (32) (7.91 %). Direct analysis of head-space vapor (with out absorbent) showed that the *Lantana* sample obtained from Kenya (sample number I in Table 22) contained sabinene (56) (37.11 %) as the major followed by β-caryophyllene (27) (12.33 %) and α-pinene (32) (8.32 %).

7. 6. 1. Chiral GC column chromatography

Head space extract sample obtained from Botanical Gardan, University of Neuchatel was subjected to chiral column chromatography in order to identify the enantiameric composition of 1-octen-3-ol (133a).

7. 7. Chemical composition of essential oils and head spaces of *P. cembra*

Essential oils and head spaces of *P. cembra* obtained from Engadin, Switzerland were analyzed by GC-MS. Table 23 illustrates chemical compositions of analyzed samples.

The essential oil sample of *P. cembra* contains α-pinene (32) (48.11 %) as the major component followed by β-phellendrene (59) (14.12 %) and β-pinene (33) (8.31%) (sample number A in Table 23). Head-space extract also contained mainly β-phellendrene (59) (28.92 %) and α-pinene (32) (19.60 %) (sample number B in Table 23). Direct head-space vapor analysis also showed α-pinene (32) (36.66 %) and β-phellendrene (59)(30.04 %) to be the major compounds (sample number C in Table 23).

Table 22. Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak No	Name	Percentage (w/w, %)									RI	Type of Identification
		A	B	C	D	E	F	G	H	I		
1.	α-Pinene (32)	2.79	2.03	3.61	0.89	3.27	6.61	7.91	1.23	8.32	1017	GC&GC/MS
2	α-Thujene (55)	0.30	0.37	0.11	0.11	0.45	0.42	0.23	0.44	1.13	1025	GC/MS
3	Thuja-2, 4 (10)-diene (281)	-	0.25	0.12	0.03	0.05	0.11	-	trace	-	1044	GC/MS
4	Camphene (26)	0.61	-	0.15	0.15	1.36	0.23	-	0.53	4.41	1067	GC/MS
5	Hexanal	-	-	0.11	0.03	trace	-	-	-	-	1093	GC/MS
6	β-Pinene (33)	1.76	0.71	2.78	0.42	1.88	4.51	3.35	0.82	4.62	1110	GC&GC/MS
7	Sabinene (56)	7.84	0.85	2.21	3.86	9.17	4.11	2.74	5.54	37.11	1127	GC&GC/MS
8	Verbenene (282)	-	-	0.11	-	-	-	-	-	-	1134	GC&GC/MS
9	Δ-3-Carene (142)	0.94	-	-	-	2.48	-	-	0.83	4.32	1150	GC-MS
10	α-Phellendrene (57)	0.11	-	-	0.02	0.45	-	0.22	trace	0.43	1164	GC&GC-MS
11	Cyclopentanol	-	-	-	-	-	0.12	trace	-	-	1166	GC-MS
12	Myrcene (31)	0.48	0.18	1.81	0.13	1.35	1.12	1.02	-	1.42	1169	GC&GC-MS
13	α-Terpinene (34)	0.06	-	trace	0.02	0.20	-	-	-	0.23	1175	GC&GC-MS
14	n-Heptanal	-	-	-	0.03	-	-	-	-	-	1185	GC&GC-MS
15	Limonene (30)	1.01	0.11	0.51	0.27	1.01	0.51	0.41	trace	1.52	1190	GC&GC-MS
16	1, 8-Cineole (28)	6.67	0.06	trace	Trace	4.67	trace	trace	2.03	6.81	1193	GC&GC-MS

Table 22 (contd.). Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak No	Name	Percentage (w/w, %)									RI	Type of Identification
		A	B	C	D	E	F	G	H	I		
17	β-Phellendrene (59)	-	-	0.12	0.05	-	0.23	trace	-	-	1195	GC&GC-MS
18	1-Octene	-	-	-	0.04	-	-	-	-	-	1202	GC-MS
19	(*E*)-2-Hexenal	-	-	0.91	0.28	0.07	-	-	-	-	1215	GC-MS
20	(*Z*)-β-Ocimene (61)	0.25	-	trace	trace	0.84	-	-	-	0.82	1236	GC-MS
21	γ-Terpinene (62)	0.42	-	0.12	0.04	0.55	trace	-	trace	0.61	1244	GC&GC-MS
22	(*E*)-β-Ocimene (38)	0.30	-	0.71	0.31	1.06	0.41	0.32	-	0.82	1256	GC&GC-MS
23	3-Octanone	-	-	-	-	-	-	trace	-	-	1259	GC-MS
24	*p*-Cymene (29)	2.03	-	trace	trace	0.09	trace	trace	-	0.22	1271	GC&GC-MS
25	Terpinolene (65)	0.08	-	0.11	0.04	0.33	trace	trace	-	0.31	1284	GC&GC-MS
26	n-Hexanol	-	-	-	0.06	-	-	-	-	-	1355	GC&GC-MS
27	(*E*)-3-Hexenol	-	-	-	0.02	-	-	-	-	-	1364	GC-MS
28	2, 4, 5-trimethyl thiazole (283)	-	-	-	trace	-	-	-	-	-	1372	GC-MS
29	(*Z*)-3-Hexenol	-	0.03	trace	0.07	-	2.02	0.32	-	-	1381	GC-MS
30	3-Octanol	-	0.05	0.31	0.22	0.05	0.11	-	-	-	1389	GC&GCMS
31	*trans*-2-Hexenol	-	-	trace	0.13	-	-	-	-	-	1399	GC-MS
32	1-Octen-3-ol (133a)	0.04	0.69	6.22	0.83	0.06	1.61	trace	-	-	1449	GC&GC-MS

Table 22 (contd.). Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak No	Name	Percentage (w/w, %)									RI	Type of Identification
		A	B	C	D	E	F	G	H	I		
33	α-Cubebene (88)	0.09	-	-	trace	0.05	-	-	-	-	1452	GC-MS
34	*trans*-Sabinenhydrate (67)	0.44	0.08	0.21	0.07	1.07	0.31	-	0.22	0.22	1461	GC-MS
35	δ-Elemene (87)	0.34	-	-	trace	0.21					1466	GC-MS
36	α-Copaene (92)	0.75	0.20	0.22	0.19	0.78	1.02	1.12	0.93	0.83	1486	GC-MS
37	1, 7-di-*epi*-α-Cedrene (284)	-	-	trace	0.07	-	0.12	trace	-	-	1492	GC-MS
38	Camphor (41)	0.74	0.02	0.11	0.08	1.32	0.13	-	0.34	0.64	1511	GC&GC-MS
39	β-Bourbonene (93)	0.09	-	-	0.10	0.06	-	-	-	-	1514	GC-MS
40	α-Gurjunene (174)	0.05	0.05	trace	-	0.05	0.21	0.22	trace	trace	1524	GC-MS
41	β-Copaene (285)	-	0.27	0.42	0.51	0.58	1.41	1.31	0.93	0.51	1534	GC-MS
42	*endo*-Fenchol (157)	-	-	-	-	0.32	-	-	-	-	1544	GC-MS
43	Linalool (68)	0.24	0.09	0.21	0.43	0.59	-	-	-	-	1546	GC&GC-MS
44	Sesquithujene (286)	-	0.78	0.31	1.64	-	0.62	0.82	-	-	1554	GC-MS
45	*exo*-Fenchol (221)	-	-	-	-	0.04	-	-	-	-	1557	GC-MS
46	α-Cedrene (261)	-	0.50	0.23	0.83	-	0.63	0.64	-	-	1558	GC-MS
47	Umbellulone (287)	-	-	0.21	-	-	-	-	-	-	1561	GC-MS

Table 22 (contd.). Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak	Name	Percentage (w/w, %)									RI	Type of
No		A	B	C	D	E	F	G	H	I		Identification
48	Daucene (287a)	-	-	-	0.14	-	-	-	-	-	1571	GC-MS
49	β-Elemene (94)	1.06	-	-		0.07	-	-	-	-	1573	GC-MS
50	*trans*-α-Bergamotene (208)	0.09	-	-	0.09	-	-	-	-	-	1580	GC-MS
51	β-Cubebene (173)	0.71	0.33	0.72	trace	trace	1.54	1.83	1.73	0.72	1581	GC-MS
52	β-Caryophellene (27)	27.51	18.20	10.21	9.85	13.42	29.56	22.81	30.67	12.33	1585	GC&GC-MS
53	β-Gurgunene (97)	1.12	-	-	-	-	-	-	-	-	1588	GC-MS
54	Terpin-4-ol (73)	0.80	-	-	-	0.99	-	-	-	0.12	1591	GC-MS
55	Acora-3, 5-diene (288)	-	0.76	0.31	1.26	-	0.81	0.72	-	-	1593	GC-MS
56	α-Guaiene (176)	0.11	-	-	-	0.19	0.12	trace	0.25	trace	1617	GC-MS
57	Aromadendrene (100)	0.39	0.31	0.13	-	0.48	tace	-	0.63	0.24	1635	GC-MS
58	(*Z*)-β-Farnesene (245)	-	0.40	0.11	0.98	-	0.41	0.53	-	-	1638	GC-MS
59	*trans*-Sabinylacetate (289)	-	-	0.12	-	-	0.12	-	-	-	1645	GC-MS
60	α-Himachalene (290)	-	-	-	-	-	0.21	0.21	-	-	1652	GC-MS
61	α-Humulene (101)	8.69	15.60	2.56	8.06	5.43	5.31	3.92	8.74	2.53	1662	GC&GC-MS
62	(*E*)-β-Farnesene (291)	-	0.35	1.61	0.83	-	1.01	2.62		-	1680	GC-MS
63	γ-Muurolene (104)	0.53	-	-	0.83	0.63	-	-	0.63	0.21	1684	GC-MS

Table 22 (contd.). Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak No	Name	Percentage (w/w, %)									RI	Type of Identification
		A	B	C	D	E	F	G	H	I		
64	γ-Curcumene (150)	-	11.60	4.92	13.50	-	4.43	8.52	-	-	1687	GC-MS
65	α-Terpineol (1)	0.47	0.43	0.12	-	0.45	-	-	-	-	1694	GC&GC-MS
66	Borneol (42)	0.26	-	-	0.20	0.48	-	-	-	-	1697	
67	Germacrene D (105)	7.50	1.41	0.71	4.83	5.71	1.80	1.84	9.74	2.30	1704	GC-MS
68	α-Zingiberene (152)	-	10.27	5.22	10.60	-	3.21	8.08	-	-	1718	GC-MS
69	α-Muurolene (180)	0.83	-	-	-	0.71	-	-	0.83	0.21	1721	GC-MS
70	β-Bisabolene (210)	-	-	0.64	0.71	-	-	0.91	-	-	1723	GC&GC-MS
71	(*Z, E*)-α-Farnesene (209)	-	-	-	0.03	-	-	-	-	-	1727	GC-MS
72	Bicyclogermacrene (149)	4.51	3.68	6.12	2.55	11.30	11.03	8.71	23.37	5.62	1728	GC-MS
73	Geranyl acetate (141)	-	-	-	0.44	-	-	-	-	-	1734	GC-MS
74	β-Curcumene (153)	-	2.76	2.43	6.36	-	1.42	3.82	-	-	1738	GC-MS
75	4-ethyl Benzyldehyde	-	-	-	-	-	-	-	trace	-	1740	GC-MS
76	(*E, E*)-α-Farnesene (167)	-	-	-	0.26	-	-	-	-	-	1748	GC-MS
77	δ-Cadinene (115)	0.56	0.45	0.42	0.72	0.50	0.51	0.62	trace	trace	1752	GC-MS
78	Germacrene A (111)	0.79	0.36	0.82	0.74	1.52	1.74	1.31	2.21	0.42	1755	GC-MS
79	β-Sesquiphellandrene (292)	-	0.27	0.13	0.95	-	-	-	-	-	1762	GC-MS

