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Characterization and Evaluation of Repellent Effect of Essential Oil of *Mangifera indica* L. from Kenya

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Abstract: Ethnopharmacologically, the mango has a lot of applications in life in human health and ethnoveterinary medicines since ancient times. The study aimed at characterizing the essential oil of *Mangifera indica* L. leaves and evaluating its repellent effect on the host-seeking female *Anopheles gambiae*, the vector of African malaria. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The repellent effect of the essential oil was evaluated using the human-bait technique to simulate field situation. Of the 26 major hydrocarbon compounds identified, α-pinene occurred in the largest amount (33.3 %), followed by α-phellandrene (22.6 %), Limonene (13.2 %), p-cymene (6.1 %), Heptane (3.8 %), β-pinene (2.6 %), Ledene (1.3 %), (-)-α-gurjunene (1.2 %), β-myrcene (1.1 %), γ-terpinene (1.0 %), (+)-2-carene (0.9 %) and *trans* (β)-caryophyllene (0.9 %) in that order. The oil showed a significant dose-dependent repellent effect on host-seeking female *Anopheles gambiae s.s.* The oil showed a complex composition of hydrocarbon compounds and may be richer in monoterpenes than in any other type of compounds. It showed the potential to repel mosquitoes.

Key word index: *Mangifera indica*; Anacardiaceae; Volatiles; Essential oil constituents; Repellents; *Anopheles gambiae.*

Introduction: *Mangifera indica* L. is a large evergreen tree of tropical and sub-

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tropical regions belonging to the order Sapindales in the family Anacardiaceae which is notorious for embracing a number of highly poisonous plants [1]. The exact origins of the plant are unknown but it is believed that the plant is native to Southern and Southeast Asia owing to the wide range of genetic diversity of the plant in the region and fossil records dating back 25 to 30 million years. For over 4000 years, the plant has been cultivated in the Indian subcontinent where its fruits are highly valued at national level and are known as the 'King of Fruits.' The plant is now found naturalized in most tropical countries. The Persian traders are believed to have introduced the mango plant to East Africa (Kenya) about the 10th Century A.D [1].

The mango plant manifests as one of the most important source of nutraceutical and functional food [2]. The plant has a lot of applications in life ranging from traditional and modern applications in human health to those in ethnoveterinary and Ayurvedic medicines [3,4]. Culturally, mango plants and their fruits and leaves are associated with fortune, plenty and fertility in South Asian folklore. They are represented in religious themes across South Asia, whether Hindu, Buddhist, Muslim or Christian [3,5]. The barks from the mango plant also contain tannins, which are used for the purpose of dyeing. At 3-10 % of mango oil in formulations, are used in cosmetic applications requiring moisturization and revitalization of dry skin. Mango oil is also good for baby creams, suncare balms and haircare products [6]. Hypoglycaemic, anti-inflammatory, anticancer, antimicrobial, antioxidant, hepatoprotective, immunomodulatory, antiviral, antifungal, antispasmodic, antipyretic, dyslipidemia, and antidiarrheal activities of various mango extracts and pure compounds (mainly mangiferin) have been demonstrated [4,7,8,9,10,11,12].

Because of the economic importance and widespread consumption of the mango fruit, the volatiles of mango fruit have been a subject of considerable study [13,14,15,16,17,18,19,20,21,22,23,24,25]. Major chemical constituents from *M. indica* have been shown to range from saponins, hydrocarbons, triterpenes, xanthones, phenolics, fatty acids, vitamins and carotenoids (vitamins A and C, β-carotene and xanthophylls) to many other essential oil compounds such as humulene, elemene, ocimene, linalool, nerol etc.[4,26, 27]**.** Pino and Mesa [28] have evaluated mango volatiles in 20 cultivars and found the following compounds to be important to mango aroma: ethyl 2-methylpropanoate, ethyl butanoate, (E,Z)-2,6-nonadienal, (E)-2-nonenal, methyl benzoate, (E)-β-ionone, decanal and 2,5-dimethyl-4-methoxy-3[2H]-furanone (mesifurane). In further studies, they found that the major mango volatiles were the terpene hydrocarbons δ-3-carene, limonene, terpinolene and α-phellandrene [29]. However, up to now, few studies have been carried out using the leaves of the mango plant but no paper has hitherto been published on the chemical characterization of the essential oil from the leaves of *M. indica* in Kenya.

