academicJournals

Vol. 14(13), pp. 1158-1166, 1 April, 2015
DOI: 10.5897/AJB2014.13966
Article Number: 882443F52134
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

GC-MS analysis of pesticidal essential oils from four Kenyan plants

Gladys Kemunto Onyambu[1]*, Rosebellah Maranga[1], Mary Ndungu[2], Gerald M. Mkoji[3], Kareru P. G.[2] and Anthony Wanjoya[4]

[1]Zoology Department, Jomo Kenyatta University of Agriculture and Technology (JKUAT) P.O Box 62000-00200 Nairobi, Kenya.
[2]Chemistry department, Jomo Kenyatta University of Agriculture and Technology (JKUAT) P.O Box 62000-00200 Nairobi, Kenya.
[3]Center for Biotechnology Research and Development (CBRD), Kenya Medical Institute(KEMRI), P.O Box, 54840-00200, Nairobi Kenya.
[4]Statistics and Actuarial Science Department, Jomo Kenyatta University of Agriculture and Technology (JKUAT) P.O Box 62000-00200 Nairobi, Kenya.

Received 6 June, 2014; Accepted 22 December, 2014

Essential oils are complex mixtures of odours and steam volatile compounds which are deposited by plants in the subcuticular space of glandular hairs, in excretory cavities and canals or exceptionally in heart wood. Essential oils have been found to have no specific biological functions in plants, but constitute many compounds that are insect repellents or act to alter insect feeding behavior, growth and development, ecdysis (moulting) and behavior during mating and oviposition. Others possess antifungal, insecticidal and antiseptic properties. Essential oils of leaves of *Tagetes minuta* L. (Asteraceae), *Fuerstia africana* T.C.E. Friers (Labiaae), *Tephrosia vogelii* Hook. f. (Leguminosae) and *Sphaeranthus ukambensis* were obtained by steam distillation using the Clevenger apparatus (Guenter, 1949). Compounds in the essential oils were identified by gas chromatography-mass spectrometry (GC-MS). The characteristic volatiles isolated from the four plants were identified as follows: major constituents of the essential oils from *F. africana* included the following: limonene (39.1%), (Z)-β-ocimene (30.5%), targetone<dihydro> (26.2%), E -targetone (59%), λ-terpinene (26.2 %), peripitenone 23.7%, ocimene allo (22.8%) and Z-targetone (100%). The compounds with the highest relative abundance value in the essential oils included Z-targetone, E-targetone and (Z)-β-Ocimene. Analysis of T*. minuta* also revealed a number of compounds which included mycene (20.1%), verbinene (8.0%), Z-ocimenone (35.2%), E-caryophylene (25.5%), α-Humelene (11.8%), germacrene D (21.4%) and camphene (2%). The major constituent of *T. minuta* was Z-ocimene, caryophylene E and mycene. Essential oils from *T. vogelii* as revealed by the GC-MS analysis were pinene α (32.7%), limonene (35.1%), copaene α (22.9%), β -elemene (36.7%), Z-nerolidol (77.7%), δ-cadinene (67.6%), α humelene (69.6%) and -4-α-ol-(β)copaene (65.7%). The highest proportion of the essential oils constituted Z- nerolidol δ-cadinene, α-humelene and 4-α-ol-β. Copaene. The compounds in essential oils obtained from *S. ukambensis* were α-copaene (23.8%), β-bourbonene (25.5%), α-gurjunene (14.3%), cymene<2, 5-dimethoxy-para (87.7%), α -humelene (100%), λ- muurolene (17.9%), λ-cadinene (77.3%), caryophylene oxide (54.7%) and δ-cadinene (61.9%). The major compounds contained in essential oils from *S. ukambensis* were α-humele, λ-cadinene, δ-cadinene and cymene<2, 5-dimethoxy-para. The compounds which were common in the four test plants included: α-pinene, α-humele, ocimene allo and (E) β-ocimene. Each of the test plant secreted essential oils constituting numerous volatiles known to exhibit acaricidal, insecticidal and/or arthropod repellent properties. These plants may be useful sources of compounds for use in the control of arthropods of medical, veterinary and agricultural importance.