Table 22 (contd.). Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak No	Name	Percentage (w/w, %)									RI	Type of Identification
		A	B	C	D	E	F	G	H	I		
80	*ar*-Curcumene (151)	-	2.49	0.81	4.59	-	0.72	2.21	-	-	1769	GC-MS
81	Methyl Salicylate (193)	-	-	0.22	0.17	-	-	-	-	-	1773	GC-MS
82	Geraniol (37)	-	-	-	0.07	-	-	-	-	-	1785	GC-MS
83	Farnesol isomer	-	-	-	0.75	-	-	-	-	-	1793	GC-MS
84	Unknown	-	-	-	-	0.21	-	-	0.24	-	1802	GC-MS
85	Germacrene B (119)	3.28	-	-	-	1.58	-	-	1.42	0.31	1819	GC-MS
86	Acetophenon-2, 4-dimethyl	-	-	-	-	-	-	-	0.32	-	1830	GC-MS
87	Acetophenon-4-ethyl	-	-	-	-	-	-	-	0.24	-	1869	GC-MS
88	*epi*-Cubebol (107)	-	0.10	trace	-	0.35	-	-	trace	-	1877	GC-MS
89	Cubebol (113)	0.18	0.22	0.41	0.39	1.15	0.11	-	0.32	-	1932	GC-MS
90	Davanone isomer	-	-	-	-	1.15	-	-	-	-	1956	GC-MS
91	Caryophyllene oxide isomer	1.00	0.13	0.12	0.21	0.10	-	-	-	-	1961	GC-MS
92	β-Caryophyllene oxide (122)	-	0.37	0.11	0.46	-	-	-	trace	-	1969	GC-MS
93	Davanone B (294)	-	-	-	-	1.23	-	-	-	-	1973	GC-MS
94	Davanone (295)	-	-	-	-	0.48	-	-	-	-	1993	GC-MS
95	(*E*)-Nerolidol (120)	-	7.07	27.95	-	2.26	2.92	6.73	0.43	-	2028	GC&GC-MS

Table 22 (contd.). Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak No	Name	Percentage (w/w, %) A	B	C	D	E	F	G	H	I	RI	Type of Identification
96	Germacrene D-4-ol (230)	-	0.77	0.81	1.59	0.50	0.51	0.61	trace	-	2037	GC-MS
97	Humulene epoxide II (124)	0.44	5.52	0.42	-	1.24	-	-	-	-	2045	GC-MS
98	Cubenol (126)	-	-	-	0.12	trace	-	-	-	-	2055	GC-MS
99	*trans*-Sesquisabinene hydrate (296)	-	0.69	0.21	1.81	-	-	-	-	-	2101	GC-MS
100	Cedrol (297)	-	-	-	trace	-	-	-	-	-	2110	GC-MS
101	Spathulenol (121)	1.76	0.37	0.24	0.20	0.61	-	-	trace	-	2112	GC-MS
102	Valerianol (218)	-	0.25	0.16	0.50	-	-	-	-	-	2121	GC-MS
103	β-Bisabolol (265)	-	-	-	0.08	-	-	-	-	-	2146	GC-MS
104	(*Z*)- *trans*-α-Bergamotol (298)	-	-	0.33	-	-	-	-	-	-	2156	GC-MS
105	*epi*-α-Cadinol (216)	0.53	0.51	0.31	0.94	0.40	-	-	trace	-	2158	GC-MS
106	Eugenol (21)	-	0.11	0.41	0.25	trace	-	-	-	-	2163	GC&GC-MS
107	*epi*-α-Bisabolol (299)	-	0-16	-	0.63	-	-	-	-	-	2172	GC-MS
108	Cadinol isomer	-	0.20	0.22	0.21	0.08	-	-	-	-	2173	GC-MS
109	3-ethyl Phenol	-	-	-	-	-	-	-	-	-	2181	GC-MS
110	Muurolol isomer	-	0.54	0.13	0.13	0.11	-	-	-	-	2186	GC-MS
111	14-hydroxy-α-Humulene (300)	-	-	-	1.05	0.61	-	-	-	-	2195	GC-MS

Table 22 (contd.). Chemical composition of essential oils and head space samples of *L. camara* from different geographical areas

Peak No	Name	Percentage (w/w, %)									RI	Type of Identification
		A	B	C	D	E	F	G	H	I		
112	α-Bisabolol (266)	-	-	-	0.10	-	-	-	-	-	2207	GC-MS
113	14-Hydroxy-α-Muurolene (267)	-	1.98	0.52	-	2.14	-	-	0.52	-	2218	GC-MS
114	α-Muurolol (127)	0.16	-	-	1.05	-	-	-	-	-	2223	GC-MS
115	3, 5-dimethyl Benzyldehyde	-	-	-	-	-	-	-	trace	-	2239	GC-MS
116	Farnesol isomer	-	-	trace	-	-	-	-	-	-	2277	GC-MS
117	14-hydroxy-9-*epi*-Caryophyllene (184)	-	0.38	0.23	0.57	0.64	-	-	-	-	2299	GC-MS
118	Sesquiterpen alchohol (M^+ 220)	2.39	0.52	1.41	0.80	3.45	-	-	-	-	2328	GC-MS
119	2, 4, 6-trimethyl Benzyldehyde	-	-	-	-	-	-	-	0.71	-	2340	GC-MS
120	2, 4, 5-trimethyl Benzyldehyde	-	-	-	-	-	-	-	0.22	-	2378	GC-MS
121	14-hydroxy-δ-Cadinene (301)	-	-	0.14	-	-	-	-	-	-	2411	GC-MS
122	Phytol (302)	-	-	2.56	1.12	1.21	-	-	-	-	2599	GC-MS

RI – Retention Index

- - Not detected

A- *L. camara* leaf from Sri Lanka, Kottawa (essential oil)

B- *L. camara* leaf from Switzerland, Papiliorama, Neuchatel (essential oil)

C**-** *L. camara* leaf from Switzerland, Botanical garden, Neuchatel (essential oil)

Table 22 (contd.).

D- *L. camara* from France, Perpignon (essential oil)

E- *L. camara* from Kenya, Nairobi (essential oil)

F- *L. camara* from Switzerland, Botanical Garden, University of Neuchatel (head space extract)

G- *L. camara* from Switzerland, Botanical Garden, University of Neuchatel (head space vapor)

H- *L. camara* from Kenya, Nairobi (head space extract)

I – *L. camara* from Kenya, Nairobi (head space vapor)

GC column: DB wax (FFAP), i.d. 0.25 mm, length 30 m; Oven: isotherm 5 min at 40°C, 5 °C/min gradient to 230°C.

Table 23. Chemical composition of essential oil and headspace samples of *P. cembra* from the Engadin, Switzerland

Number	Name of the compound	Percentage (w/w, %)			RI	Method of Identification
		A	B	C		
1	Tricyclene (187)	0.11	-	0.11		GC/MS
2	α-Pinene (32)	48.00	19.60	36.66	1015	GC & GC/MS
3	Camphene (26)	1.31	0.11	1.03	1066	GC & GC/MS
4	β-Pinene (33)	8.32	9.90	18.73	1110	GC & GC/MS
5	Sabinene (56)	0.21	0.31	1.83	1126	GC & GC/MS
6	Δ-3-Carene (142)	0.74	-	-	1150	GC & GC/MS
7	α-Phellendrene (57)	0.23	0.22	0.11	1164	GC & GC/MS
8	Myrcene (31)	2.01	1.61	2.23	1169	GC & GC/MS
9	α-Terpinene (34)	0.11	-	-	1176	GC & GC/MS
10	Limonene (30)	8.82	6.61	7.82	1190	GC & GC/MS
11	β-Phellendrene (59)	14.12	28.92	30.04	1196	GC & GC/MS
12	γ-Terpinene (62)	0.13	0.11	Trace	1243	GC & GC/MS
13	*p*-cymene (29)	trace	trace	Trace	1271	GC & GC/MS
14	Terpinolene (65)	0.61	0.53	0.11	1284	GC & GC/MS
15	Unknown	0.12	0.21	0.10	1360	-
16	α-Cubebene (88)	trace	-	-	1451	GC/MS

Table 23 (contd.). Chemical composition of essential oil and headspace samples of *P. cembra* from the Engadin, Switzerland

Number	Name of the compound	Percentage (w/w, %)			RI	Method of Identification
		A	B	C		
17	α-Copaene (92)	trace	-	Trace	1486	GC/MS
18	Linalool (68)	0.12	-	-	1547	GC/MS
19	Bornyl acetate (169)	0.71	0.42	Trace	1573	GC/MS
20	β-Cubebene (173)	0.13	0.11	Trace	1581	GC/MS
21	β-Caryophellene (27)	0.21	0.52	0.12	1583	GC & GC/MS
22	Methyl thymol (235)/Methyl carvacrol (303)	0.52	0.82	0.12	1589	GC/MS
23	Terpin-4-ol (73)	0.31	0.21	-	1592	GC/MS
24	*cis*-*p*-Menth-2-en-1-ol (70)	0.11	-	-	1619	GC/MS
25	*Trans*-Pinocarveol (160)	trace	-	-	1649	GC/MS
26	α-Humulene (101)	0.22	0.72	Trace	1662	GC/MS
27	β-Cedrene (304)	0.11	-	-	1664	GC/MS
28	Methyl chavicol (146)	0.32	0.12	-	1670	GC & GC/MS
29	*cis*-Piperitol (75)	0.11	-	-	1675	GC/MS
30	γ–Muurolene (104)	0.12	0.21	-	1683	GC/MS
31	α-Terpineol (1)	1.01	13.62	-	1694	GC/MS
32	Borniol (42)	trace	-	-	1697	GC/MS

Table 23 (contd.). Chemical composition of essential oil and headspace samples of *P. cembra* from the Engadin, Switzerland