In the current study, we report the chemical composition of the essential oil extracted from the leaves of mango plant obtained from Nairobi, Kenya and its effect on host-seeking adult female *Anopheles gambiae s.s.*

Experimental

Plant materials: Fresh leaves of *M. indica* were collected from Kasarani division, Nairobi province, Kenya during the months of June/July, between 9.00 am and 11.00 am

in the morning. This place is located between geographical coordinates 1° 17' 0" South and 36° 49' 0" East. The location of the place is 1,661 metres (5,450 ft) above sea level with temperatures ranging from 10°C (50°F) in the June/July season to 24°C (75°F) in the sunniest and warmest part of the year from December to March.

The identity of the collected leaves of *M. indica* (Fig. 1) was confirmed in the herbarium at the School of Biological Sciences, University of Nairobi, Kenya.

The plant grows to a height of 35-40 m and is topped with a rounded canopy of foliage forming a crown radius of about 10 m. The leaves of the plant are evergreen, alternate, simple, 15-35 cm long and 6-16 cm wide. When the leaves are young, they are flaccid and pendulous in morphology and orange-pink in colour but the colour rapidly changes to a dark glossy red and then to dark green as they mature. The leaves appear in flushes and have fibers and crackle when crushed. They strongly smell of turpentine (some cultivars do not smell). The leaves contain considerable amounts of mangiferin (xanthone). The flowers are produced in terminal panicles 10-40 cm long. Each flower is small and white in colour with five petals that are 5-10 mm long.

The flowers produce a mild sweet odour suggestive of lily of the valley. Fruits develop from flowers and take three to six months to ripen. The mango plants may live for more than 300 years once planted [5].

In Kenya, there are two varieties of mango plant that are grown: the local and exotic (improved) varieties. The latter is usually grafted on local variety and is grown for the export market where Kenya ranks second to South Africa in terms of export of mango fruits from the continent of Africa. The local mango varieties are usually left to grow naturally without much crop husbandry. In this study, the local varieties of mango plants were purposively chosen to reflect the Kenyan situation. The dark green mature leaves were collected from the local varieties of mango plants growing in the wild.

Fig. 1. Mango leaves and fruits: The leaves are long and leathery. They have fibres which ‘crackle’ when they are crushed. The fruit is a well-known large drupe, but shows a great variation in shape and size. It contains a thick yellow pulp, single seed and thick yellowish-red skin when ripe. (Adopted from Morton, 1987) [1].

Extraction of essential oils: Following the preliminary studies, the extraction of the essential oils was done in accordance with the operating standard procedures laid down in the chemistry laboratory at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. The identified and confirmed plant materials (about 20 Kg), were put in a well-ventilated room for about 1-2 weeks before hydrodistillation. The materials were cut into small pieces and about 1 Kg was hydrodistilled using a clevenger-type apparatus for 8 h [30,31]. Pure oil was collected into 2 ml sealed glass vials and stored at -20°C in a deep freezer until required for analysis and bioassay studies.

Synthetic chemicals: Synthetic standard chemicals (authentic samples) used in GC co-injections were obtained from Sigma Chemical Company Limited, Poole, Dorset BH17 7TG, UK and Aldrich Chemical Company Limited, Gillingham, Dorset SP8 4JL, UK. All the authentic samples used were over 95 % pure.

Test material: One gram of neat essential oil was dissolved in 10 ml acetone to give 10 g/ml (10 %) solution. By serial dilution with acetone, 0.01 %, 0.1 %, 1 %, 2 % and 6 % solutions were prepared for bioassay studies of the repellent effect.

Assay of the repellent effect of the essential oil: The female *Anopheles gambiae s.s*. used were obtained from a colony reared according to the WHO [32] protocol at ICIPE, Nairobi, Kenya. The larvae were reared at 32-36°C and fed on TetraMin® (manufactured by Tetra GmbH, Germany). The adults were maintained on 6 % glucose solution and the females fed on human blood thrice a week. Rearing temperatures and relative humidity were 26 - 28°C and 70 - 80 %, respectively, for the adults.