Key words: Essential oils, GC-MS, *Tagetes minuta*, *F. africana*, *T. vogelii*, *S. ukambensis*

INTRODUCTION

Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants. Steam distillation of aromatic plants yields essential oils, long used as fragrances and favoring in the perfume and food industries, respectively (Bakkali et al., 2008). More recently they have become popular as agents for aromatherapy. Essential oils are characterized by a strong odor and have a generally lower density than water. Among higher plants, there are 17,500 aromatic plant species (Bruneton, 1999) and approximately 3,000 essential oils are known out of which 300 are commercially important for cosmetics, perfume, and pharmaceuticals industries apart from pesticidal potential (Chang and Cheng, 2002; Bakkali et al., 2008). Several plant families, for example, Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae, and Piperaceae, have been examined for anti-insect activities. To defend themselves against herbivores and pathogens, plants naturally release a variety of volatiles including various alcohols, terpenes, and aromatic compounds. These volatiles can deter insects or other herbivores from feeding, have direct toxic effects, or involve in recruiting predators and parasitoids in response to feeding damage. They may also be used by the plants to attract pollinators, protect plants from disease, or help in interplant communication (Pichersky and Gershenzon, 2002). Since the middle-ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, parasiticidal, insecticidal, medicinal, and cosmetic applications, especially in the pharmaceutical, sanitary, and cosmetic applications. Aromatic plants produce many compounds that are insect repellents or act to alter insect feeding behavior, growth and development, ecdysis (moulting), and behavior during mating and oviposition.

Monoterpenoids (90% of the essential oils) have a great variety of structures with diverse functions. They are ten carbon hydrocarbon compounds or related compounds such as acyclic alcohols (linalool, geraniol, citronellol), cyclic alcohols (e.g. menthol, isopulegol, terpeniol), bicyclic alcohols (borneol, verbenol), phenols (thymol, carvacrol), ketones (carvone, menthone, thujone), aldehydes (citronellal, citral), acids (chrysanthemic acid) and oxides (cineole). The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents all characterized by low molecular weight terpenes mainly the monoterpenes (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) also exist. Aromatic compounds occur less frequently than the terpenes and are derived from phenylpropane for example aldehyde: cinnamaldehyde; alcohol: cinnamic alcohol; phenols: chavicol, eugenol; methoxy derivatives: anethole, elemicine, estragole, methyl eugenols; methylene dioxy compounds: apiole, myristicine, safrole (Isman, 2006; Tripathi et al., 2009).

The composition of these oils can vary dramatically, even within species according to the part of the plant from which the oil is extracted (leaf tissue, fruits, stem, etc.), the phonological state of the plant, the season, the climate, the soil type, and other factors. For example, rosemary oil collected from plants in two areas of Italy were demonstrated to vary widely in the concentrations of two major constituents; 1,8-cineole (7 to 55%) and a-pinene (11 to 30%) (Flamini et al., 2002). Such variations are common and have also been described for the oils derived from *Ocimum basilicum* (Pascual-Villalobos and Ballesta-Acosta, 2003) and *Myrtus communis* (Flamini et al., 2004). In this study, we investigated the phytochemical properties of essential oils from *Tagetes minuta*, *Fuerstia africana*, *T vogelii*, and *S. ukambensis* from Machakos county Kenya. The understanding of the chemical composition of the essential oils was essential in determining their use in arthropod control, antiseptic and food industries among others.

MATERIALS AND METHODS

Plant materials and extraction of oils

Plant material (Leaves) of *T. minuta*, *F. africana*, *T. vogelii*, and *S. ukambensis* were collected in April 2010 from the farms and fields in Machakos, Kenya. The fresh leaves were sliced into smaller pieces. The essential oil was isolated from the plant materials by steam distillation using Clevenger apparatus (Guenter, 1949). The condensed oils were collected in n-hexane solvent (Aldrich HPLC grade) and the solution was filtered using Whattmann grade 1 filter papers containing anhydrous sodium sulphate in a funnel to remove any remaining traces of water. Hexane was then removed by distillation at 60ºC by the use of 'Contes' Short Path distillation apparatus. When condensation stopped, the oil was collected and weighed into small amber colored vials.

GC-MS analysis

The GC-MS analysis was performed on a 7890A gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) linked to a 5975 C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA) by using the following conditions: inlet temperature of 270°C, transfer line temperature of 280˚C, and column oven temperature programmed from 35 to 285°C with the initial temperature maintained for 5 min then 10 C/min to 280°C

*Corresponding author. E-mail: glaonya@yahoo.com.

Table 1. Compounds obtained from GC-MS analysis of essential oils from *Fuerstia africana (*Labiatae).

