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Comparative studies of Chemical Constituents and Antimalarial Activity of Essential oils Extracted from the Stem, Root and Fruit peel of *Citrus paradisi* Grown in Nigeria

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Abstract *Three parts of Citrus paradisi grown in Nigeria were chosen with the aim of investigating the chemical compositions of the essential oils and their antimalarial effects. Two-dimensional Gas Chromatography coupled to Time of Flight Mass Spectrometry (GC-TOFMS) was used for compositional profiling and an in vitro anti-plasmodial activity against Plasmodium falciparum (CQS) NF54 was used for malarial screening of the oils. Pulverized plant samples were hydrodistilled using all-glass Clevenger-type apparatus to obtain the essential oils. The compositional profile of the stem oil revealed 36 components (93.28 %). A total of 179 compounds totaling 78.11 % were identified in the root oil while 126 components amounting to 90.51 % were detected in the fruit peel oil. The major components of the stem oil were 2-heptanone (24.18 %), 3(Z)-hexen-1-ol (23.04 %), hibaene (12.61 %) and naphthalene-1,2,3,4,4a,5,6,8-dimethyl-2-(1-methylethyl)-, [2R-(2a,4aa,8aa)] (10.26 %). α-cadinol (6.51 %) and α-phellandrene (6.32 %) were detected as the major components in the root oil meanwhile α-myrcene (13.08 %), limonene (11.15 %), caryophyllene (8.81 %) and (2R,5S)-2-methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran (8.36 %) were the major components in the fruit peel oil. C. paradisi root oil showed a moderate activity (IC_{50} =22.2 μg/mL), stem oil revealed weak activity (IC_{50} = 48.1 μg/mL) while fruit peel oil has no significant activity against P. falciparum strain. The compositional pattern of the oils obtained revealed significant qualitative and quantitative differences and these might have resulted in the observed differences in the antimalarial activity.*

Keywords: *Citrus paradisi, Plasmodium falciparum, constituents, hibaene*

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I. Introduction

There is an increase in the level of malaria parasite drug resistance nowadays. Malaria which is caused by a bite from a mosquito infected with parasites remains a major public health problem in Sub-Saharan Africa [1]. The most severe form of malaria is caused by *P. falciparum*. This causes death among children less than 5 years in Sub-Saharan African [2]. Further researches are requested on medicinal plants so as to detect new antimalarial drugs [3]. *Citrus* genus belongs to the large family *Rutaceae*, containing 130 genera in the seven subfamilies with many important fruit and essential oil producers [4].

The major constituents of Floridian *Citrus* oils was limonene (33.7 %) and other components included sabinene (7.8 %), *γ*-terpinen (7.4 %), *β*-ocimene (7.3 %), linalool (5.3 %), cironellal (7.3 %) and (*E*)-*β*-ocimene (5.0 %) [5]. Limonene and *α*-pinene were the main compounds in the peel oils of sour orange (grape fruit) [6]. The main components of Cretan *Citrus* (grape) leaf essential oils were limonene (54.02 %), *β*-pinene, myrcene, neral, geranial, neryl acetate and *β*-caryophyllene [7]. Essential oils were extracted from the peels of Malta (*C. sinensis*), Mousami (*C. sinensis*), Grapefruit (*C. paradisi*) and Eureka lemon (*C. limon*) and the main constituents detected in Malta peel oil were limonene (61.08 %), citral (7.74 %), borneol (7.63 %), citronellol (4.18 %), *α*-terpinolene (2.06 %) and linalool (1.28 %) [8]. The principal compounds in Turkish grapefruit peels oil were limonene (76.28 %), *β*-pinene (5.45 %), linalool (2.32 %), citral (1.74 %), and *α*-pinene (1.26 %) [9], while limonene (86.27 %), myrcene (6.28 %), γ-terpinene (2.11 %) and *α*-pinene (1.26 %) were present in Saudi Grape fruit peels essential oil [10]. It is obvious that the leaf and fruit peel oils of this plant shows extremely variable compositions in different localities.

Lemon essential oils are complex mixtures of chemical compounds like limonene, γ-terpinene, citral, linalool and β-caryophyllene among others, which can be represented by three main classes, namely terpenes, oxygenates and sesquiterpenes [11]. The major component of the *Citrus paradisi* fruit peel oil grown in Pakistan was limonene (50.8 %) [12]. Limonene (74.4 %) was also reported as the major component in Sudan *C. paradisi* fruit peel essential oils followed by myrcene (12.85 %) [13], they stated further that the Turkish grape fruit peels (*Citrus paradisi*) oil contains limonene (92.5 %) and myrcene (2.6 %). In the case of *C. paradisi* grown in Pakistan, its limonene (50.8 %) concentration, was very small compare to concentration of limonene (74.4 %) in Sudan *Citrus paradisi* oil.

This study was embarked on, due to the paucity of information on the antimalarial effects and compositional profile of the chemical constituents of the stem, root and fruit peels essential oils of *Citrus paradisi* grown in Nigeria. Therefore, this present study investigates the chemical constituents of the stem; root and fruit peel essential oils of *C. paradisi* and the effect of these oils on *Plasmodium falciparum*.

II. Experimental

Samples Collection

The selected plant parts (stem, root and fruit peels) of *Citrus paradisi* were collected from National Horticultural Research Institute (NIHORT) Ibadan, Nigeria. *Citrus paradisi* plant samples were identified and authenticated at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Voucher specimens were deposited with herbarium number FH110390.

Extraction of the Essential Oils

The freshly collected plant samples were immediately air dried in the laboratory at room temperature for four days. The air dried plant samples were pulverized and five hundred (500 g) of each sample was subjected to hydrodistillation. Each essential oil was obtained by hydrodistillation in an all-glass Clevenger-type apparatus [14].