The repellent effect of the essential oil was evaluated using the human-bait technique to simulate the condition of human skin to which repellents will be eventually applied [32,33]. Six human volunteers were selected from those who showed mild or no allergic reaction to mosquito bites or candidate oils. They had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. Evaluation was carried out in a 7 m × 5 m × 3 m room, at 30 - 32°C and relative humidity of 65 - 80 % using 5 - 7 days old female *An. gambiae* that had been starved for 18 h, but previously fed on 6 % glucose solution. Bioassay of the essential oils was carried out in aluminium-frame cages (50 cm × 50 cm × 50 cm), with aluminium sheet bottom, window screen (mesh size 256) on top and back, clear acrylic (for viewing) on the right and left sides, and a cotton stockinet sleeve for access on the front, at 0.01 %, 0.1 % , 1 % , 2 %, 6 % and 10 % concentration levels [32]. Briefly, test solutions (0.5 ml), in HPLC grade acetone, were dispensed on one of the forearms of a volunteer from the wrist to the elbow. The rest of the hand was covered with a glove. HPLC grade acetone (0.5 ml) was dispensed on the other forearm to serve as control. The control and test arms of one person were interchanged regularly (in the any succeeding observations, a control arm could become a test arm and a test arm could become a control arm) following thorough cleaning and drying of both arms to eliminate any bias. Experimental mosquitoes (100) were released into the bioassay cage in paper cups and left for 3 min to settle. By gently tapping the sides of the experimental cages, the mosquitoes were activated, the control arm introduced into the cage first and kept there for 3 min. The

mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood. Subsequently, the test arm was introduced into the cage for the same duration and the number of landing insects recorded. The different sample concentrations were tested sequentially starting with the lowest one. For each concentration, both the test and control arms were exposed to the experimental mosquitoes 20 times and an average of protection efficacy obtained. The repellent effect of the essential oil was evaluated according to the formula adopted by Odalo *et al*.[34] namely:

$$\text{protection efficacy (PE)} = ((C-T)/C) \times 100\ \%$$

where C and T represent the number of mosquitoes landing on control and treated arms, respectively.

Determination of the composition of the essential oils: Both qualitative and quantitative characteristics of the various essential oils were studied using gas-chromatography (GC) and gas-chromatography/Mass Spectrometry (GC-MS) techniques [35]. The constituents of the essential oils were identified by analysis of their mass spectra, direct comparison of their mass spectra to the Wiley NBS and NIST databases or library of mass spectra, and co-injection with authentic standards on the GC.

GC analyses were performed with a Hewlett Packard HP 5890A Gas Chromatography equipped with a flame ionization detector (at 230°C). A fused silica capillary column (Hewlett Packard, 50 m × 0.22 × 0.33 mm CD) coated with methyl silicon (0.3 μm film thickness) was used with nitrogen as the carrier gas. All GC analyses were performed in the splitless mode with the injector temperature at 270°C. The oven temperature was programmed from 60°C isothermal for 7 min, to 120°C at 5°C per min, then to 180°C at 10°C per min, and finally to 220°C at 20°C per min, where it was maintained for l0 min. Peak areas were calculated using a Hewlett Packard 3393 B series integrator and together with their GC retention times, compared to those of authentic samples.

GC-MS analyses were performed with a VG Masslab 12-250 quadruple gas chromatography-mass spectrometer. Chromatographic separations were achieved using a fused silica capillary column (Hewlett Packard, 50 m × 0.32 mm ID) coated with Carbowax 20M (0.3 μm film thickness) with helium as the carrier gas. All the GC-MS analyses were made in the splitless mode with helium as the carrier gas. The GC column temperature was programmed as in the case of GC analysis. Compounds were identified by their electron impact (El) mass spectral data, order of elution and relative GC retention times, and by comparison of their mass spectra and GC retention times to those of authentic samples.

The computer on the GC-MS system records a mass spectrum for each scan and has a library of spectra that can be used to identify an unknown chemical in the sample. The library compares the mass spectrum from a sample component with mass spectra in the library. It then reports a list of likely identifications along with the statistical probability of the match.

Data management and analysis: Data were entered in excel, database structure constituted, cleaned and entered into an SAS database and made usable as SAS data sets

for analysis. The data were transformed and subjected to analysis of variance. (ANOVA) [36]. Student-Newman-Kuels (SNK) test was used to compare the mean values of repellency obtained of different doses expressed as protection efficacy [36].