Compound[a]	RT[b] (min)	Percentage maximum
α -Pinene	9.403	0.84
Sabinene	10.299	5.14
β-Pinene	10.680	0.68
β-Phellandrene	10.904	1.54
δ-2-Carene	11.150	0.52
Ortho-Cymene	11.329	39.08
Limonene	11.396	39.08
(Z)-β-Ocimene	11.576	30.51
(E)-β-Ocimene	11.755	1.63
Dihydro-Tagetone	11.867	26.19
λ-Terpinene	11.934	26.19
Linalool	12.695	6.31
Allo-Ocimene	13.143	22.75
E-Tagetone	13.479	59.05
Z-Tagetone	13.636	100
Terpinen-4-ol	13.994	4.06
Z-Ocimenone	14.778	16.9
14. Piperitenone	14.913	23.71
g- Gurjunene	16.077	1.58
Isobazzanene	16.257	1.58
Silphiperfol-6-ene	16.391	0.79
Modheph-2-ene	16.906	1.51
α -Humulene	17.869	2.42
Bicyclogermacrene	18.407	2.2
Spathulenol	19.459	4.49

[a]Compounds are listed in order of elution from an SE-52 column.
[b]RT, Retention time.

held at this temperature for 10.5 min and finally 50°C/min to 285°C and held at this temperature for 29.9 min. The GC was fitted with a HP-5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25 μm) (J&W, Folsom, CA, USA). Helium at a flow rate of 1.25 ml/min served as the carrier gas. The mass selective detector was maintained at ion source temperature of 230°C and a quadruple temperature of 180°C. Electron impact (EI) mass spectra were obtained at the acceleration energy of 70 eV. A 1.0 µl aliquot of extract was injected in the split/splitless mode using an auto sampler 7683 (Agilent Technologies, Inc., Beijing, China). Fragment ions were analyzed over 40-550 *m/z* mass range in the full scan mode. The filament delay time was set at 3.3 min.

Due to unavailability of most of the authentic standards, relative quantification was achieved through the use of two authentic compounds [1, 8-cineole and β-caryophyllene (Sigma, St. Louis, MO, USA) whose calibration curves were used to quantify monoterpenes and sesquiterpenes respectively]. GC-MS in full scan mode was used to detect the terpenes in the distillate. Serial dilutions of authentic standards of 1, 8-cineole and β-caryophyllene (1-100 pg/µl) were also analyzed by GC-MS in full scan mode to generate linear calibration curves (peak area vs. concentration) with the following equations; 1, 8-cineole [$y = 0.7694x + 3.6807$ (R^2=0.9991)], and β-caryophyllene [$y = 0.5999x + 2.3004$ (R^2=0.9327)] which served as the basis for the external quantification of the terepenes identified.

RESULTS AND DISCUSSION

Major constituents of the essential oils from *F. africana* are presented in Table 1 and included the following: limonene (39.1%), (Z) β-ocimene (30.5%), Targetone <dihydro> (26.2%), E -targetone (59%), λ- terpinene (26.2 %), peripitenone (23.7%), Allo-ocimene (22.8%) and Z-targetone (100%). The compounds with the highest relative abundance value in the essential oils included Z-targetone, E- targetone and (Z) β- ocimene (Figure 1).

GC-MS analysis of *T. minuta* also revealed a number of constituent compounds which included the following: mycene (20.1%), verbinene (8.0%), Z-ocimenone (35.2%), E-caryophylene (25.5%), α-humelene (11.8%), D-Germacrene (21.4%) and camphene (2%) (Table 2). The major constituent of *T. minuta* was Z-ocimene, E-caryophylene and mycene (Figure 2).

The major constituents of *T. vogelii* essential oils are presented in Table 3 and they include: α-pinene (32.7%), limonene (35.1%), α-copaene (22.9%), β-elemene (36.7%), Z-nerolidol (77.7%), δ-cadinene (67.6%), α-