GC-MS Analysis using a LECO Pegasus 2D GC-TOFMS

GC-MS analyses were carried out on the three essential oils obtained from plant parts of *Citrus paradisi*. They were all dissolved in n-hexane before the injection. Volatile compounds were identified and quantified using LECO Pegasus 2D GC-TOFMS. The GC was equipped with a DB-5 (30 m x 0.25 mm x 0.25 µm). The flow rate of the helium carrier gas was set up at 1.40 mL min^{-1} in the constant flow mode. The injection volume was of 1 µL. The GC inlet was maintained at 250 ^{0}C and was operated in slitting modes (50:1 split ratio). The GC oven temperature program was 40 ^{0}C (2 min) and then at 8 ^{0}C min^{-1} to 280 ^{0}C (5 min). The MS transfer line temperature was set up at 310 ^{0}C and the ion source temperature at 310 ^{0}C. The electron energy was 70 ev in the electron impact ionization mode (EI+), while the mass acquisition range was 40-450 atomic mass units (amu), and the detector voltage was set at 1650 V. Identification of volatile organic components was confirmed using their MS data compared to those from the NIST mass spectral library and published mass spectra [15,16]. The Compounds were further confirmed by comparison of their retention indices with those of known compounds [17]. The Kovats Index has been calculated for each sample peak. It is useful because the retention time may vary as columns age [18-20]. The formula used for calculation of KRI is Regression Analysis Method as follows:

$$Kovat\ Retention\ Index = \frac{100[\log tR\,(A) - \log tR\,(n)]}{[\log tR\,(n+1) - \log tR\,(n)]} + 100Cn$$

Where C_n is the carbon number of the alkane eluting just before the analyte
tR(n) is the retention time of the alkane eluting just before the analyte
tR(n+1) is the retention time of the alkane eluting just after the analyte
tR(A) is the retention time of the analyte.

III. Antimalarial screening

The essential oils obtained were screened for malaria parasite. *Citrus paradisi* essential oils were carried out in triplicate against chloroquine –sensitive (CQS) strain of *Plasmodium falciparum* (NF54). Continuous *in vitro* cultures of asexual erythrocyte stages *of Plasmodium falciparum* were maintained using a modified method of [21]. Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using modified method described by [3].

The oil samples were prepared as 20 mg m^{-1} stock solution in Dimethylsulfoxide. Oil samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -20 ^{0}C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) and artesunate were used as the reference drugs in all experiments. A full dose-response was performed for all compounds to determine the concentration inhibiting 50 % of parasite growth ($1C_{50}$ value). Test samples were tested at a starting concentration of 100 µg mL^{-1}, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 µg mL^{-1}. The same dilution technique was used for all samples. Reference drugs were tested at a starting concentration of 1000 µg mL^{-1}. The highest concentration of solvent to which the parasites were exposed to had no measurable effects on the parasite viability. The IC_{50} values were obtained using a non-linear dose-response curve fitting analysis via Graph pad prism v.4.0 software.

IV. Results and Discussion

Citrus paradisi stem essential oil yields (1.10 %w/v), *Citrus paradisi* root (0.80 %w/v) and *Citrus paradisi* fruit peel (1.30%w/v); these were in agreement with % yield values of *Citrus* plants (0.2 to 1.5 % w/v), which was recorded by [22]. The yields of essential oils in *Citrus paradisi* fruit peel were higher than the rest of the oil samples followed by *Citrus paradisi* stem. Tables 1, contain lists of the chemical components identified in the essential oils of stem, root and fruit peels of *Citrus paradisi* respectively. In the *C. paradisi* stem oil, 60 components were detected by GC-TOFMS but only 36 components were identified which make up 93.28 % of the total oil. On the contrary, 608 components were detected in the *C. paradisi* root essential oil while 179 (78.11 %) components were identified. In the case of the essential oil obtained from *C. paradisi* fruit peel, 200 components were detected while 126 components were identified which make up 90.51 % of the total oil. Only compounds with similarity of 80 % and above were identified (see supporting documents for mass spectra of the major components) from the compounds detected by GC-TOFMS; these are in line with [23] which reported that compounds with similarity of 80 and 90 % above indicate that an acquired mass spectrum is a good match with the library spectrum. The results observed were also in agreement with the result of *Citrus reshni* leaf oil by [22]; who reported that over 140 components were observed in GC/MS analysis, of which 123 could be identified. One hundred and thirty-nine (139) chemical components were identified in the *Citrus jambhiri* leafy oil [23]. The use of two-dimensional GC-TOFMS improved peak capacity by identifying up to 1200 compounds [24]. The present report is similar to their respective reports of the present study.

V. Proportion of classes of terpenoids identified in the oil of *Citrus paradisi*

The proportion of different classes of components identifies in the oils of *Citrus paradisi* are shown in Figure 1. Essential oils of odoriferous plants were characterized by monoterpenoids, oxygenated monoterpenoids, sesquiterpenoids, oxygenated sesquiterpenoids, diterpenoids, triterpenoids, polyterpenoids and other components. The chemical composition (%) of hydrocarbon monoterpenoids, oxygenated monoterpenoids, hydrocarbon sesquiterpenoids, oxygenated sesquiterpenoids and hydrocarbon diterpenoids present in stem, root and fruit peel of *Citrus paradisi* oils are shown in Figure 1. The essential oil obtained from *Citrus paradisi* stem reveals 36 components which represent 93.28 %, of which hydrocarbon sesquiterpene accounts for 15.85 %, diterpene (16.24 %) while the non-terpenoid components constitute 61.19 % of the total oil; hydrocarbon monterpenoids and oxygenated monoterpenoids were absent. One hundred and seventy-nine components (78.11 %) identify from the essential oil of *Citrus paradisi* root; it constitutes hydrocarbon monoterpenoids (18.2 %), oxygenated monoterpenoids (14.49 %), hydrocarbon sesquiterpenoids (18.16 %), oxygenated sesquiterpenoids (11.98 %), hydrocarbon diterpenoids (0.22 %), oxygenated diterpenoids (0.08 %) and non-terpenoids (14.98 %) components. The oil of *Citrus paradisi* fruits peel reveals the presence of 126 components which represent 90.51 % in which hydrocarbon monoterpenoids accounts for 31.27 %, oxygenated monoterpenoids (33.72 %), hydrocarbon sesquiterpenoids (16.74 %), oxygenated sesquiterpenoids (0.75 %), hydrocarbon diterpenoids (0.25 %) and non-terpenoids component (7.78 %). It is worthy to note that diterpene which are rare in essential oil constituents were detected in the oils of the stem, root and fruit peel of *C. paradisi.* Hydrcarbon diterpenes were detected in the stem, root and fruit peel oils while oxygenated diterpenes were only detected in the root oil of *C. paradise*