Number	Name of the compound	Percentage (w/w, %)			RI	Method of Identification
		A	B	C		
33	Germacrene D (105)	1.51	11.32	0.51	1704	GC/MS
34	α- Muurolene (180)	0.31	0.21	-	1720	GC/MS
35	Bicyclogermacrene (149)	0.61	1.02	0.11	1728	GC/MS
36	*Trans*-Piperitol (77)	0.12	-	-	1742	GC/MS
37	δ-Cadinene (115)	1.81	0.50	0.12	1752	GC/MS
38	Cadina-1,4-diene (116)	trace	-	-	1774	GC/MS
39	Cubebol (113)	trace	trace	-	1931	GC/MS
40	β-Caryophyllene oxide (122)	trace	trace	-	1972	GC/MS
41	Humulene epoxide II (124)	0.11	0.10	-	2029	GC/MS
42	Germacrene D-4-ol (230)	0.12	0.22	trace	2039	GC/MS
43	1, 10-di- *epi*-Cubenol (263)	0.21	-	-	2048	GC/MS
44	*epi*-1-Cubenol (125)	0.22	-	-	2054	GC/MS
45	Spathulenol (121)	0.31	-	-	2115	GC/MS
46	*epi*-α-Cadinol (216)	0.92	-	-	2161	GC/MS
47	Cadinol isomer	0.91	-	-	2177	GC/MS

Table 23 (contd.). Chemical composition of essential oil and headspace of *P. cembra* from the Engedin, Switzerland

Number	Name of the compound	Percentage (w/w, %)			RI	Method of Identification
		A	B	C		
48	Muurolol isomer	0.31	-	-	2188	GC/MS
49	α-Bisabolol (266)	0.32	-	-	2209	GC/MS
50	*epi*-α-Muurolol (183)	1.91	-	-	2223	GC/MS

A- *P. cembra* leaf essential oil

B- *P. cembra* leaf headspace extract

C- *P. cembra* headspace vapor

GC column: DB wax (FFAP), i.d. 0.25 mm, length 30 m; Oven: isotherm 5 min at 40°C, 5 °C/min gradient to 230°C

8. STRUCTURE ELUCIDATION AND DISCUSSION

8. 1. Isolation of compound 154 and flavesone (155) from *C. citrinus* oil

The major GC-EAG response for *G. Pallidipes* was recorded for compound 154 followed by limonene (30), α-terpineol (1), spatulenol (121), α-humulene (101) and β-caryophyllene oxide (122) from the essential oil of *C. citrinus*. (Table 10 & Figure3). Since compound 154 could not be identified from the available mass spectral libraries and available mass spectral references, we underlook the isolation of this compound together with the structurally similar compound, flavesone (155) by liquid chromatography. Structure elucidation was done by spectroscopic methods.

8. 1. 1. Isolation of 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4. 3. 0]-non-1(6)-ene-2, 4-dione (154) from *C. citrinus* oil

Fraction CC/6 obtained from medium pressure liquid chromatography on *C. citrinus* subjected to HPLC (MeOH:Water) gave white solid compound 154. Its high resolution mass spectrum (HRMS) indicated its molecular formula to be $C_{14}H_{20}O_3$. Its ^{1}H NMR showed it as having only four singlets with 6:6:6:2 H ratio.^{13}C NMR and APT experiments confirmed that four signals correspond to one at δ 39.96 due to one methylene group and three signals at δ 28.50, 25.05 and 24.64 due to six methyl groups. Other ^{13}C-NMR signals at δ 214.44 and 195.33 indicated it to have two carbonyl groups, signals at δ 176.95 and 109.82 confirmed it as having a tetra substituted double bond. The down-field signal at δ 176.95 indicates one double bond carbon attached to the electro negative atom. Signals at δ 55.48 and 45.73 can be attributed to two quaternary carbons. A peak at δ 91.24 indicated that one carbon is attached to the electronegative atom.The molecular formula of $C_{14}H_{20}O_3$ suggested it to be oxygen. The molecular formula of $C_{14}H_{20}O_3$ suggested it to have an un-saturation number of 5. Two carbonyl carbons and one double bond fulfilled 3. Therefore the presence of 2 additional numbers confirmed the compound to be bicyclic. Combination of all the data together with APT and 2D NMR long rang C-H COSY experiments (Table 22 and

Figure 15) confirmed the structure to be 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4. 3. 0]-non-1(6)-ene-2, 4-dione (154). The base peak at m/z 166 in its mass spectrum due to its retro Diels-Alder fragmentation (Scheme 11) also confirmed the structure to be 154.

Comparison of the spectral and physical data of compound 154 with those reported for the compound with this structure showed them to be identical, confirming it to be 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo[4. 3. 0]-non-1(6)-ene-2, 4-dione (154).[118] Scheme 11 illustrates mass spectral fragmentations of the compound 154.

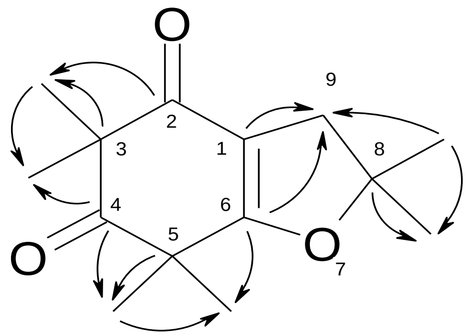


Figure 15. Correlations observed for compound 154 by 2-D NMR long rang (C → H) COSY.

8. 1. 2. Isolation of flavesone (155)

The late eluting fraction of CC/6 was collected as flavesone (155) (2mg). Its mass spectrum confirmed a molecular formula of $C_{14}H_{20}O_4$. Its ^1H NMR spectrum showed an absorption corresponding to one proton at δ 18.48 which is characteristic of enolic proton in a very strong chelated bond (intra molecular hydrogen bond)[119, 120] (Figure 16). A multiplet centered at δ 3.82 due to one methylen proton and doublet absorbed at δ 1.18 due to the six adjacent gem-dimethyl protons can be assigned to its isobutyl side chain. Two signals of almost equal intensity at δ 1.45 and 1.37 corresponding to twelve protons were assigned to the ring methyls. Two signals appeared for four methyl groups instead of one signal also explained that the tautomeric enols, (155a) & (155b) are stable and would interchange at a negligible rate.

Table 22. Observed 2-D NMR long rang (C→H) COSY correlations and APT signals for compound 154

Position	δ_C	APT	δ_H			
			C-9 CH_2, 2.76	C-8 2xCH_3, 1.49	C-5 2xCH_3, 1.40	C-3 2xCH_3, 1.36
C(4)	214.44				√	√
C(2)	195.33					√
C(6)	176.95		√		√	
C(1)	109.82		√			
C(8)	91.24			√		
C(3)	55.48					√
C(5)	45.73				√	
C(9)	39.96	CH_2				
C(8), (2xCH_3)	28.50	CH_3	√	√		
C(3), (2xCH_3)	25.05	CH_3				√
C(5), (2xCH_3)	24.40	CH_3			√	