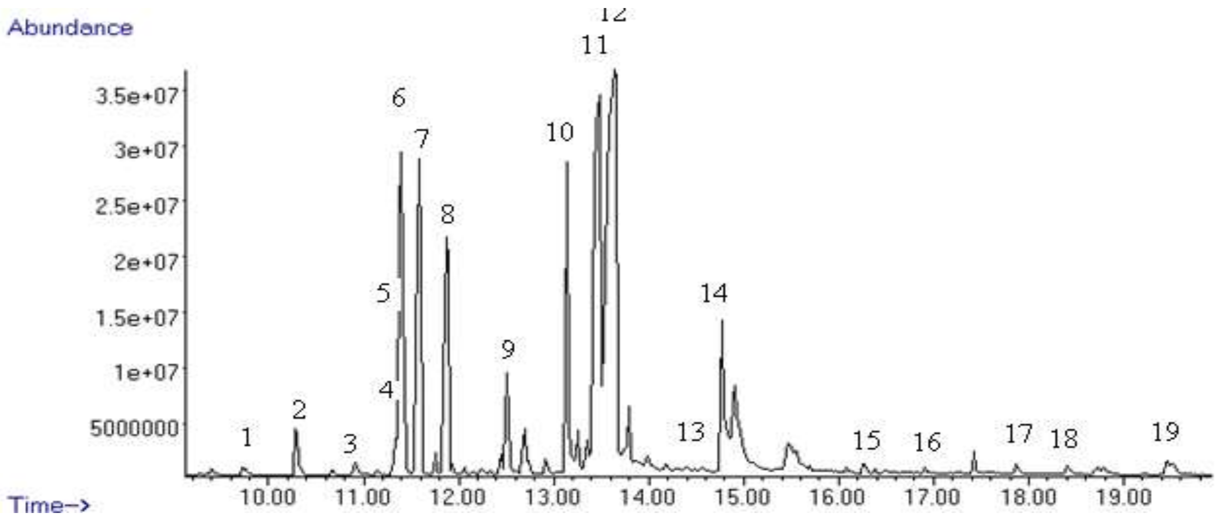

Figure 1. GC-MS chromatogram of essential oils obtained from *Fuerstia africana (*Labiatae).

Table 2. Compounds obtained from GC-MS analysis of *T. minuta.*

Compound[a]	RT[b] (min)	Percentage maximum
α-Pinene	9.425	1.07
Camphene	9.739	2.24
Sabinene	10.321	1.36
Verbenene	11.239	8.01
yrcene	10.702	20.05
Sylvestrene	11.419	
(E)-β-Ocimene	11.732	6.12
Dihydro-Tagetone	11.866	1.18
Santolina Triene	12.695	
Allo-Ocimene	13.143	3.38
Myrcenone	13.658	
α-Terpineol	14.218	
Z-Ocimenone	14.801	35.2
Isophorone	15.450	
Dehydro-Sabina ketone	16.279	
Piperitenone	16.458	5
β-Bourbonene	16.951	4.7
E-Caryophyllene	17.443	25.54
β-Copaene	17.555	7.6
α-Humulene	17.869	11.78
Muurola-4(14),5-diene<trans->	17.961	4.98
Germacrene D	18.227	21.43
λ-Muurolene	18.429	9.86
λ-Cadinene	18.608	2.39
Caryophyllene oxide	19.504	3.72

[a]Compounds are listed in order of elution from an SE-52 column. [b]RT, Retention time

humelene (69.6%) and 4-α-ol-(β)-copaene (65.7%). The highest proportion of the essential oils constituted the following compounds; nerolidol-Z-, δ-cadinene, α-humelene and 4-α-ol-(β)-copaene (Figure 3).