VI. Comparison of the chemical components present in the oil samples of Citrus paradisi

The major components identify in the oil of *Citrus paradisi* stem were 2-heptanone (24.18 %), 3(*Z*)-hexen-1-ol, (23.04 %), hibaene (12.61 %) and naphthalene, 1,2,3,4,4a,5,6, 8-dimethyl-2-(1-methylethyl)-, [2R-(2a,4aa,8aa)] (10.26 %) but absent in the essential oils of *C. paradisi* root and *C. paradisi* fruits peel. The major components identify in the essential oil of *C. paradisi* root were humulene (5.63 %) whereas humulene (0.78 %) was present as minor constituent in the oil of *C. paradisi* fruits peel. α-cadinol (6.51 %) is a major compound in *C. paradisi* root oil while it was absent in the remaining essential oils. In the same vein, the major components present in the *C. paradisi* fruit peel essential oil are α-myrcene (13.08 %), limonene (11.15 %), (2R,5S)-2-methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran (8.36 %) and caryophyllene (8.81 %) meanwhile α-myrcene was found to be moderate (2.89 %) constituent in the root oil of *C. paradisi*. Moreso, caryophyllene was identified as minor constituent (0.47 %) in the oil of *C. paradisi* root. Only *C. paradisi* fruit peel oil revealed the presence of (2R,5S)-2-methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran (8.36 %) but absent in the remaining essential oils samples. Limonene (50.80 %) was reported as main constituents in the oil obtained from Pakistan *C. paradisi* peels followed by myrcene (3.51 %) and linalool oxide (1.07 %) [12], this is similar to the results of present investigation where myrcene (2.89 and 13.08 %) identified in the oils of *C. paradisi* stem and fruit peel respectively. Moreso, in the present study, trans-linalool oxide (3.39 and 0.42 %) was observed in the *C. paradisi* fruit peels and root oils respectively. Also, limonene (28.5 %) was reported as the major component of Pakistan *C. paradisi* peels oil followed by α-terpinolene (2.06 %), γ-terpinolene (2.11 %) and myrcene (6.28 %) [8]. In the same vein, the present study identifies γ-terpinolene (4.26, 1.79, and 0.31 %) in the oils obtained from *C. paradisi* stem, root and fruit peel respectively. Limonene (74.45 %), myrcene (12.85 %), – (+)-carvone (0.93 %), copaene (0.96 %), trans-caryophellene (1.15 %) and dececanal (1.18 %) were identified by [13]. The results were also similar to our investigation in which (-)-carvone (0.01 and 0.31 %) and copaene (1.34 and 0.97 %) were identified in the *C. paradisi* root and fruit peel respectively. It was observed that limonene contents in the Egyptian *Citrus sinesis* leaf and flower accounts for 10.18 % and 16.90 % respectively and other chemical constituents includes (*Z*)-*β*-ocimene (7.63 and 6.35 %), *β*-pienene (3.55 and 3.09 %), *γ*-terpinene (3.86 and 1.27 %) and *α*-pienene (2.82 and 1.55 %), terpinen-4-ol (5.31 and 2.05 %), *α*-terpineol (1.44 and 2.05 %) *β*-elemene (2.27 and 1.04 %) and indole [22]. Moreover, limonene (7.18 %) was also the major constituents of Algerian *Citrus paradisi* leaf oil [26]. The results of limonene from Egypt and Algeria limonene results were similar to the result of limonene in the present investigation which reports limonene (11.15 %) in the *C. paradisi* fruits peel oil and as one of the major components in the oil samples.

VII. Antimalarial Activities of Citrus paradisi

Malaria is a major parasitic disease in the world, especially in Africa. There are needs for developing new drugs or drug combinations for the treatment of malaria [27]. *In vitro* antiplasmodial activities against *P. falciparum* (CQS) NF54 strain for the essential oils and DCM/methanol extracts obtained from selected parts of *Citrus paradisi* is reported in Table 2. Chloroquine (CQ) (Sigma) and Artesunate were used as the standard reference drugs (positive control). Antimalarial IC_{50} value of essential oil obtained from *C. paradisi* root (22.2±1.43) was found active against the strain (moderate active) according to Council for Scientific and Industrial Research (CSIR) criteria. In the same vein, the oil obtained from *C. paradisi* stem (48.1±2.29) value showed weak activity against the strain whereas essential oil obtained from *Citrus paradisi* fruit peel was inactive against the strain (IC_{50} ≥100 μg/mL). Essential oil extracted from *C. limon* (Citrus species) was effective in killing mosquito larvae [28]. We are yet to come across literature data on the antiplasmodial properties of *Citrus paradisi* as at the time of this literature survey; meanwhile [29] reported antiplasmodial properties on *Citrus limon* leaves and their report is similar to this present investigation. The essential oil of *C. paradisi* root showed moderate potency against the plasmodial strain, therefore the oil sample could be used to prevent or help in fighting malaria. Malaria is one of the ailments that the plants selected can cure or prevent according to the traditional practitioner consulted before the commencement of this research. Although, traditional practitioner explained further, that those plant always work well when combined with other plants.

VIII. Conclusion

Hydrocarbon monoterpenoids and oxygenated monoterpenoids constitute the main groups of compounds detected in the essential oils obtained from *C. paradisi* fruit peel (31.27 and 33.72 % respectively). Also, hydrocarbon monoterpenoid (18.20 %) and hydrocarbon sesquiterpenoid (18.16 %) constitute the root oil of *C. paradisi*. Meanwhile non-terpenoids (61.19 %) compounds dominated *C. paradisi* stem oil. There is a qualitative and quantitative difference in the compositional pattern of the oils obtained from stem, root, and fruit peel essential oils of *C. paradisi*. The root oil revealed good antimalarial potency towards *P. falciparum*. This shows correlation between the chemical components and antimalarial activities. The essential oil of *C. paradisi* root could find use in the fight against malaria parasite. Further studies are needed to carry out the toxicity, antioxidant and antimicrobial activities of the stem, root and fruit peel essential oils of *C. paradisi*.