√ - C→H long rang couplings

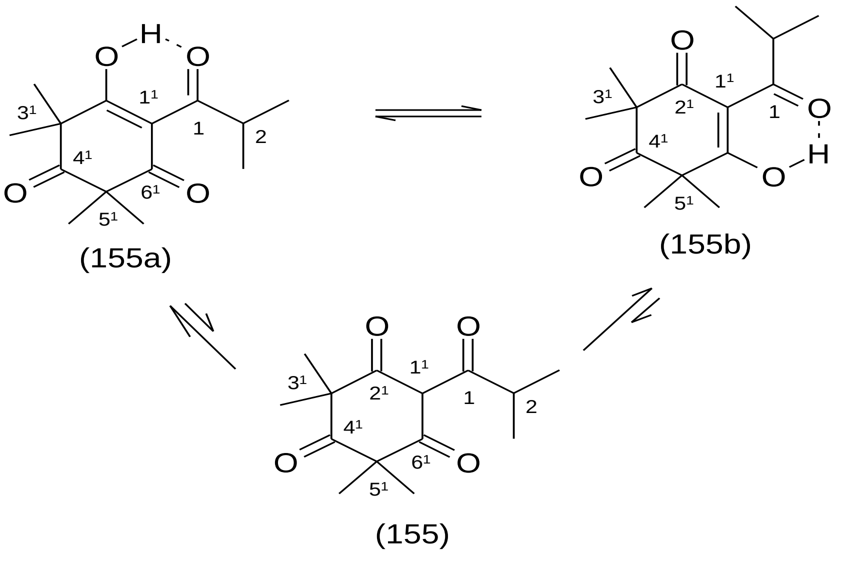
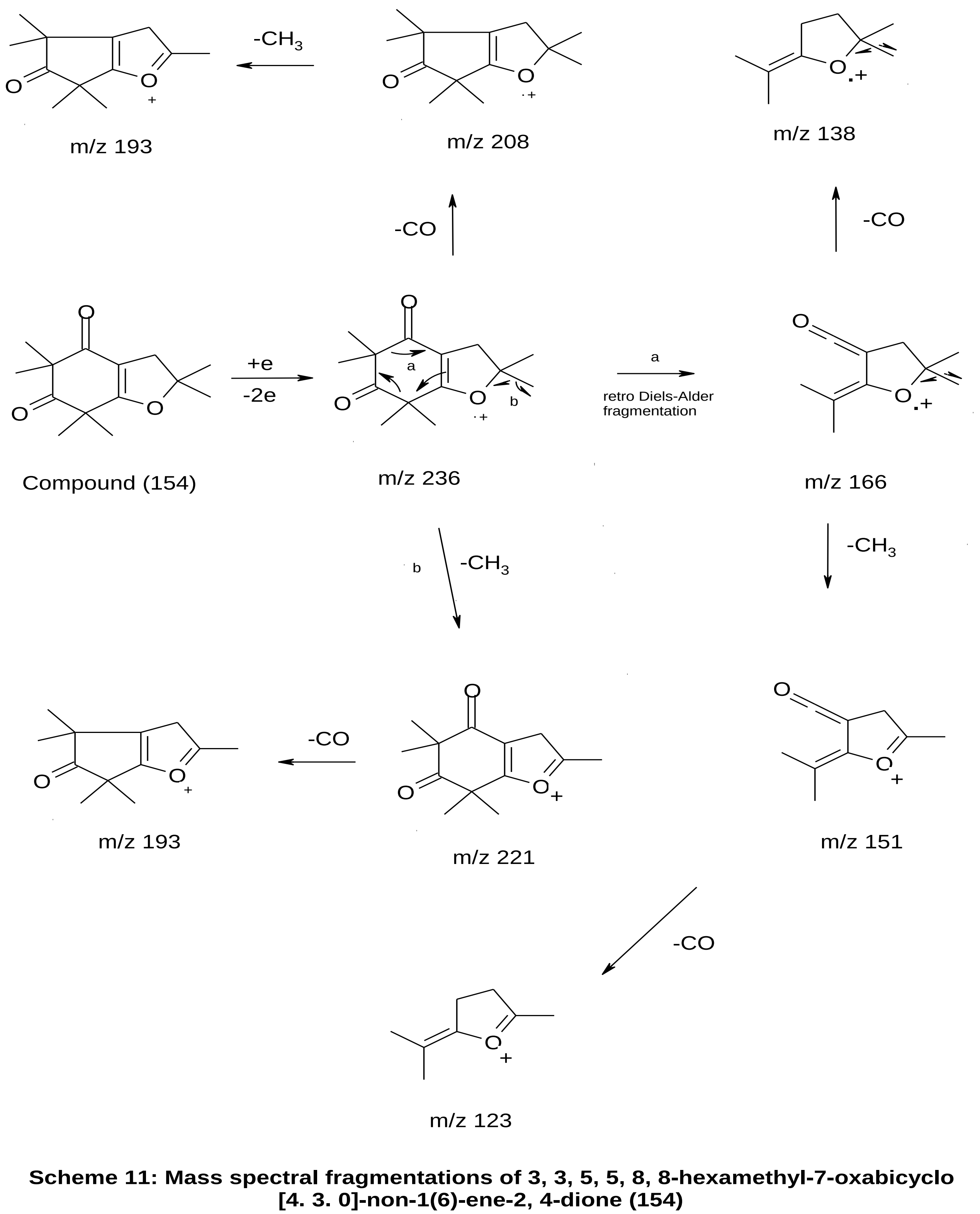
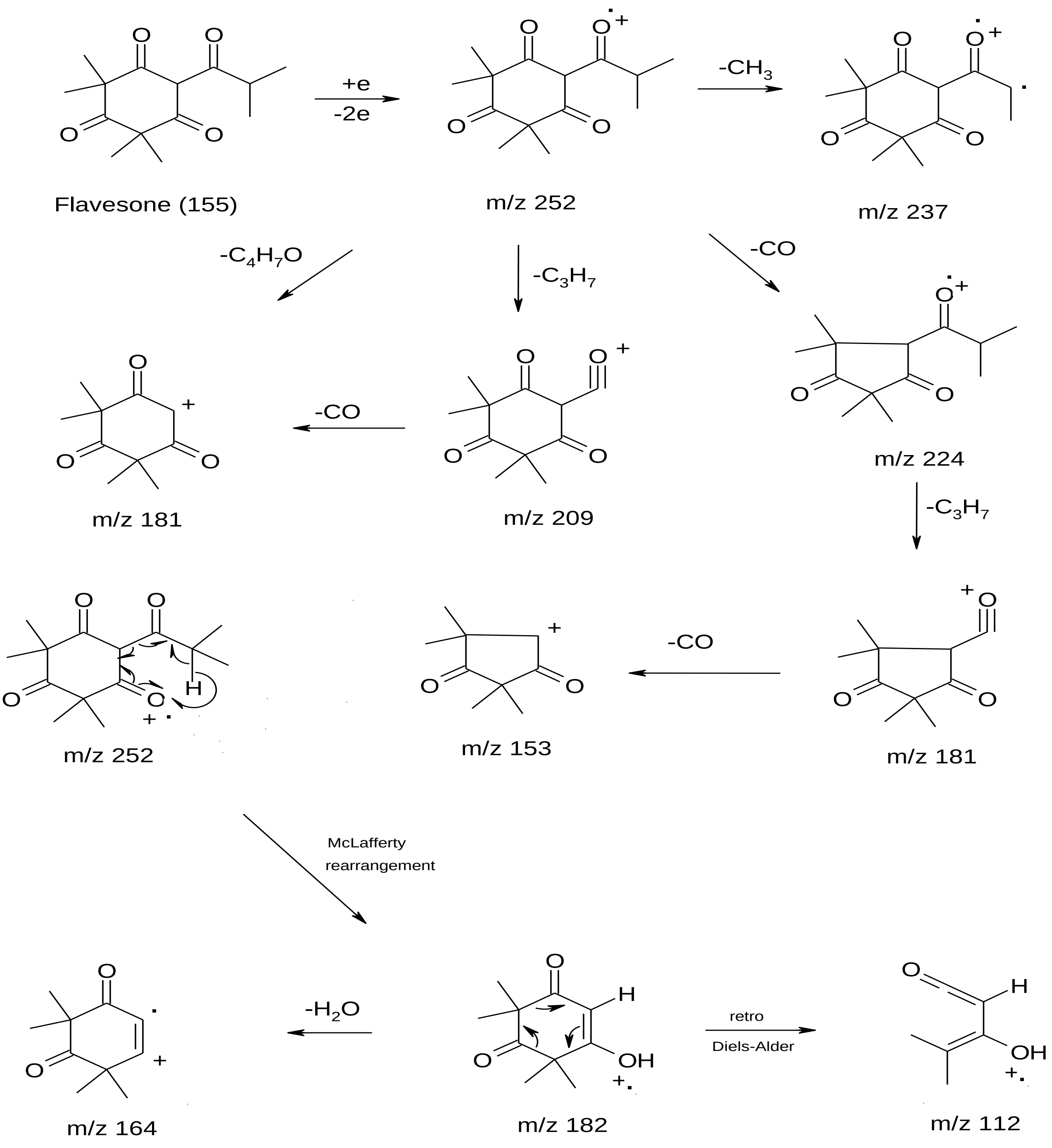


Figure 16. Flavesone (155) tautomers

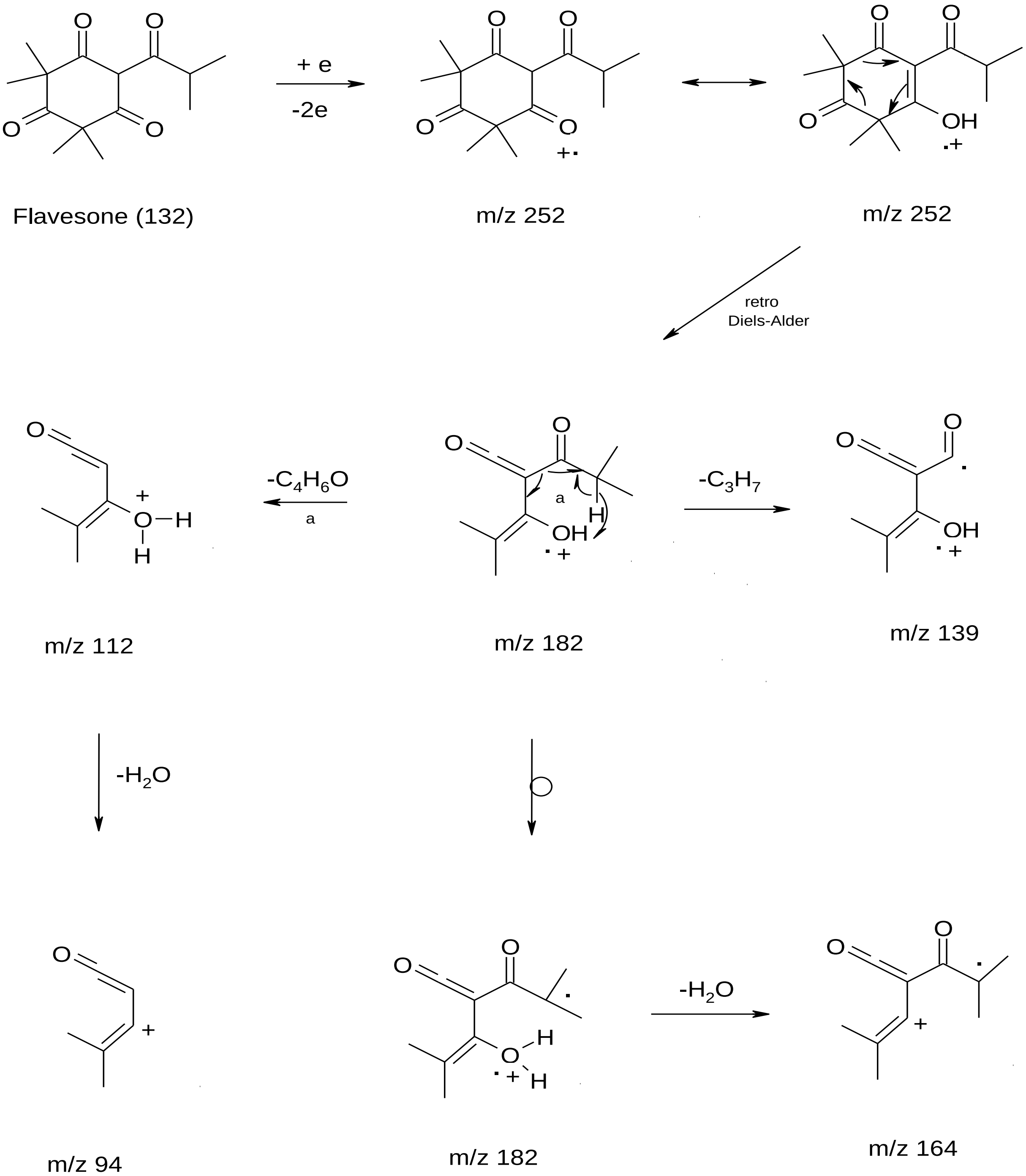


Scheme 11: Mass spectral fragmentations of 3, 3, 5, 5, 8, 8-hexamethyl-7-oxabicyclo [4. 3. 0]-non-1(6)-ene-2, 4-dione (154)



Scheme 12: Mass spectral fragmentations of flavesone (155) ***(continued overleaf)***

Scheme 12 ***(continued from previous page)***



Scheme 12: Mass spectral fragmentations of flavesone (155)

Comparison of the reported ^{1}H NMR,[119, 120] mass [42a] and GC retention indices[42a] with this structure showed them to be identical and confirmed it to be flavesone (155). Scheme 12 illustrates mass spectral fragmentations of flavesone (155).

In our GC-MS analysis we found that the major compound present in Sri Lankan *C. citrimus* oil to be 1,8-cineole (28) (53.70 %). This finding is in agreement with Pakistani and Indian *C. citrinus* leaf oil where the major constituent is also 1,8-cineole (28). But the secondary major component was bicyclic β diketone 154, which was previously isolated from *Myrtus communis* L.,[118] is new to the *C. citrinus*. Two other known monocyclic β-diketones, flavesone (155) and leptuspermone (156) were also identified for the first time in *C. citrinus*.