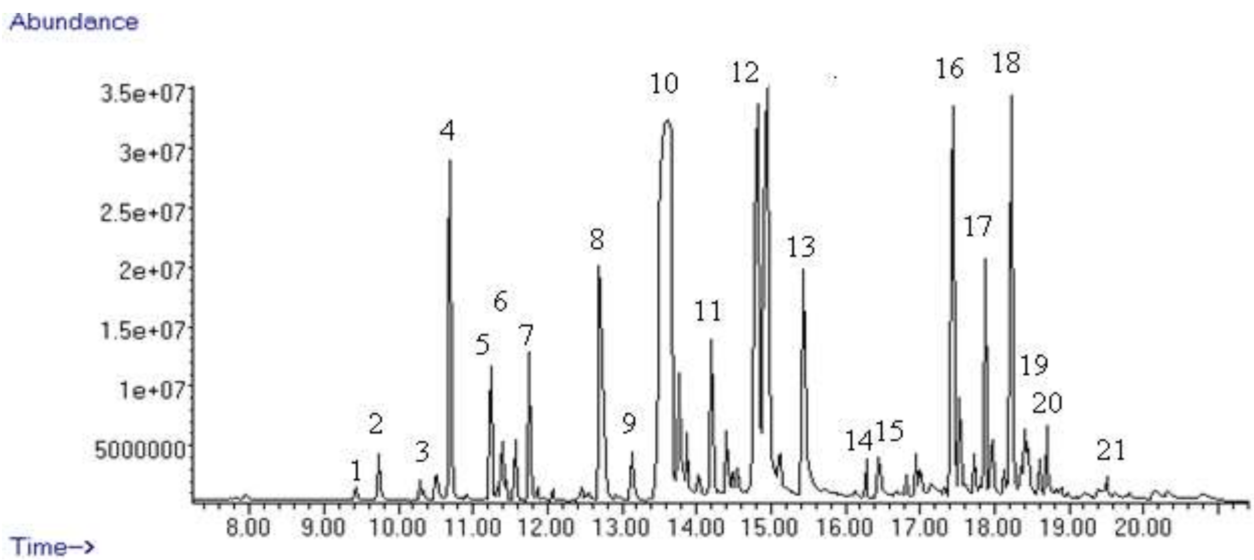

Figure 2. GC-MS chromatogram of essential oils obtained from *T. minuta*

Table 3. Compounds obtained from GC-MS analysis of essential oils from *Tephrosia vogelii*.

Compound[a]	**RT[b] (min)**	**Percentage maximum**
α-Pinene	9.448	32.65
Camphene	9.739	0.58
α-Phellandrene	10.299	
Myrcene	10.679	
α-Thujene	9.313	1.5
β-Pinene	10.344	
δ-2-Carene	11.150	0.96
Limonene	11.441	35.09
(Z)-β- Ocimene	11.575	3.16
(E)-β-Ocimene	11.755	8.62
λ-Terpinene	11.956	1.67
Linalool	12.673	7.55
Terpinolene	12.471	2.64
Cis-Rose oxide	12.852	0.78
Allo-Ocimene	13.143	3.1
Citronellal	13.546	
Terpinen-4-ol	13.972	3.31
α-Terpineol	14.218	3.54
Cis-Calamenene	16.122	
α-Cubebene	16.436	6.58
Trans-Calamenene	16.615	
α-Copaene	16.816	22.98
β-Elemene	17.063	38.67
α-trans-Bergamotene	17.175	
β-Vetivenene	17.242	
Germacrene D	17.466	
β- Copaene	17.555	
α-Humulene	17.891	69.56
Calamenen-10-one<10-nor->	17.981	

Table 3. Contd.

λ-Gurjunene	18.474	65.75
δ-Cadinene	18.742	67.62
Z-Nerolidol	19.213	77.71
4-α-ol-β-Copaen	19.885	65.71
2E, 6Z-Farnesol	21.005	

[a]Compounds are listed in order of elution from an SE-52 column. [b]RT, Retention time.