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Table1: Chemical comparison of the components identified from *Citrus paradisi* Stem, Root and Fruit peel Essential Oils

Compounds	Kovat Index	Area (%) CPS	CPR	CPP
Boric acid	126	0.43	-	-
Furfural	165	0.18	-	-
Octanal	554	-	-	0.46
p-Cymen-7-ol	580	-	0.01	-
1H-Tetrazole, 5-methyl-	581	-	-	0.04
3-Hexen-1-ol, (Z)	631	23.04	-	-
(+)-4-Carene	641	-	1.34	-
Benzene, 2,4-dichloro-1-methoxy-	652	-	4.85	-
2-Heptanone	667	24.18	-	-
Fenchone	667	-	0.53	-
Heptane, 2,4-dimethyl-	723	-	-	0.36
Octane	735	-	0.03	-
Indole	755	-	-	0.73
trans-2-(2-Pentenyl)furan	770	-	0.07	-
Heptane, 2-bromo-	773	-	0.03	-
Cyclohexane, 1,4-dimethyl-, cis-	774	-	0.02	0.02
Phenol, 2-methyl-5-(1-methylethyl)-	786	-	3.80	-
Cyclopentanol, 1-methyl-	800	-	0.01	-
2-Hexanone	800	-	0.01	0.02
Hexanal	800	1.94	0.01	0.56
Cyclohexane, 1,3-dimethyl-, cis-	800	-	0.02	0.14
Cyclohexane, 1,4-dimethyl-	800	-	0.02	-
Cyclotrisiloxane, hexamethyl-	800	0.67	-	-
Heptane, 2,5-dimethyl-	800	-	0.01	-
Benzene, 1-methyl-3-(1-methylethenyl)-	800	-	-	0.13
Isogeranial	800	-	-	0.02
Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, [1S-(1à,3á,5à)]-	848	-	0.05	-
Cyclohexane, ethyl-	850	-	0.01	0.01
Cyclohexane, 1,2-dimethyl- (cis/trans)	855	-	0.03	-
Acetic acid, octyl ester	857	-	0.04	-
Benzene, 1,3-dimethyl-	865	-	-	0.09
Pentalene, octahydro-, cis-	870	-	-	-
Toluene	878	0.55	-	-
Cyclohexane, 1,1,3-trimethyl-	882	-	-	0.01
Heptanal	893	0.24	0.03	-
Bicyclo[4.2.0]octa-1,3,5-triene	893	-	0.03	0.02
o-Xylene	893	-	0.07	0.05
Cyclopentane, 1-ethyl-2-methyl-, cis-	897	-	0.01	-
Ethylbenzene	900	-	0.01	-
Heptane, 3-methyl-	900	-	0.01	0.01
Nonane	900	-	-	0.05
(E)-4,8-Dimethylnona-1,3,7-triene	900	-	-	0.08
3-Hexen-1-ol, acetate, (Z)	917	0.59	-	-
(2R,5S)-2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran	918	-	-	8.36

Butanoic acid, 2-methyl-, hexyl ester	921	-	0.01	-
Myroxide	925	-	0.13	-
p-Mentha-1,5-dien-8-ol	933	-	0.02	-
5-Hepten-2-one, 6-methyl-	941	-	1.44	0.02
1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	950	-	0.02	-
Dill ether	950	-	0.04	-
trans-á-Ionone	950	-	0.31	-
Cyclooctane, methyl-	954	-	-	-
Benzene, 1,2,3-trimethyl-	957	-	0.07	-
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	957	-	0.54	0.42
α-Myrcene	958	-	2.89	13.08
1,3-Dioxolane, 2-heptyl-	962	-	-	0.91
4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate	973	-	-	0.06
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	978	-	0.67	0.01
Cyclopentasiloxane, decamethyl-	982	0.76	-	-
α-Ocimene	983	-	0.75	1.11
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aá,8aà)-	984	-	0.28	-
Acetophenone	989	-	0.01	-
Ethanone, 1-(3-methylphenyl)-	992	-	0.03	-
1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	996	-	1.11	-
Benzaldehyde	1000	-	0.03	0.01
2-Cyclopenten-1-one, 3,4,4-trimethyl-	1000	-	0.08	-
4-Acetyl-1-methylcyclohexene	1000	-	-	0.01
2-Nonenal, (E)-	1000	-	0.04	-
1,6-Dioxaspiro[4.4]nonane, 2-ethyl-	1000	-	0.08	-
Nonanal	1000	-	-	0.69
p-Mentha-1,5,8-triene	1000	-	0.14	-
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	1000	-	1.56	-
1,5-Cyclooctadiene, 3,4-dimethyl-	1000	-	1.72	-
α-Pinene	1000	-	-	1.12
2,4,6-Octatriene, 2,6-dimethyl-, (E,E)-	1000	-	0.34	0.48
Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	1000	-	0.20	-
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	1000	-	-	0.03
cis-(-)-1,2-Epoxy-p-menth-8-ene	1000	-	0.05	0.18
cis-p-Mentha-2,8-dien-1-ol	1000	-	-	0.14
2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	1000	-	0.36	-
Limonene	1000	-	-	11.15
2,6-Octadiene, 2,6-dimethyl-	1000	-	-	0.06
Benzaldehyde, 4-(1-methylethyl)-	1000	-	0.01	-
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	1000	-	-	0.13
Limonene oxide, trans-	1000	-	-	0.14
3-Cyclohexene-1-acetaldehyde, à,4-dimethyl-	1000	-	-	0.09
2,6-Octadienal, 3,7-dimethyl-, (E)-	1000	-	0.10	0.75
Camphor	1000	-	0.67	-
Isopulegol	1000	-	-	0.12
6-Octenal, 3,7-dimethyl-, (R)-	1000	-	0.67	-
trans-Linalool oxide (furanoid)	1000	-	0.42	3.39
Decanal	1000	-	0.11	3.29
Butanoic acid, hexyl ester	1000	-	-	0.02
n-Valeric acid cis-3-hexenyl ester	1000	-	-	0.01
Undecane, 5-methyl-	1000	-	-	0.12
Butanoic acid, 2-methyl-, methyl ester	1021	-	0.01	-
1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	1026	-	-	0.16
Octane, 2,4,6-trimethyl-	1026	-	-	0.01
(-)-Carvone	1027	-	0.01	0.31
Undecanal	1027	-	-	0.05
Aristol-1(10)-en-9-yl isovalerate	1036	-	0.08	-
cis-3-Hexenyl isovalerate	1045	-	0.02	-
α-Phellandrene	1050	-	6.32	-
2,4,6-Trimethyl-1,3,6-heptatriene	1052	-	0.13	-
(1S,2R,4R,7R)-4-Isopropyl-7-methyl-3,8-dioxatricyclo[5.1.0.02,4]octane	1053	-	0.07	-
Terpinen-4-ol	1055	-	1.62	0.73
o-Cymene	1061	-	0.08	-
Hexadecanal	1073	-	0.01	-
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	1100	-	0.42	3.39
2,6-Octadienal, 3,7-dimethyl-, (Z)-	1100	-	0.04	0.69
Linalool	1100	-	3.74	4.53
α-Terpinyl acetate	1104	-	0.14	-
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	1133	-	0.07	0.01
Cyclohexasiloxane, dodecamethyl	1134	0.21	-	-
1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4á,4aá,7à,7aá,7bà)]-	1146	-	0.03	-
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	1177	-	-	0.72