8. 2. GC-EAG responses on *G. pallidipes* (tsetse) antenna to essential oils

Previous GC-EAD work on tsetse antenna with animal host odor extracts revealed that 1-octen-3-ol (133a), CO_2, acetone, *p*-cresol (131), 3-n-propylphenol, (*E*)-2-heptenal, octanal, nonanal, decanal, undecanal, (*E*)-2-undecenal and dodecanal as chemostimuli.[60-68] Single cell recordings of the tsetse antenna revealed that 1-octen-3-ol (133a), CO_2, acetone and alkylphenols as specific chemostimuli for four different receptor cells present in the tsetse antenna sensilla.[121, 122]

In our studies we also found that 1-octen-3-ol (133a) was present in essential oils of *C. flexuosus* (lemon grass), *C. nardus* (citronella), *T. erecta* (marigold), *E. odoratum*, *P. zeylanicus* and *H. indicus* and it evoked the highest response from the tsetse (*G. pallidipes)* antenna compared to the other identified compounds in these oils. We found that decanal present in the *C. flexuosus* oil was also active to the tsetse antenna. Low response to decanal may be attributed to its low quantity present in the oil. We also found that β-caryophyllene (27) also evoked a strong response from tsetse antennae. In addition to β-caryophyllene (27), its isomers such as γ-caryophyllene (251), 2-*epi*-caryophyllene (247), α-humulene (α-caryophyllene) (101) and its oxygenated derivative, β-caryophyllene oxide (122) were also active to the tsetse antennae. This same class of compounds may fit in to one type of receptor cell site and therefore be responsible for the opening of the ion channels of the particular cell in order to depolarizes it. Comparison of the active monoterpene hydrocarbons and oxygenated monoterpens with the same basic carbon skeletons, limonene (30), isopulegole (185), terpin-4-ol (73), α-terpineol (1), piperitone (147) can be considered also as one group of compounds which affect a single type of receptor. Citronellal (54), citral b (neral) (139), geraniol, citral a (geranial) (138), methyl geranate (170), geranyl acetate (141), neryl isovalerate (254) can also be considered as another group of compounds affecting the same receptor site or another olfactory cell.

The GC-EAD active aromatic compounds safrole (25), eugenol (21), methyl eugenol (24), myristicin (44) and elemicin (43) can be classed as phenyl propanoids. Each of these five molecules has an oxygen substituent at the 3rd position in the aromatic ring with respect to its propene group. 3-n-propyl phenol also has an oxygen substituent at 3rd position in its aromatic ring. 3-n-propyl phenol is reported to have activity on tsetse fly antennae. [121, 122] Since safrole (25), eugenol (21), methyl eugenol (24), myristicin (44) and elemicin (43) have a very much similar structural skeleton to that of 3-n-propyl phenol these five phenyl propanoids can also be assumed to activate the same receptor type.

In our screening of essential oils for tsetse antenna we found 1-octen-3-ol (133a) to be the best electrophysiologically active compound. We also found that 1-octen-3-yl-acetate (253) activates the tsetse fly antenna. Since 1-octen-3-yl-acetate (253) and 1-octen-3-ol (133a) have the same basic carbon skeleton this may be the reason for the activity of acetate substituent.

Since β-caryophyllene (27) and geranyl acetate (141) appeared to be very good stimulants over the tsetse fly antennae, we studied the dose response relation ships (Figure 14a) for these products in order to find the threshold values. These studies revealed that at high doses (~0.5 μg) both β-caryophyllene (27) and geranyl acetate (141) showed almost equal responses. But at low doses (~2 ng) β-caryophyllene (27) showed a better response than geranyl acetate (141). The dose response study of isolated bicyclic β-diketone 154 with β-caryophyllene (27) revealed that at high doses (~0.5 μg) β-caryophyllene (27) evoked almost a 1.7 times higher response than compound 154. At low doses (~10 ng) β-caryophyllene (27) evoked a 62 times higher response than compound 154.

Since retention indices suggested that an unknown compound (peak c in Figure 10 & Table 17 and peak f in Figure 9 & Table 16) to be allylhexanoate, we tested the synthetic compound on tsetse antenna. This compound showed a moderate response but not a response as high as we expected and as recorded for the product in the oils of *T. erecta* and *E. citrodara.* This observations suggests that this is not the compound present in the two oils of *T. erecta* and *E. citrodara* which evoked good responses from tsetse antennae. Comparison on β-caryophyllene (27) revealed that β-caryophyllene (27) was 1.3 times more active than allylhexanoate at high doses (~0.5 μg) and 10 times better at low doses (10ng).

In our dose response experiments we observed responses from tsetse antennae at very low odor doses. This is near the threshold value for the particular product. Small increment from this dose results in a high increment in response from the antenna. But when the odor dose increses further, the response of the antenna flattened out. The reason for this can be attributed to antennal saturation at high doses.

In our GC-MS studies we identified terpenoids, phenyl propanoids, short/long chain hydrocarbons and oxygenated hydrocarbons as essential oil volatiles. It is known that the terpinoids are derived from mevalonate and deoxy-xylulose biosynthetic pathway, phenyl propanoids from the shikimic acid pathway, whereas simple hydrocarbons and oxygenated hydrocarbons are direved from lypoxygenase pathway. In our GC-EAD experiments on tsetse antennae we identified active responses from volatiles from each of these biosynthetic pathways (table 21). This confirms that the tsetse fly is responding to volatiles derived from all the major biosynthetic pathways of plants.

As a continuation of these studies it is necessary to study the behaviour on tsetse fly with respect to EAD active compounds in order to correlate neurophysiological activity and behaviour (actractants or repellents).

8. 3. EAG responses on *A. aegypti* (mosquito) antenna to essential oils

Stewart *et al*[123] and Lacher[124] reported that they have identified four types of olfactory receptor cells designated as A1, A2, A3, and A4 on mosquito antenna. Stewart *et al* also indicated that the A1 and A3 receptor cells are associated with attraction and the A2 with a repellent. Single cell recordings done by Lacher indicated that A1 cells are inhibited by α-terpineol (1), geraniol (37), eugenol (21), citral and citronellol (140) and excited by fatty acids. Type A2 cells are inhibited by C_{2-5} fatty acids and excited by C_{7-10} fatty acids. A3 and A2 cells were excited by citral. A3 cells were also excited by α-terpineol (1). In our EAG studies on *A. aegypti* antennae we found that *C. flexuosus* (lemon grass) oil was one of the best stimulants for the antenna among the other oils tested (Table 9 and Figure 1). Our GC-MS studies revealed that this oil mainly contains citral a (geranial) (138) (37.93 %) and citral b (neral) (139) (27.95 %). According to the reported data by Lacher,[124] cell type A2 and A3 are excited by citral. High response from lemon grass oil by the mosquito antenna may be attributed for having its high percentages of citral a (138) and citral b (139).

8. 4. Chemistry of essential oils

It was reported that an Indian *P. nigram* leaf oil contained the sesquiterpene alcohols elemol (135) (11.52 %) caryophyllene alcohol (4.85 %) and (*Z, E*)-farnesol (137) (4.59%).[89] But the oil sample which we analyzed from Sri Lanka contains (*E*)-nerolidol (120) (17.90 %) and bicyclogermacrene (149) (16.32 %).

Reported data on Sri Lankan *C. flexuosus* (lemon grass) oil reveled that the oxygenated monoterpenes citral a (geranial) (138) (47 %) and citral b (neral) (139) (31 %) were the major compounds present in the oil.[90] Our data is in agreement with their findings recording citral a (138) (37.93 %) and citral b (139) (27.95 %) as major compounds.

Our GC-MS analysis of Ceylon citronella (*C. nardus*) oil revealed that this oil contains geraniol (37) (22.60 %) and (*E*)-methyl isoeugenol (186) (15.50 %) as major compounds. Paranagama's[90] analysis of Ceylon citronella (*C. nardus*) oil also reported geraniol (37) (18.77 %) and methyl isoeugenol (9.74 %) in high propotions. But Regnault-Roged *et al*[92]reported that plant materials of *C. nardus* obtained from either a local market or research plot at the Université de Pau et des Pays de l'Adour, Pau, France contained citronellal (54) (33.8 %) as a major component, followed by geraniol (37) (21.6 %), citronellol (140) (9.2 %) and geranyl acetate (141). This compositional change may be due to geographical conditions of the place where the plant materials were harvested.

Sri Lankan mace (*M. fragrans*) oil is reported to contain sabinene (56) (24.45 %), terpin-4-ol (73) (14.63 %), β-pinene (33) (10.89 %), α-pinene (32) (10.00 %), myristicin (44) (5.18 %) and elemicin (43) (3.37 %) as major components.[90] In our studies, Sri Lankan essential oil of mace was given almost the same chemical composition as that of the previous studies[90] indicating sabinene (56) (22.7 0%) followed by terpin-4-ol (73) (18.50 %), α-pinene (32) (10.10 %), β-pinene (33) (9.57 %), myristicin (44) (7.73 %) and elemicin (43) (3.93 %) as major components.

Reported data on chemical composition of *E. globules* indicated 1,8-cineole (28) (74.80 %) as the major component followed by α-pinene (32) (16.9 %).[90] But our studies indicated α-phellendrene (57) (28.40 %) as the major component followed by 1,8-cineole (28) (22.10 %).

It is reported that *T. patula* and *T. erecta* usually contain limonene (30), terpinolene (65), piperitone (147) and caryophyllene as components.[97] In our analysis we also found piperitone (147) (63.60 %) as a major component followed by limonene (30) (13.60 %).

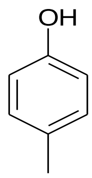
Study of the literature on the chemical composition of *L. camara* oil revealed that its composition was dramatically different from each, suggesting that the composition may depends on cultivated areas of plants.[111-114] Therefore we also studied the chemical compositions of *L. camara* obtained from different continents namely, Asia (Sri Lanka), Africa (Kenya) and Europe (Switzerland and France) (Table 22). Our results also agree with the reported data on *L. camara*, i.e. chemical composition was noticeably different from different locations. But, our results revealed that β-caryophyllene (27) which evoked electrophysiologically high responses from tsetse fly antennae is present at high quantities in all the analyzed samples of *L. camara*. Published data also reveales that β-caryophyllene (27) is one of the major constituents of *L. camara* essential oil. 1-octen-3-ol (133a), which evoked highest EAG response from the tsetse fly antennae in the oils tested, was also present in this plant in good yields. Considering these two compounds, *L. camara* may be regarded as a potential attractant for tsetse flies. Therefore we suggest that the *L. camara* is a promising candidate to study the behavior of tsetse-plant interactions.

Study of chiral column chromatographic separation of 1-octen-3-ol (133a) from head space extract sample of *L. camara* obtained from Botanical Gardan, University of Neuchatel revealed that 1-octen-3-ol is present in the plant as its (R) isomer.

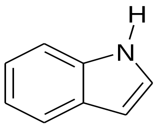
We observed differences in the chemical composition of the essential oil and head-space vapours from the same plant, *L. camara*. It is known that almost all the volatiles are being extracted from the plant by hydro distillation whereas the head-space extraction method may not be collecting all the volatiles from the plant. This may be the reson for having different chemical constituents with these two extraction methods even with the same plant material.