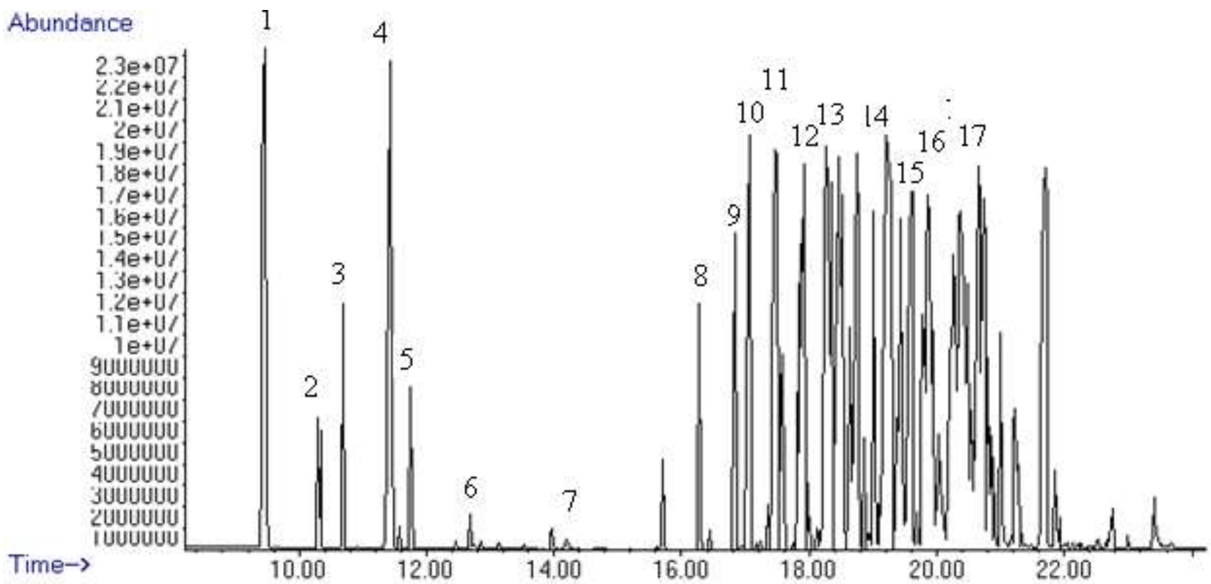

Figure 3. GC-MS Chromatogram for essential oils obtained from *T. vogelii*

The following compounds constituted the essential oils obtained from *S. ukambensis*: α-copaene (23.8%), β-bourbonene (25.5%), α-gurjunene (14.3%), cymene<2, 5-dimethoxy-para (87.7%), α- humelene (100%), λ-muurolene (17.9%), λ-cadinene (77.3%), caryophylene oxide (54.7%) and δ- cadinene (61.9%) (Table 4). The major compound contained in essential oils from *Sphaeranthus ukambensis* were α- humele, λ-cadinene, δ-cadinene and cymene<2, 5-dimethoxy-para (Figure 4).

Previous studies have shown that the main components of *T. minuta* oil are α-terpineol, (Z)-β-ocimene, dihydrotagetone, (E)-ocimenone, (Z)-tagetone, and (Z)-ocimenone (Dubey et al., 2011). These findings agree in with the current study where all these compounds were identified. Additionally humulene α and Germacrene D were found to be abundant.

GC-MS analysis of the essential oils revealed compounds toxic to pests and parasites. Linalool for instance toxic to eggs and larvae of insects (Liu et al., 2011) was found in *F. africana* and *T. vogelii*. Humelene, a strong repellent against *R. appendiculatus* was found in all the four study plants. Terpeneol a repellant of both *R. appendiculatus* and *I. ricinis* was a constituent compound of essential oils from *T. manuta* and *T. vogelii*. α-Pinene was found in all test plants except *S. ukambensis* and has repellent properties against arthropod pests (Tapondjou et al., 2005).

Essential oils interfere with basic metabolic, biochemical, physiological, and behavioral functions of Arthropods. They inhale, ingest or absorb essential oils. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (Enan, 2005) or GABA-gated chloride channels (Priestley et al., 2003; Khater 2011).

Essential oils have been found to be useful to man in various ways. Some essential oils have larvicidal effects and the capacity to delay development and suppress emergence of adult insects of medical and veterinary importance (Khater and Shalaby, 2008; Koul et al., 2008; Khater, 2011). Thyme oil and monoterpenoids including thymol, anethole, eugenol, and citronellal combinations have been patented for pesticidal activity against cockroaches and the green peach aphid. Similarly,

Table 4. Compounds obtained from GC-MS analysis of essential oils obtained from *Sphaeranthus ukambensis.*

Compound[a]	**RT[b] (min)**	**Percentage maximum**
Limonene	11.374	5.88
(E)-β-Ocimene	11. 754	1.11
(E)-β-Ocimene	11.777	1.7
Terpinolene	12.471	1.02
Linalool propanoate	12.695	
Borneol	13.793	2.96
Terpinen-4-ol	13.972	2.32
α-Terpineol	14.173	
Myrtenal	14.263	3.5
Nerol	14.733	17.44
α-Cubebene	16.436	
Neryl propanoate	16.615	2.86
Cyclosativene	16.727	
α-Copaene	16.839	23.84
β-Bourbonene	16.951	25.47
Germacrene D	17018	
Cyperene	17.175	12.71
α-Gurjunene	17.287	14.29
Cymene<2,5-dimethoxy-para-	17.421	87.68
E-Caryophyllene	17.488	
β-Copaene	17.555	
α-Humulene	17.936	100
Dauca-5,8-diene	18.003	
λ-Muurolene	18.138	17.91
Trans- Muurola-4(14),5-diene	18.384	
δ-Cadinene	18.720	61.91
Cadina-1,4-diene<trans->	18.832	13.33
α-Muurolene	18.899	
E-Nerolidol	19.168	42.37
Caryophyllene oxide	19.526	54.66
Humulene epoxide II	19.817	
Zonarene	19.997	
λ-Cadinene	20.176	77.3
Geranyl tiglate	20.489 min	34.79

[a]Compounds are listed in order of elution from an SE-52 column. [b]RT, Retention time.

citronellal, cotronellol, citronellyl or a mixture of these have been patented as pest treatment composition against the human louse (Ping, 2007). Nutmeg oil has been determined to significantly impact both the maize weed, *Sitophilus zeamais* and the red-flour beetle, *Tribolium castaneum* and demonstrates both repellent and fumigant properties. Essential oils of *Cinnamomum camphora*, *C. cassia,* and *C. zeylanicum* repel mosquitoes (Kim et al., 2003).