Acetic acid, (dodecahydro-7-hydroxy-1,4b,8,8-tetramethyl-10-oxo-2(1H)-phenanthrenylidene)-,2-(dimethylamino)ethyl ester	1179	-	0.73	-
Limonene oxide, cis-	1200	-	-	0.62
Dodecanal	1200	-	-	0.19
alfa.-Copaene	1200	-	1.34	0.97
(-)-α-Bourbonene	1210	0.37	-	-
Caryophyllene	1216	-	0.47	8.81
Trisiloxane,1,1,1,5,5,5-hexamethyl-3-[(trimethysilyl)oxy]-	1223	1.43	-	-
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	1229	-	1.11	-
α-Longipinene	1250	-	-	0.04
α-Muurolene	1250	-	-	1.41
Neointermedeol	1250	-	0.73	-
Octane, 1,1'-oxybis-	1250	-	-	0.04
Humulene	1252	-	5.63	0.78
Terpineol	1267	-	0.20	2.13
2-Octen-1-ol, 3,7-dimethyl-	1284	-	0.04	-
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1à,2á,5à)-	1311	-	1.00	-
Tridecane, 5-methyl-	1326	-	-	0.06
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1338	-	0.16	0.03
2(3H)-Furanone, 5-butyldihydro-	1350	-	0.04	-
α-Cubebene	1350	-	0.10	0.06
Elemene isomer	1355	-	0.32	0.08
Benzoic acid, 2-methylpropyl ester	1358	-	0.03	-
10,18-Bisnorabieta-8,11,13-triene	1365	-	0.01	-
4-Isopropyl-6-methyl-1-methylene-1,2,3,4-tetrahydronaphthalene	1370	-	0.03	0.15
Anthracene, 9-phenyl-	1381	0.12	-	-
2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	1382	-	0.01	-
2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	1383	-	0.09	0.01
2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	1385	-	0.74	6.95
γ-Muurolene	1398	5.22	0.47	0.05
2-Pentadecanone, 6,10,14-trimethyl-	1398	-	0.45	-
Camphene	1400	-	0.07	-
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	1416	-	0.07	0.72
α-Guaiene	1425	-	-	0.03
3-Heptyne, 2,2,6-trimethyl-5-chloro-6-phenyl-	1443	-	0.09	-
α-Calacorene	1445	-	0.02	-
cis-Muurola-4(15),5-diene	1446	-	0.18	-
(E)- α-Famesene	1450	-	0.61	0.09
(-)-Aristolene	1460	-	0.21	-
(8R,8aS)-8,8a-Dimethyl-2-(propan-2-ylidene)-1,2,3,7,8,8a-hexahydronaphthalene	1464	-	0.02	-
Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-	1469	-	0.03	-
Neophytadiene	1472	-	0.17	-
(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene	1482	-	0.01	-
Lanceol, cis	1486		0.66	-
Naphthalene, 1,6-dimethyl-	1500	-	0.47	-
Acetic acid, decyl ester	1500	-	-	0.03
1-Iodo-2-methylundecane	1500	-	-	0.01
Diphenylmethane	1500	1.31	-	-
α-Corocalene	1500	-	-	0.07
trans-Calamenene	1500	-	0.02	0.08
1,1,7,7a-Tetramethyl-1a,2,6,7,7a,7b-hexahydro-1H-cyclopropa[a]naphthalene	1500	-	0.47	-
Bicyclosesquiphellandrene	1500	-	0.08	0.75
(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane	1500	-	0.15	-
(1S,4S,4aS)-1-Isopropyl-4,7-dimethyl-1,2,3,4,4a,5-hexahydronaphthalene	1500	-	0.07	0.02
1-Isopropyl-4,7-dimethyl-1,2,3,4,5,6-hexahydronaphthalene	1500	-	-	0.05
α-Bulnesene	1500	-	-	0.07
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	1500	-	-	1.65
Isoledene	1500	-	0.19	0.09
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aà,7à,8aá)]-	1500	-	0.20	-
2,6,9,11-Dodecatetraenal, 2,6,10-trimethyl-, (E,E,E)-	1500	-	-	0.03
Cubenol	1500	-	0.15	-
α-Cadinol	1500	-	6.51	-
Di-epi-1,10-cubenol	1500	-	-	0.09
Epicubenol	1500	-	0.72	0.13
Kaur-16-ene	1500	0.27	-	-
Hexadecane	1500	-	-	0.11
Tridecane	1518	-	-	2.13
5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	1526	-	0.02	-
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	1543	-	2.45	0.78
γ-Terpinene	1547	-	4.26	0.31