We also studied the chemical composition of essential oil, head-space extract and direct vapor analysis of *P. cembra* volatiles obtained from Engadin, Switzerland. Our results were in agreement with reported data[95] on *P. cembra* confirming α-pinene (32), β-pinene (33), limonene (30) and β-phyllendrene (59) as the major constituents.

ANNEX 2 (Structures 131 to 304)



p-Cresol (131)
CAS # 106-44-5



Indole (132)
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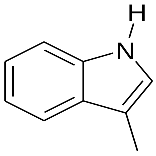
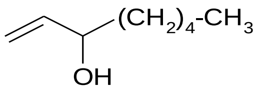
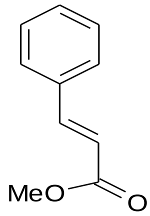


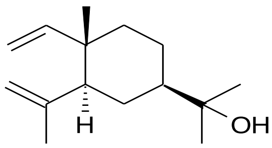
3-Methyl indole (133)



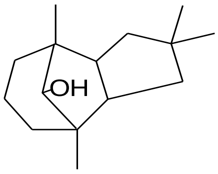
1-Octen-3-ol (133a)
CAS # 3391-86-4



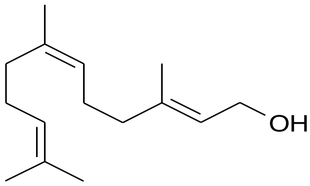
Methyl cinamate
(134)



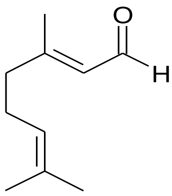
Elemol (135)
CAS # 639-99-6



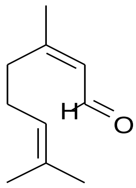
Caryophyllene
alcohol (136)
CAS # 4586-22-5



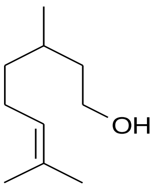
(*E, Z*)-Farnesol (137)
CAS # 3879-60-5



Geranial (138)
(citral a)
CAS # 141-27-5



Neral (139)
(citral b)
CAS # 106-26-3



Citronellol (140)
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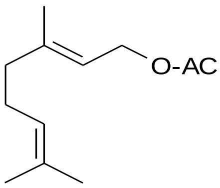
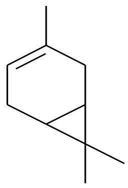
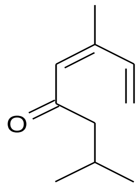


Geranyl acetate
(141)



δ-3-Carene (142)
CAS # 13466-78-9



(*Z*)-Tagetone (143)
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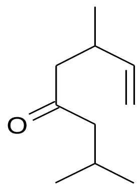
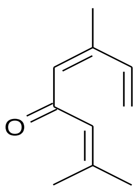
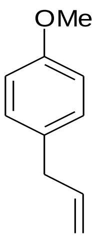


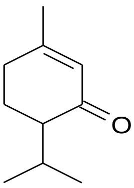
Dihydro tagetone
(144)



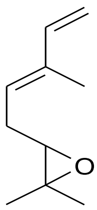
(*Z*)-Ocimenone
(145)
CAS # 33746-71-3



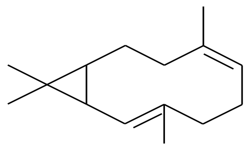
Methyl chavicol
(146)
CAS # 140-67-0



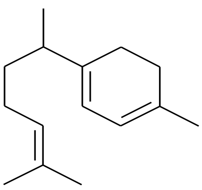
Piperitone
(147)
CAS # 89-81-6



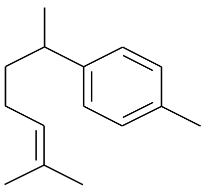
(*E*)-Myroxide
(148)
CAS # 94607-47-3



Bicyclogermacrene
(149)
CAS # 24703-35-3



γ-Curcumene (150)
CAS # 28976-68-3



ar-Curcumene (151)
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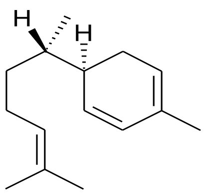

α-Zingiberene (152)
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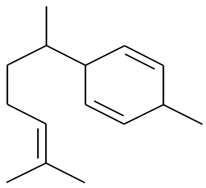

β-Curcumene (153)
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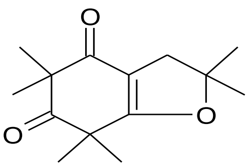
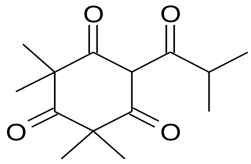
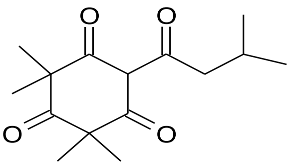


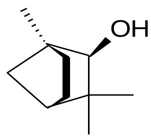
(154)



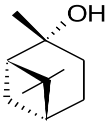
Flavesone (155)
CAS # 22595-45-5



Leptospermone (156)
CAS # 567-75-9



endo-Fenchol (157)
CAS # 14575-74-7



cis-Pinene hydrate (158)
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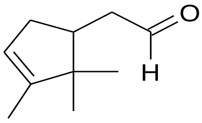

α-Campholenal (159)
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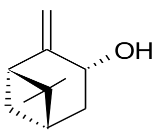
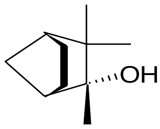
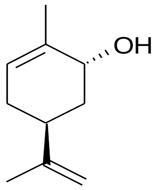


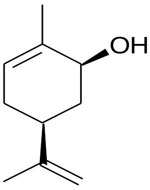
trans-Pinocarveol (160)
CAS # 1674-08-4



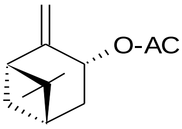
Camphene hydrate (161)
CAS # 465-31-6



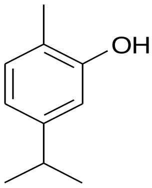
trans-Carveol (162)
CAS # 1197-07-5



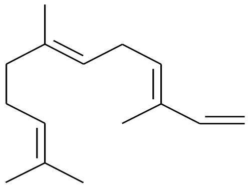
cis-Carveol(163)
CAS # 1197-06-4



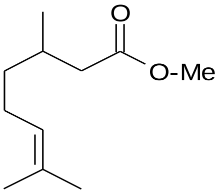
trans-Pinocarvyl acetate (164)
CAS # 1686-15-3



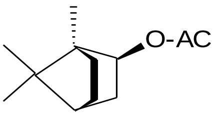
Carvacrol (165)
CAS # 499-75-2



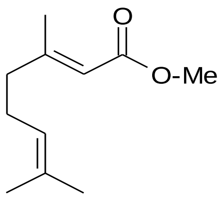
(*E*, *E*)-α-Farnesene (167)
CAS # 502-61-4-9



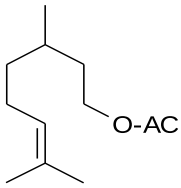
Methyl citronellate (168)
CAS # 2270-60-2



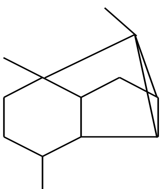
Bornyl acetate (169)
CAS # 76-49-3



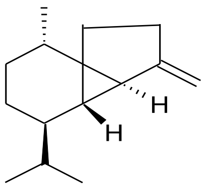
Methyl geranate (170)
CAS # 2349-14-6



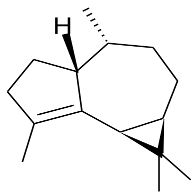
citronellyl acetate (171)
CAS # 150-84-5



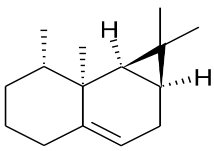
Cyclosativene (172)
CAS # 22469-52-9



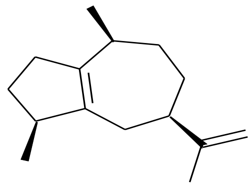
β-Cubebene (173)
CAS # 13744-15-5



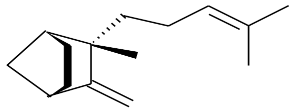
α-Gurjunene (174)
CAS # 489-40-7



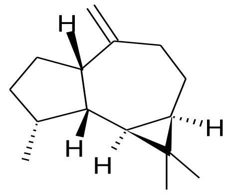
Aristolene (175)
CAS # 6831-17-0



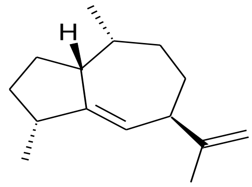
α-Guaiene (176)
CAS # 3691-12-1



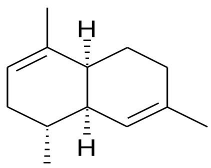
β-Santalene (177)
CAS # 511-59-1



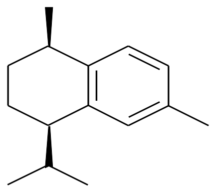
allo-Aromadendrene (178)
CAS # 25246-27-9



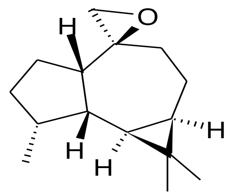
γ-Gurjunene (179)
CAS # 22567-17-5



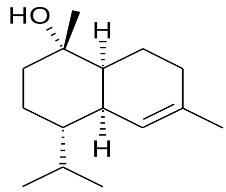
α-Muurolene (180)
CAS # 31983-22-9



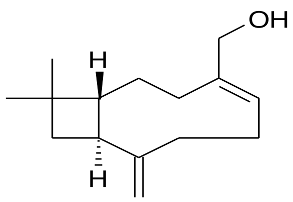
cis-Calamenene (181)
CAS # 72937-55-4



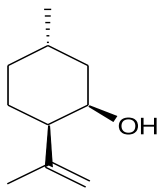
epoxy-*allo*-
Alloaromadendrene (182)
CAS # 85760-81-2



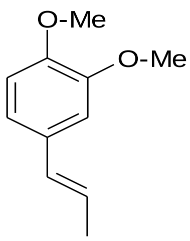
epi-α-Muurolol (183)
CAS # 19912-62-0



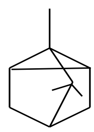
(*E*)-14-hydroxy-9-*epi*-
Caryophyllene (184)
CAS # 79768-25-5



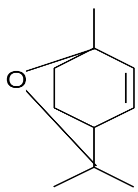
Isopulegol (185)
CAS # 121468-66-4



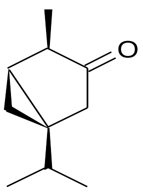
(*E*)-Methyl isoeugenol(186)
CAS # 6379-72-2



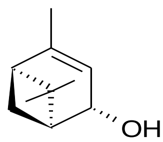
Tricyclene (187)
CAS # 508-32-7



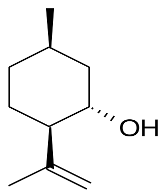
dehydro-1,8-Cineole (188)
CAS # 66113-06-2