Oils of soybean, lemongrass, cinnamon, and the compounds 3,8-*p*menthane-diol (from lemon eucalyptus), citronellal (from lemongrass), and 2-phenethylpropionate (from groundnut), are effective against mosquitoes (Fradin and Day, 2002). Lemon eucalyptus is a potent repellent. Its oil, comprising 85% citronellal, is used by cosmetic industries due to its fresh smell. Flea and tick control products for companion animals based on *d* limonene, a constituent of citrus peel oil, or oils of peppermint, cinnamon, clove, thyme, and lemongrass, have been introduced recently (Isman, 2010). *d*-Limonene is heavily used for controlling structural pests as termite in California, and other plant oils like clove and peppermint. (Liu et al., 2011).

As can be concluded from the data presented on plant volatile, each species seems to have its own unique chemical composition with little similarity. In summary,

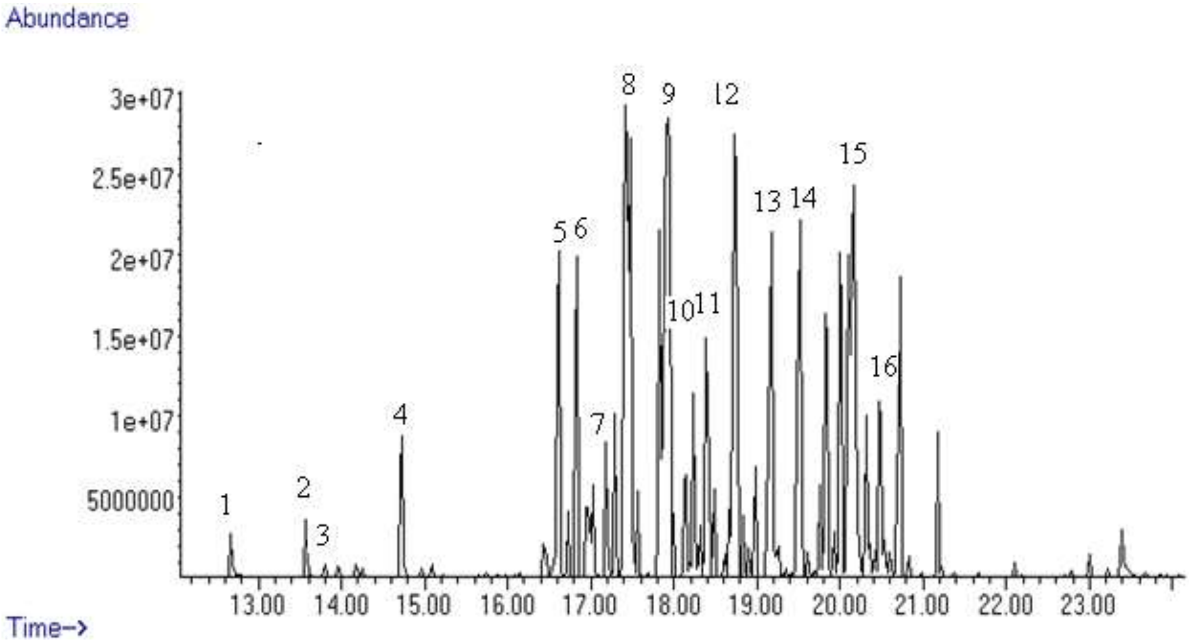

Figure 4. GC-MS chromatogram of essential oil from *Sphaeranthus ukambensis*.

Table 5. Compounds common in all the four plant species.

Compound	Quantity			
	F. africana	***T. manuta***	***T. vogelii***	***S. ukambensis***
A- Pinene	0.84	1.07	32.65	-
Sabinene	5.14	1.36	-	-
Limonene	39.08	-	35.09	5.88
(E)-β-Ocimene	1.63	6.12	8.62	1.11
Targetone <dihydro	26.19	1.18	-	-
Linalool	6.31	-	7.55	-
Ocimene <allo>	22.75	3.38	3.1	-
α-Humelene	2.42	11.78	69.56	100

the results of this study, further strengthens the view that *T. vogelii, T. minuta, F. africana* and *S. ukambensis* are potential sources of anti-arthropods agents especially insects and ticks and to some extent validates the traditional use of the plants for insect pest control by the farmers in livestock keeping areas in Kenya (Table 5).