Benzene, 1,1',1''-(1-ethanyl-2-ylidene)tris-	1547	0.13	-	-
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1550	-	0.01	-
Isoneral	1551	-	-	0.03
(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1551	-	0.20	0.07
(3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	1586	-	0.16	-
(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene	1600	-	-	0.03
.tau.-Cadinol	1600	-	0.73	-
2-Chlorodiphenylmethane	1605	0.17	-	-
Eicosane	1628	-	-	0.25
Aromandendrene	1630	-	0.28	-
Aromadendrene, dehydro-	1667	-	0.09	-
Hexadecanoic acid, methyl ester	1714	-	0.04	0.05
Caryophyllene oxide	1734	-	1.31	0.23
9H-Fluorene, 9-phenyl-	1745	0.43	-	-
2,6,11-Dodecatrienal, 2,6-dimethyl-10-methylene-	1750	-	0.92	0.19
Octadecane, 2-methyl-	1755	-	-	0.01
Hentriacontane	1784	-	0.21	-
9-Octadecenoic acid (Z)-, methyl ester	1800	-	-	0.59
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	1813	-	0.76	-
Triphenylmethane	1814	0.21	-	-
Benzene, 1,4-bis(phenylmethyl)-	1836	0.44	-	-
2,6,10-Dodecatrienal, 3,7,11-trimethyl-, (E,E)-	1853	-	-	0.01
Heptacosane	1863	-	0.04	-
Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-1,1,4b,7-tetramethyl-, [4aS-(4aà,4bá,7à,8aà)]-	1881	2.18	-	-
9,12-Octadecadienoic acid, methyl ester	1900	-	-	0.11
Methyl stearate	1918	-	-	0.02
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	1918	-	-	0.01
1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	1924	0.49	0.04	-
4-Benzylbiphenyl	1926	0.14	-	-
Naphthalene, 1,2,3,4,4a,5,6, 8-dimethyl-2-(1-methylethyl)-, [2R-(2a,4aa,8aa)]	1931	10.26	-	-
o-Terpheny	1944	0.17	-	-
Germacrene D	1950	-	1.34	0.53
Nerolidol	1988	-	0.02	-
m-Terpheny	1992	0.61	-	-
Hibaene	2000	12.61	-	-
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	2071	-	-	0.12
Bis(2-ethylhexyl) phthalate	2227	0.04	-	-
Sebacic acid, di(2-propylpentyl) ester	2560	-	0.04	-
Diisooctyl phthalate	2593	-	0.01	0.01
Total		**93.28**	**78.11**	**90.51**

Key: CPS - *Citrus paradisi* stem oil CPR - *Citrus paradisi* root oil CPP - *Citrus paradisi* peel oil

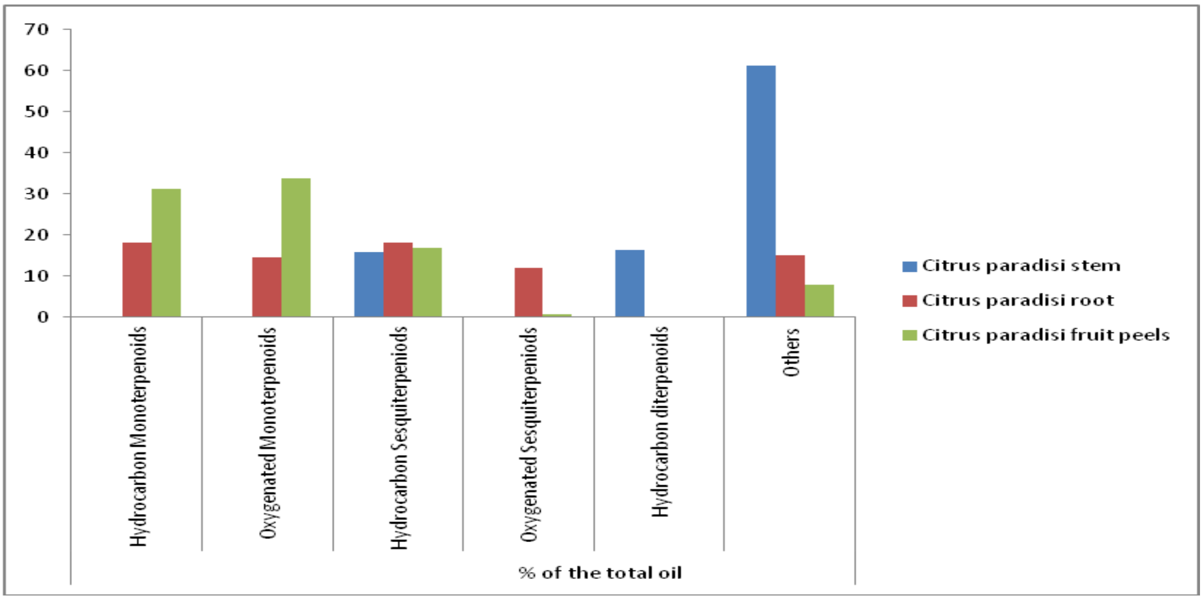


Figure 1: Different classes of components identified in the oils of *Citrus paradise*

Table 2: *In vitro* antiplasmodial activity against *P. falciparum* (CQS) NF54 strain (with standard deviations) of the essential oils from parts of *Citrus paradisi*

Samples	**NF54: IC_{50} (μg/mL)**
Citrus paradisi stem oil	48.1±2.29
Citrus paradisi root oil	22.2±1.43
Citrus paradisi peel oil	>100±0.18