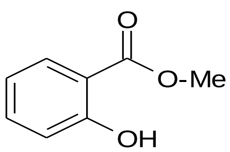
cis-Thujone (189)
CAS # 546-80-5



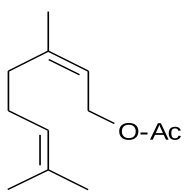
cis-Verbenol(190)
CAS # 1845-30-3



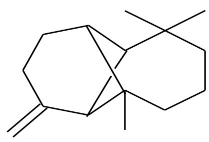
iso-Isopulegol(191)
CAS # 59905-54-3



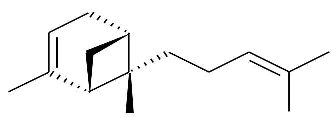
Methyl Salicylate (193)
CAS # 119-36-8



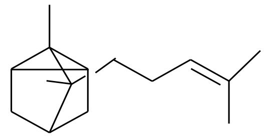
Neryl acetate (194)
CAS # 141-12-8



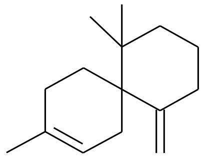
β-Longipinene (195)
CAS # 41432-70-6



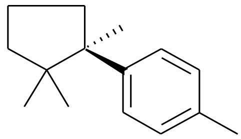
cis-α-Bergamotene (196)
CAS # 64727-43-1



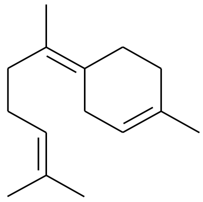
α-Santalene (197)
CAS # 512-61-8



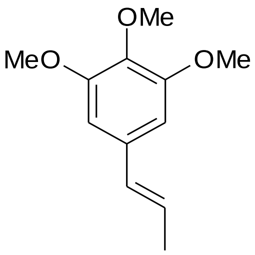
β-Chamigrene (198)
CAS # 18431-82-9



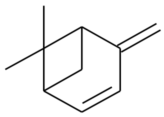
Cuparene (199)
CAS # 16982-00-6



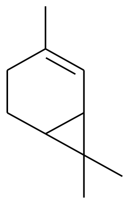
(*E*)-γ-Bisabolene (200)
CAS # 53585-13-0



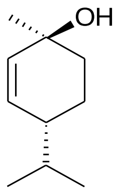
(*E*)-Isoelemicin
(201)
CAS # 5273-88-8



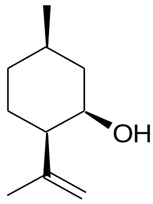
Verbenene
(202)
CAS # 4080-46-0



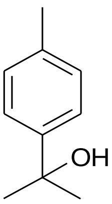
δ-2-Carene
(203)
CAS # 554-61-0



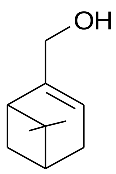
trans-*p*-Menth-2-en-1-ol (204)
CAS # 29803-81-4



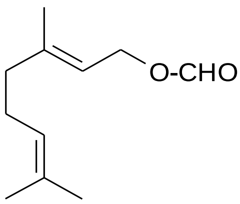
neoiso-Isopulegol
(205)
CAS # 21290-09-5



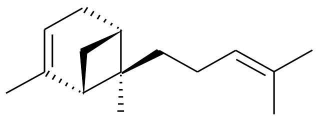
p-Cymen-8-ol
(206)
CAS # 1197-01-9



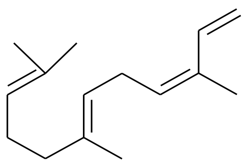
Myrtenol
(206a)
CAS # 515-004



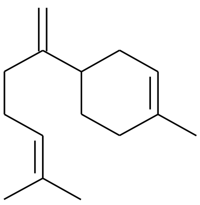
Geranyl formate
(207)
CAS # 105-86-2



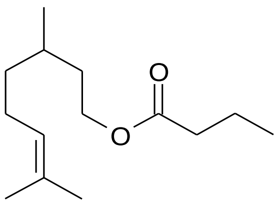
trans-α-Bergamotene
(208)
CAS # 13474-59-4



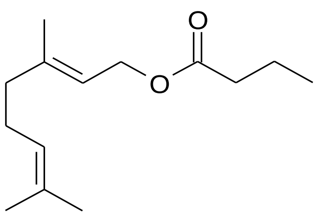
(*Z*, *E*)-α-Farnesene
(209)
CAS # 26560-14-5



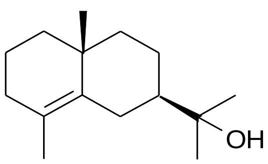
β-Bisabolene (210)
CAS # 495-61-4



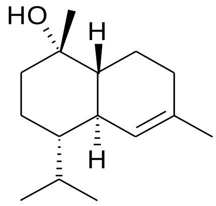
Citronellyl n-butyrate (211)
CAS # 141-16-2



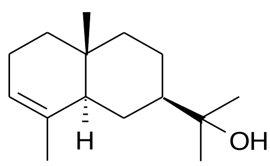
Geranyl n-butyrate (213)
CAS # 106-29-6



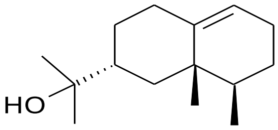
γ-Eudesmol (215)
CAS # 1209-71-8



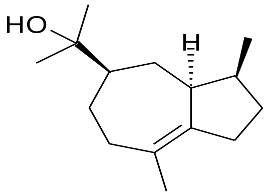
epi-α-Cadinol (216)
CAS # 11070-72-7



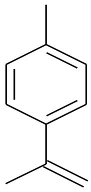
α-Eudesmol (217)
CAS # 473-16-5



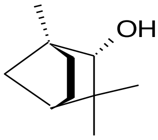
Valerianol (218)
CAS # 20489-45-6



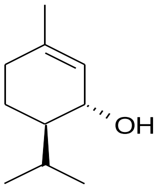
Bulnesol (219)
CAS # 22451-73-6



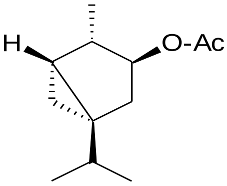
p-Cymenene
(220)
CAS # 1195-32-0



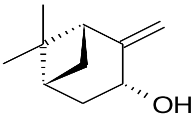
exo-Fenchol
(221)
CAS # 22627-95-8



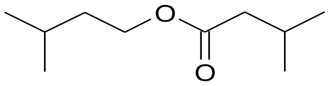
trans-Piperitol
(222)
CAS # 16721-39-4



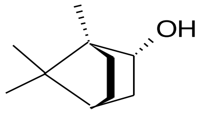
iso-3-Thujyl
acetate (223)
CAS # 62181-90-2



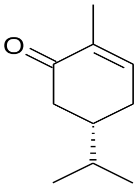
cis-Pinocarveol
(224)
CAS # 19889-99-7



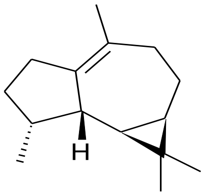
Isopentyl isovalerate
(225)
CAS # 659-70-1



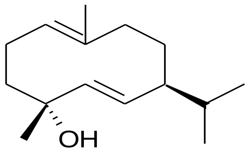
Isoborneol
(226)
CAS # 124-76-5



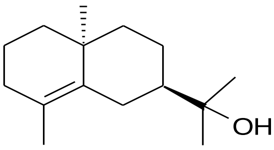
Carvotanacetone
(228)
CAS # 499-71-8



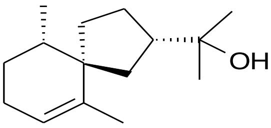
Viridiflorene
(229)
CAS # 21747-46-6



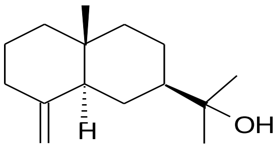
Germacrene D-4-ol
(230)
CAS # 74841-87-5



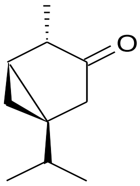
10-*epi*-γ-Eudesmol (231)
CAS # 15051-81-7



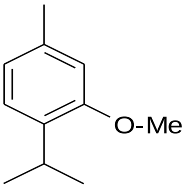
Hinesol (232)
CAS # 23811-08-7



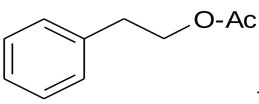
β-Eudesmol
(233)
CAS # 473-15-4



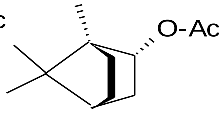
trans-Thujone
(234)
CAS # 471-15-8



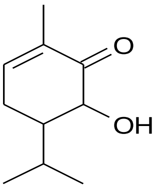
Methyl thymol
(235)
CAS # 1076-56-8



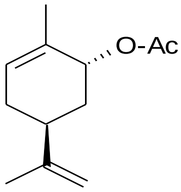
2-Phenyl ethyl
acetate (236)
CAS # 103-45-7



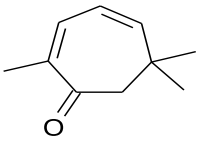
Isobornyl
acetate (237)
CAS # 76-49-3



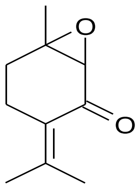
6-hydroxy-
Carvotanacetone (238)
CAS # 131486-99-2



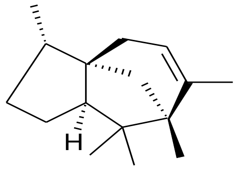
trans-Carvyl
acetate (239)
CAS # 1134-95-8



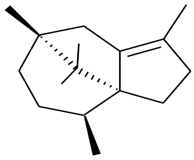
Eucarvone (240)
CAS # 503-93-5



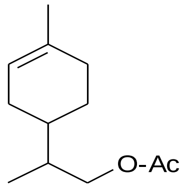
Piperitenone oxide (241)
CAS # 35178-55-3



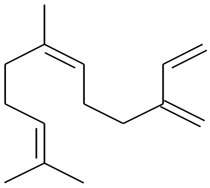
1,7-di-*epi*-α-Cedrene (242)
CAS # 50894-66-1



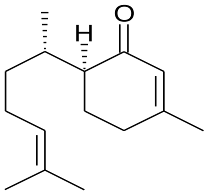
Cyperene (243)
CAS # 2387-78-2



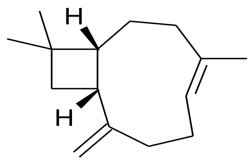
p-Menth-1-en-9-ol acetate (244)
CAS # 28839-13-6



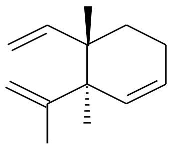
(*Z*)-β-Farnesene (245)
CAS # 28973-97-9



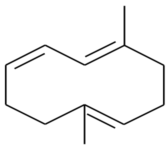
Bisabolone (246)