Author's contributions

This work was carried out in collaboration with all authors. All authors participated in drafting and revising the manuscript. They also read and approved the final manuscript.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENT

JKUAT research planning and extension (RPE) is acknowledged for the provision of funds for the research.

REFERENCES

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils -A review. Food Chem. Toxicol. 46: 446-475.

Bruneton J (1999). Pharmacognosy, Phytochemistry, Medicinal Plants: Essential Oils. 2nd ed. Lavoisier Publishing, New York. pp. 461-780.

Chang ST, Cheng SS (2002). Antitermite activity of leaf essential oils and their constituents from Cinnamomum osmophloeum. J. Agric. Food Chem. 50: 1389-1392.

Dubey NK, Shukla R, Kumar A, Singh P, Prakash B (2011). Global Scenario on the Application of Natural Products in Integrated Pest Management Programmes. In: NK Dubey (Ed.) Natural Products in Plant Pest Management, CAB International, 1: 1-20.

Enan EE (2005). Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response

to plant essential oils. Arch. Insect Biochem. Physiol. 59:161-171.
Flamini G, Cioni PL, Morelli I, Macchia M, Ceccarini L (2002). Main agronomic-productive characteristics of 2 ecotypes of *Rosmarinus officinalis* L. and chemical composition of their essential oils. J. Agric. Food Chem. 50:3512-3517.
Flamini G, Cioni PL, Morelli I, Maccioni S, Baldini R (2004). Phytochemical typologies in some populations of *Myrtus communis* L. on Caprione Promontory (East Liguria, Italy). Food Chem. 85:599-604.
Guenter E (1949). The Essential oils, Toronto: van Nostrand. 111: 399
Isman MB (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol. 51:45-66.
Isman MB (2010). Botanical insecticides, deterrents, repellents and oils. In: BP, Singh (Ed.) Industrial crops and uses CAP International. pp. 433-445.
Khater HF (2011). Ecosmart Biorational Insecticides: Alternative Insect Control Strategies. In: F Perveen (Ed.) Advances in integrated pest Management. Croatia: In Tech. pp. 17-60
Khater HF, Shalaby AA (2008). Potential of biologically active plant oils for control mosquito larvae *Culex pipiens* (Diptera: Culicidae) from an Egyptian locality. Rev. Inst. Med. Trop. S Paulo 50:107-112.
Kim SII, Park C, Ohh MH, Cho HC, Ahn YJ (2003). Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). J Stored Prod Res. 39:11-19.
Koul O, Walia S, Dhaliwal GS (2008). Essential oils as green pesticides: Potential and constraints. Biopestic. Int. 4:63–84.
Liu ZL, Yu M, Li XM, Wan T, Chu SS (2011). Repellent Activity of Eight Essential Oils of Chinese Medicinal Herbs to *Blattella germanica* L. *Rec.* Nat. Prod. 5:176-183.
Pascual-Villalobos MJ, Ballesta-Acosta MC (2003). Chemical variation in an *Ocimum basilicum* germplasm collection and activity of the essential oils on Calosobruchus maculates. Biochem. Syst. Ecol. 31:673-679.
Pichersky E, Gershenzon J (2002). The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr. Opin. Plant Biol. 5:237-243.
Ping JH (2007). Pest treatment composition. US Patent no. 7,282,211
Pitasawat B, Choochote W, Tuetun B, Tippawangkosol P, Kanjanapoth D, Jitpakdi A, Riyong D (2003). Repellency of aromatic turmeric *Curcuma aromatica* under laboratory and field conditions. Journal of Vector Ecology 28:234-240.
Priestley CM, Williamson EM, Wafford KA, Sattelle DB (2003). Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABAA receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster.* Br. J. Pharmacol. 140:1363– 7278.
Tapondjou AL, Adler C, Fontem D A, Bouda H, Reichmuth C (2005). Bioactivities of cymol and essential oils of *Cupressus semperviens* and *Eucalyptus salgina* against *Sitophilus zeamais* Motschulsy and *Tribolium confusum* du val. J. Stored Prod. Res. 41:91-102
Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR (2009). A review on prospects of essential oils as biopesticide in insect-pest management. J. Pharmacogn. Phytother. 1:052-063.