Legal use of experimental animals and humans: All the procedures requiring experimental animals were approved by ICIPE's Institutional Animal Care and Use Committee and were performed in compliance with guidelines published by the Kenya Veterinary Association and the Kenya Laboratory Animal Technician Association [37]. The research involving human volunteers used for mosquito repellency bioassays, followed guidelines of the Declaration of Helsinki and Tokyo for humans and the research was conducted in accordance with the ICIPE's ethical rules on scientific research and development. In addition, informed consent was obtained from the human volunteers used for mosquitoes' repellency bioassays.

Results and discussion

Gas Chromatography analyses of the essential oil of *Mangifera indica*: Each of the peaks in the chromatogram represents the signal created when a compound in the injected essential oil elutes from the GC column into the detector. The result in Fig. 2 shows the gas chromatogram of the essential oil of *M. indica* indicating a variety of compounds manifested in the several peaks labeled with their corresponding retention times (RTs). The corresponding compounds to the RTs are shown in Table 1, respectively. However, some of the compounds existing in very minute amounts of less than the lowest percentage (0.2 %) shown in Table 1, were not shown in Fig. 2.

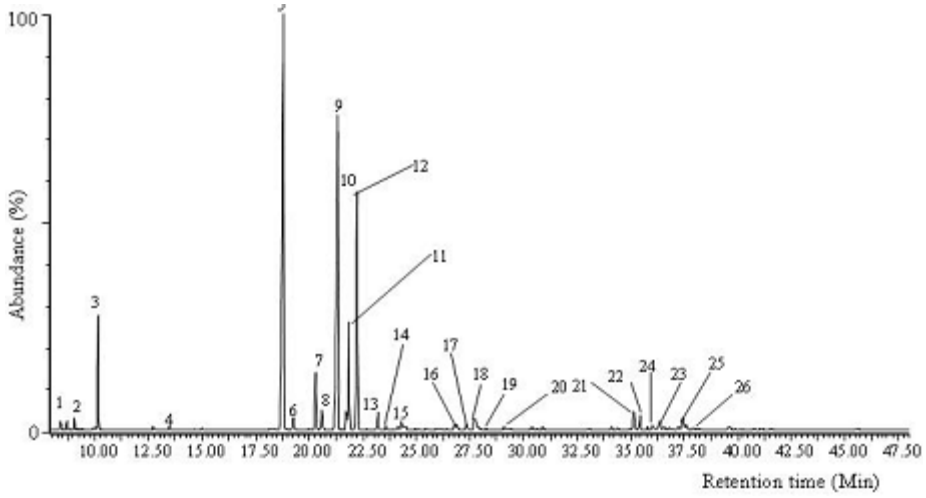

Fig. 2. Chromatogram generated by gas chromatography for the essential oil of *Mangifera indica*. Each of the peaks in the chromatogram represents the signal created when a compound in the injected essential oil elutes from the chromatographic column into the detector.

Mass spectroscopy of the essential oil of *Mangifera indica*: The yield of the essential oil of the fresh aerial parts of *M. indica* was 0.000369 % w/w. Individual components in the sample mixture of the essential oil of *M. indica* were characterized and revealed natural mixes of constituent chemicals (Table 1). In the essential oil, 26 major compounds were identified. Of these 26 compounds, 76.9 % (20 compounds) were monoterpene hydrocarbons while the rest 23.1 % (6 compounds) were sesquiterpene hydrocarbons. All the monoterpene hydrocarbons were separated within the first 30 min while the sesquiterpene hydrocarbons followed in the next 8 min. Of these 26 hydrocarbon compounds, α-pinene occurred in the largest amount (33.3 %), followed by α-phellandrene (22.6 %), Limonene (13.2 %), p-cymene (6.1 %), Heptane (3.8 %), β-pinene (2.6 %), Ledene (1.3 %), (-)-α -gurjunene (1.2 %), β-myrcene (1.1 %), γ-terpinene (0.9 %), (+)-2-carene (0.9 %) and *trans* (β)-caryophyllene (0.9 %) in that order (Table 1). Of these 12 top compounds, only three are sesquiterpene hydrocarbons (Ledene, (-)-α-gurjunene and *trans* (β)-caryophyllene) while the rest are monoterpene hydrocarbons.