CQ: 0.004 Artesunate: <0.002

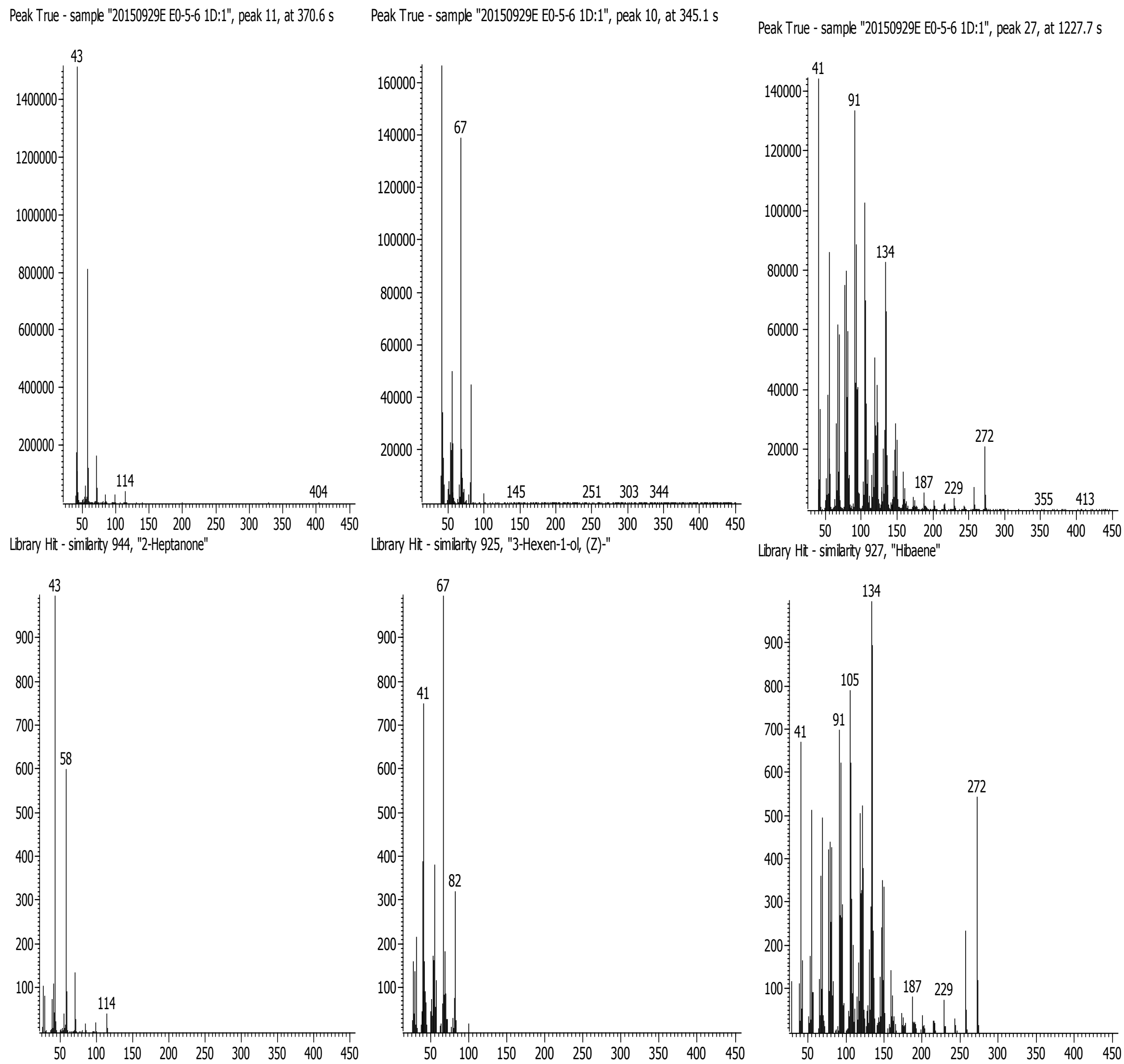
Standard:

IC_{50}, g/Ml	Status	**IC_{50}, μM**	Status
> 100	Inactive	> 100	Inactive
< 100	Weak	< 100	Weak
> 50		>15	
< 50	Moderate	< 15	Moderate
>10		> 6.25	
< 10	Potent	< 6.25	Potent