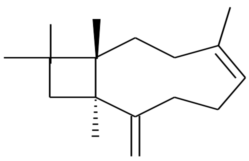
2-*epi*-Caryophyllene (247)
CAS # 68832-35-9



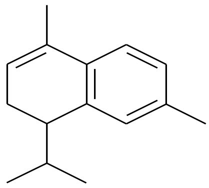
Geijerene (249)
CAS # 6902-73-4



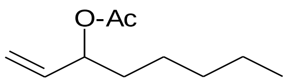
Pregeijerene (250)
CAS # 20082-17-1



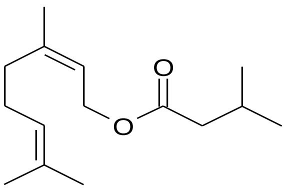
γ-Caryophyllene (251)
CAS # 118-65-0



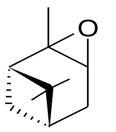
α-Calacorene (252)
CAS # 21391-99-1



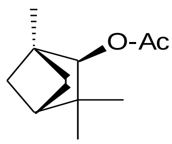
1-Octen-3-yl-acetate (253)
CAS # 2442-10-6



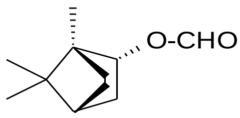
Neryl isovalerate (254)
CAS # 3915-83-1



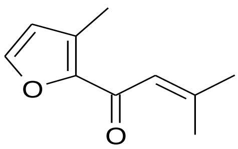
α-pinene oxide (255)
CAS # 1686-14-2



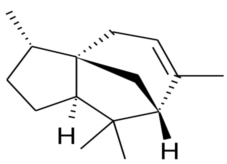
endo-Fenchyl acetate (256)
CAS # 4057-31-2



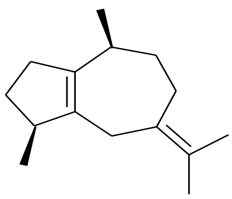
Isobornyl formate (257)
CAS # 1200-67-5



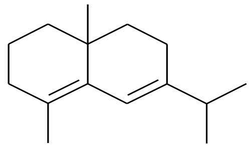
Dehydro elsholtzia ketone (258)
CAS # 6138-88-1



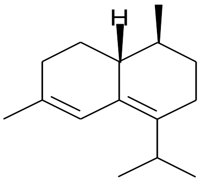
α-Cedrene (259)
CAS # 469-61-4



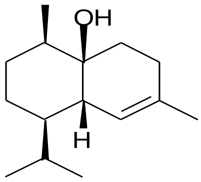
cis-β-Guaiene (260)
CAS # 88-84-6



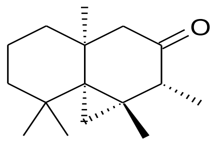
Selina-4, 6-diene (261)
CAS # 62358-43-4



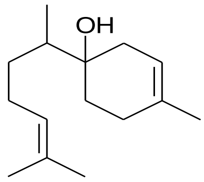
Epizonarene (262)
CAS # 41702-63-0



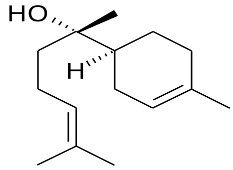
1,10-di-*epi*-Cubenol (263)
CAS # 73365-77-2



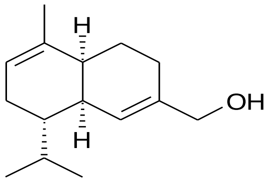
3-*iso*-Thujopsanone (264)
CAS # 25966-81-8



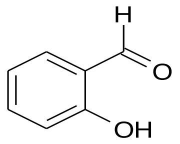
β-Bisabolol (265)
CAS # 15352-77-9



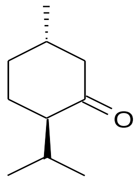
α-Bisabolol (266)
CAS # 25428-43-7



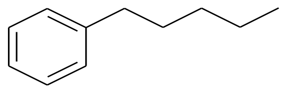
14-hydroxy-α-Muurolene (267)
CAS # 135118-51-3



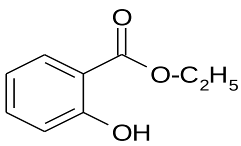
Salicyldehyde (268)
CAS # 90-02-8



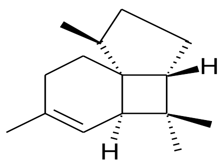
Menthone (269)
CAS # 89-80-5



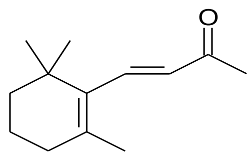
Pentyl benzene (270)
CAS # 538-68-1



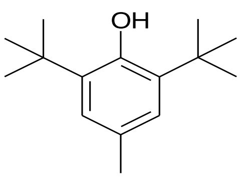
Ethyl salicylate (271)
CAS # 118-61-6



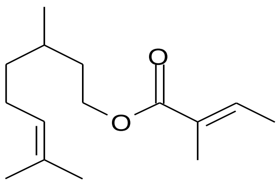
Italicene (272)
CAS # 94535-52-1



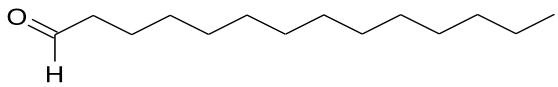
(*E*)-β-Ionone (274)
CAS # 79-77-6



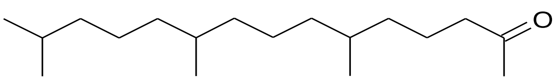
Butylated hydroxy toluene (275)
CAS # 128-37-0



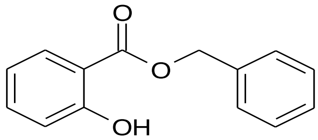
(*E*)-Citronellyl tiglate (276)
CAS # 24717-85-9



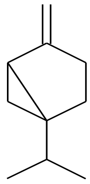
Myristaldehyde (277)
CAS # 124-25-4



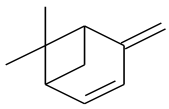
Hexahydrofarnesyl acetone (278)
CAS # 502-69-2



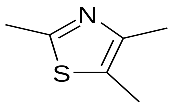
Benzyl salicylate (279)
CAS # 118-58-1



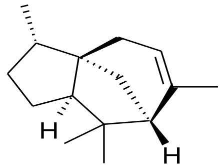
Thuja-2,4 (10)-diene (281)
CAS # 36262-09-6



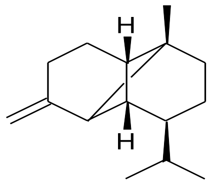
Verbenene (282)
CAS # 4080-46-0



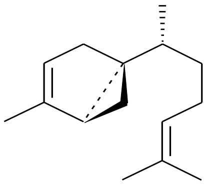
2, 4, 5-trimethyl Thiazole (283)
CAS # 13623-11-5



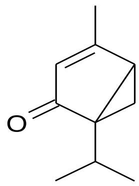
1, 7-di-*epi*-α-Cedrene (284)
CAS # 50894-66-1



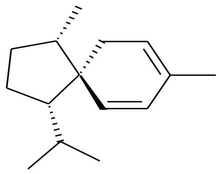
β-Copaene (285)
CAS # 18252-44-3



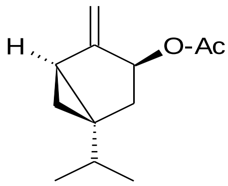
Sesquithujene (286)
CAS # 58319-06-5



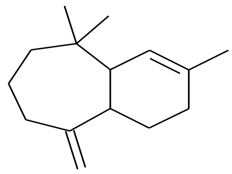
Umbellulone (287)
CAS # 24545-81-1



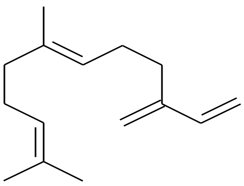
Acora-3,5-diene (288)
CAS # 89955-08-8



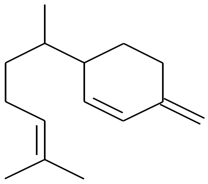
trans-Sabinyl acetate (289)
CAS # 53833-85-5



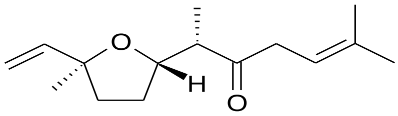
α-Himachalene (290)
CAS # 3853-83-6



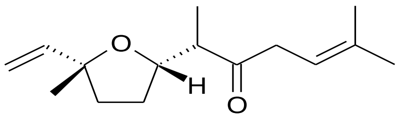
(*E*)-β-Farnesene (291)
CAS # 18794-84-8



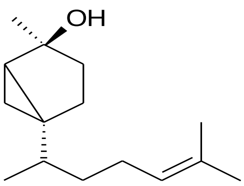
β-Sesquiphellandrene (292)
CAS # 20307-83-9



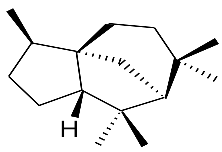
Davanone B (294)
CAS # 134107-13-4



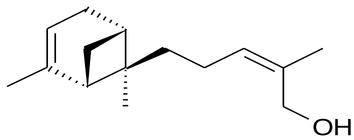
Davanone (295)
CAS # 20482-11-5



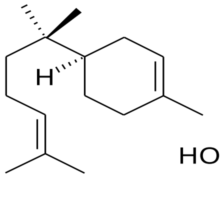
trans-sesquisabinene hydrate (296)
CAS # 145512-84-1



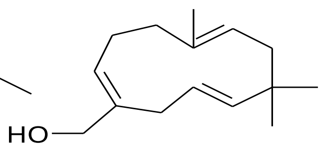
Cedrol (297)
CAS # 77-53-2



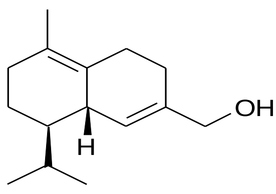
(*Z*)-α-*trans* Bergamotol (298)
CAS # 88034-74-6



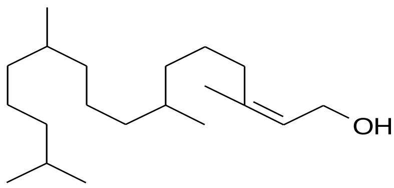
epi-α-Bisabolol (299)
CAS # 78148-59-1



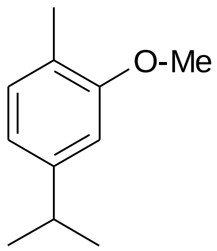
14-hydroxy-α-Humulene (300)
CAS # 75678-90-9



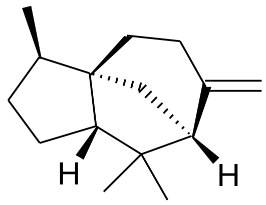
14-hydroxy-δ-Cadinene (301)
CAS # 135118-52-4



Phytol (302)
CAS # 150-86-7



Methyl carvicrol (303)
CAS # 6379-73-3



β-Cedrene (304)
CAS # 546-28-1

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