Repellency assays of the volatile oils: The essential oil from *M. indica* showed repellent effect against host-seeking female *An. gambiae*. The results of this effect are shown in Fig. 3 and manifest dose-dependent responses.

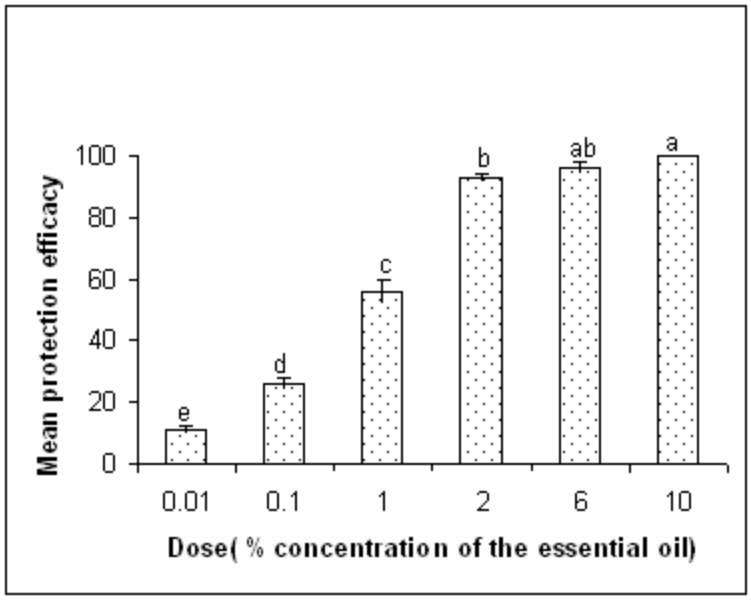

Fig. 3. Mean repellency percentage of different doses of the essential oil of *M. indica* against adult female *Anopheles gambiae* s. s. Means (±SE) with the same letter(s) are not significantly different from one another at α = 0.05 level of significance (Students-Newman-Keuls H test)

Discussion and conclusions: In the present study, gas-chromatography (GC) and

gas-chromatography/Mass Spectrometry (GC-MS) techniques have revealed natural mixes of chemicals constituent in the essential oil of *M. indica*. Combined GC-MS and GC is a very useful tool for the analysis of mixtures, especially when using chemical ionization in positive and negative modes. However, when the GC-MS-GC technique is used sloppily by untrained operators, the interpretation of results may be of limited value [38]. By using GC-MS-GC technique, this study reports a total of 26 major hydrocarbon chemical components in the essential oil of *M. indica* from Kenya. As previously reported, these hydrocarbon chemical components may be contributing to mango volatiles and aroma chemistry [16,19,39]. Three (limonene, terpinolene and α-phellandrene) of the four (δ-3-carene, limonene, terpinolene and α-phellandrene) previously noted to be the most important oxygenated aroma compounds in mango[29] were also reported by this study. Our results are also comparable to other studies by MacLeod and Pieris [16] and MacLeod *et al*. [19] conducted on the African mango where eremophilene along with minor amounts of δ-3-carene, ethyl-3-hydroxybutyrate and butyric acid were reported to be present.

Of the 26 compounds identified from the essential oils of *M. indica* in this study, 15 were also constituents of another set of plants, which had been previously assayed and reported of their repellent effects against *An. gambiae* [34,40]. These studies support the current results obtained on the repellent effects of the essential oil of *M. indica* against *An. gambiae*. The results showed dose-dependent responses against the host-seeking female *An. gambiae*, similar to previous studies [34,40,41]. The essential oil of *M. indica* therefore represents a potential candidate to exploit as mosquito repellent at an individual level [42]. The feasibility of such preventive measures against bites of vectors has been shown by the use of repellents [43]. Repellents provide a practical means of protection against bites of vectors and along with preventive measures provided for by the use of vaccines, they may be a fundamental resource for minimizing the transmission of vector-borne diseases at an individual level [44,45].

Being one of the most important sources of nutraceutical and functional food [2], the study of the essential oil of mango and its constituent compounds and the search for their bioactivities therefore increases our knowledge of the potential use of the oil in food and therapeutic industries. Further studies therefore on the various chemical components of the essential oil of *M. indica* are needed in order to exploit the full potential of the volatile phytochemical blends available.