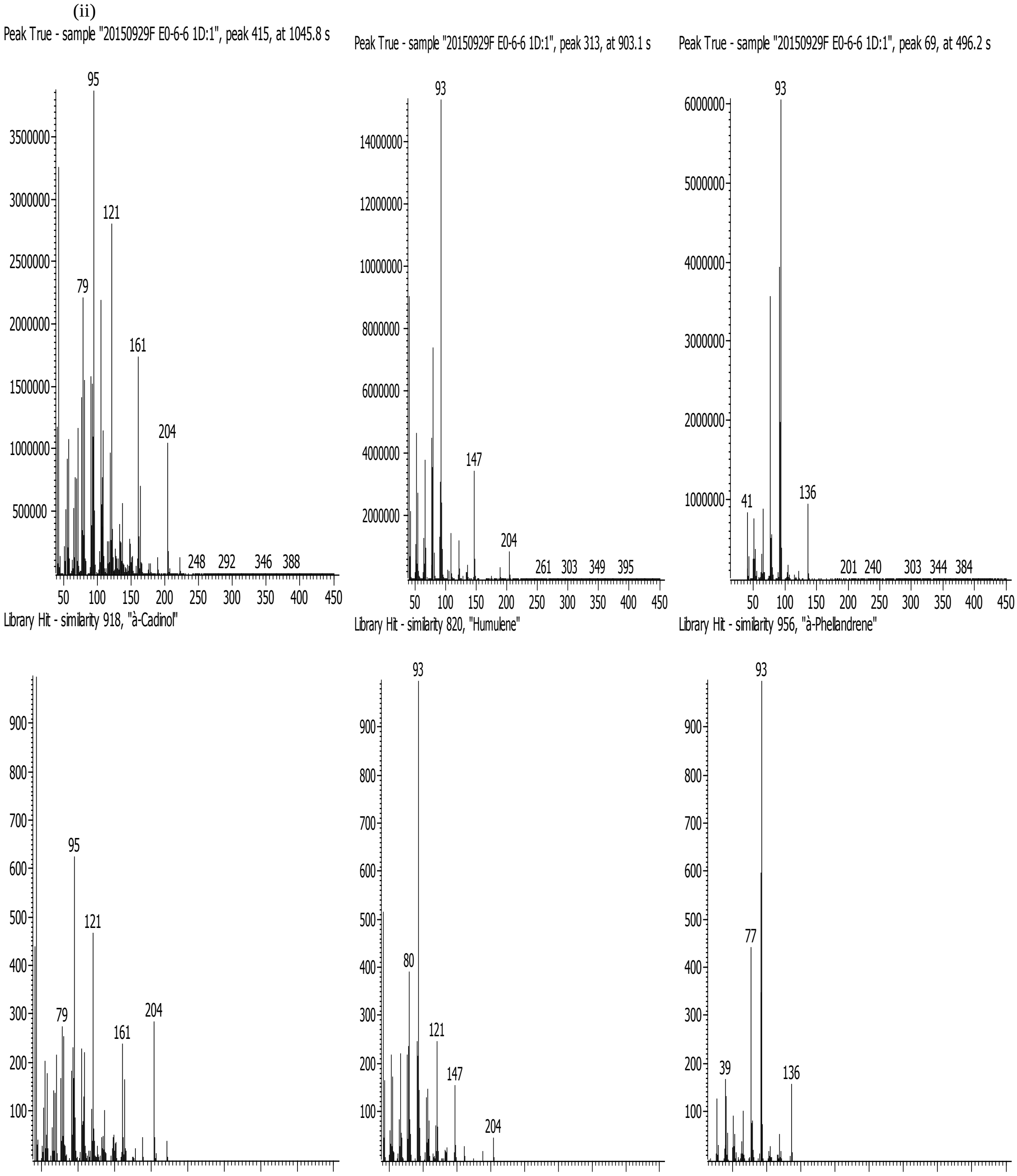
Supporting Documents

Mass Spectra of the major compounds identified in the essential oils of *Citrus paradisi* (i) stem, (ii) root and (iii) fruit peels.

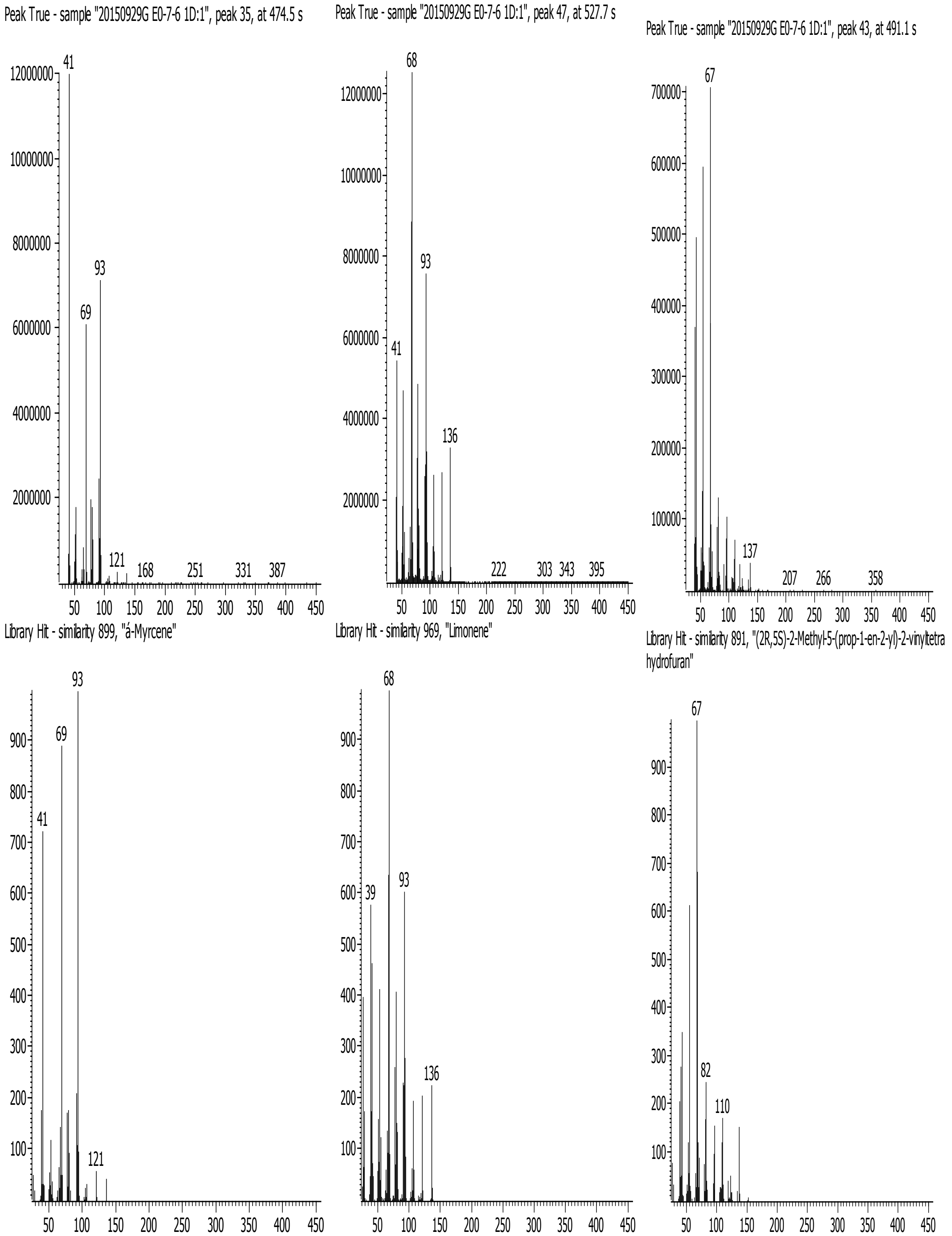
(i)



(ii)


Peak True - sample "20150929F E0-6-6 1D:1", peak 415, at 1045.8 s
95
121
79
161
204
248 292 346 388
Library Hit - similarity 918, "à-Cadinol"
95
121
79
161
204
Peak True - sample "20150929F E0-6-6 1D:1", peak 313, at 903.1 s
93
147
204
261 303 349 395
Library Hit - similarity 820, "Humulene"
93
80
121
147
204
Peak True - sample "20150929F E0-6-6 1D:1", peak 69, at 496.2 s
93
41
136
201 240 303 344 384
Library Hit - similarity 956, "à-Phellandrene"
93
77
39
136

(iii)



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