In conclusion, the essential oil of *M. indica* leaves shows a complex composition of hydrocarbon compounds and may be richer in monoterpene hydrocarbons than in any other type of compounds. The results indicate that the oil from *M. indica* show the potential to repel mosquitoes to a variable degree and the bioactivity of the essential oil increases with concentration. There seems to be a direct correlation between concentration and bioactivity against the *An. gambiae*. The repellent property of the oil in addition to its use in the perfume, food and therapeutic industries, presents useful property that help to add potential economic value to the plant for its conservation.

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Table 1. The GC and GC-MS identified major constituent in the essential oil of *Mangifera indica*

No.	Compound	Molecular formula	M^+ (g/mol)	RT (min)	Relative (%)	Identification of compounds
1.	2-methyl-1-pentene	C_6H_{12}	84.2	8.40	0.5	GC-MS,GC-MS-CO
2.	3-methylbutan-2-ol	$C_5H_{12}O$	88.2	8.70	0.3	GC-MS,GC-MS-CO
3.	Heptane	C_7H_{14}	98.2	10.15	3.8	GC-MS,GC-MS-CO
4.	Octane	C_8H_{18}	114.2	13.47	0.2	GC-MS,GC-MS-CO
5.	α-pinene	$C_{10}H_{16}$	136.2	18.80	33.3	GC-MS,GC-MS-CO
6.	Camphene	$C_{10}H_{16}$	136.2	19.25	0.7	GC-MS,GC-MS-CO
7.	β-pinene	$C_{10}H_{16}$	136.2	20.30	2.6	GC-MS,GC-MS-CO
8.	β-myrcene	$C_{10}H_{16}$	136.2	20.60	1.1	GC-MS,GC-MS-CO
9.	α-phellandrene	$C_{10}H_{16}$	136.2	21.35	22.6	GC-MS
10.	(+)-2-carene	$C_{10}H_{16}$	136.2	21.73	0.9	GC-MS,GC-MS-CO
11.	p-cymene	$C_{10}H_{14}$	134.2	21.88	6.1	GC-MS,GC-MS-CO
12.	Limonene	$C_{10}H_{16}$	136.2	22.25	13.2	GC-MS,GC-MS-CO
13.	γ-terpinene	$C_{10}H_{16}$	136.2	23.20	0.9	GC-MS
14.	*cis*-Linalool oxide	$C_{10}H_{18}O_2$	170.2	23.60	0.3	GC-MS,GC-MS-CO
15.	Terpinolene	$C_{10}H_{16}$	136.2	24.33	0.6	GC-MS,GC-MS-CO
16.	2-cyclohexene-1-one	C_6H_8O	96.1	26.78	0.3	GC-MS
17.	Carvenone	$C_{10}H_{16}O$	152.2	26.88	0.4	GC-MS,GC-MS-CO
18.	4-terpineol	$C_{10}H_{18}O$	154.3	27.33	0.7	GC-MS,GC-MS-CO
19.	α-terpineol	$C_{10}H_{18}O$	154.3	27.75	0.6	GC-MS,GC-MS-CO
20.	Bornylene	$C_{10}H_{16}$	136.2	29.10	0.3	GC-MS
21.	(-)-α-Gurjunene	$C_{15}H_{24}$	204.4	35.18	1.2	GC-MS
22.	*trans* (β)-caryophyllene	$C_{15}H_{24}$	204.4	35.43	0.9	GC-MS
23.	Alloaromadendrene	$C_{15}H_{24}$	204.4	36.00	0.3	GC-MS
24.	α-humulene	$C_{15}H_{24}$	204.4	36.35	0.5	GC-MS
25.	Ledene	$C_{15}H_{24}$	204.4	37.45	1.3	GC-MS
26.	δ-guaiene	$C_{15}H_{24}$	204.4	37.60	0.5	GC-MS

M^+ = Molecular weight
RT = Retention time (min.)
GC-MS = identification based on comparison of mass spectra in NIST/NBS and Wiley libraries only
GC-MS Co = identification based on comparison of mass spectra in NIST/NBS and Wiley libraries followed by a comparison with retention time identical to authentic compounds