CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF AFRICAN BLUE BASIL (*Ocimum Kilimandscharicum*Guerke) AS A PYRETHRUMSYNERGIST

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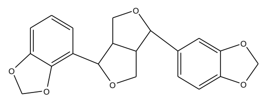
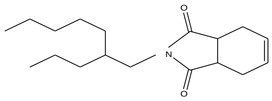
ABSTRACT
Concern on quality and safety of life in managing mosquitoes, has shifted steadily from the use of conventional chemicalstowardsalternative botanicalinsecticidesthataretarget-specific,biodegradableandenvironmentallysafe. Pyrethrins are natural plant compounds used in commercial vector control. They are usually formulated with synergists to improve quality, increase efficacy, mitigate resistance and make them cost effective. This discovery of insecticidal synergists initiated the use of insecticide synergist, Piperonylbutoxide(PBO), which is obtained from Safrole a main component of sassafras. *Ocimumkilimandscharicum*of the family Lamiaceae, commonly known as African Blue Basil and 'okita' in native Luo language, is a perennial, under shrub with simple ovate-oblong leaves. Earlier findings showed that the plant consists of linalool, camphor and 1,8-cineole. Literature review revealed no report available on the chemical composition of the essential oil and its economic importance fromKenya. In lightof this observation, this article presents identified essential oil composition from the leaves of*kilimandscharicum*collected from the Kipkaren in Nandi county(Kenya), as alternative recipe that can be used as pyrethrum synergist. The essential oil was obtained by hydro-distillation of the leaves of *kilimandscharicum*, and then analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Seventeen constituents were identified, most of which were mainly phenylpropane and their derivatives. Most of whose functional group is methylenedioxyphenyl which form the binding site for the insecticide during the reaction of insecticide against insects. The findings of this research could enable further exploration for large scale production and exploitation of the biological synergists for commercial application; which could go along revitalizing agriculture and environment. Biological products are biodegradable as opposed to convectional pyrethrum synergist, PBO currently being used by the Pyrethrum Boardof Kenya. Further research to identify the active component of the seventeen constituents is inevitable.
Keywords*:Ocimumkilimandscharicum,*Guerke,Lamiaceae,essentialoilcomposition,Synergists

INTRODUCTION:BACKGROUNDINFORMATION
The history of insecticidal synergists originated with attempts to enhance potency of pyrethrins (B-Benard & Philogene, 1993). This discovery initiated use of insecticide synergists in search for better compounds. The discovery of *methylenedioxyphenyl* synergist started with realization that, the synergistic activity of sesame oil was due to the *sesamin*(**1**) and *sesamolin*(**2**) components. Synthesis and testing of related compounds led to sulfoxide, propylisome, tropital and piperonylbutoxide (**3**). *Propynylphosphonates*and certain amides such as MGK264 *(N-Octylbicycloheptenedicarboximide)* (**4**) were also effective. The shifting of compound for effectiveness, economics and toxicology led only to two major synergists for practical use, piperonylbutoxide and MGK264 (Casida & Quistad,1995). Safrole (**5**) a main component of sassafras oil obtained from *Sassafras albiduma* plant of Lauraceae family is known for its synergist activity. Safrole (**5**) is the precursor for piperonylbutoxide, a synergist commonly used with pyrethrum (Dewick, 2002).



1 **2** **3**



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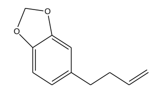

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Figure1Structuresofinsecticidalsynergists,1,2,3,4,and5

Manyplant speciesincluding pyrethrum, *Artemisia* and essentialoilsfromplantssuchas *Ocimum*havebeenproved to have mosquicidal properties (Kimbaris*et al.,* 2012). The insecticidal action of these plants is based on the active ingredientsproducedbytheplants.Whentheseplantbiocidesarecombinedwithpyrethrinstheymayhave synergistic or additive effects which may have bettereffects on the mosquitoes. Piperonylbutoxide (**3**), a synergist,is often used in combination with pyrethrins, making the mixture more effective by not allowing the insect's system to detoxify the pyrethrins. However, the latest information regarding toxicity of piperonylbutoxide (**3**) has determined that it can pose a distinct health risk when it becomes airborne (Kumar *et al.,* 2002). In addition, when used as a synergist or as pyrethrum formulation is very costly, toxic and its continuous supply is not guaranteed (Kumar *et al.,* 2002). The cost of PBO is three times that of pyrethrins (Romero *et al.,* 2009). This researchidentified a replacement of PBO from naturally occurring sources (natural oils and essential oils) from leaves of higher plants. A synergist which is cheap, readily available and environmentally friendly is needed and many botanicals are readily available. Piperonylbutoxide is commonly used synergist for pyrethrum and pyrethroids and has a unique mode of action **(**Casida, 1970).Insects have in-built, complex systems that always attack an insecticide once itentersthe insectbody.Mixed FunctionOxidases(MFO's) isone ofthe insect'sdefense mechanisms.MFO's work by binding with the insecticide active site thereby rendering it ineffective. When PBO is present in a compound, it binds with the MFO's, thus making the insecticide available to do its job (Metcalf *et al.,* 2002).

The genus *Ocimum*, of the family Lamiaceae, has tropical distribution with nearly two-third of the 160 species reported fromWest Africa and the remaining one-third from Asia and America. India is represented by nine species of *Ocimum*, mainly confined to tropical and peninsular regions. *O. kilimandscharicum*commonly known as African Blue Basil and '*okita'* (Kokwaro, 1976) in native luo language is a perennial, under shrub with simple ovate-oblong leaves. Flowers are light purplish or white. Seeds are oblong, black to brown. *O. kilimandscharicum*is an exotic West African species. The plant has carminative, stimulant, antipyretic, anti-fungal and anti-bacterial properties Camphor is a major component of essential oil of *O. kilimandscharicum*. The biological activity of camphor against the beetles, *Sitophilus granarius, S. zeamais*, *Triboliumcastaneum*and *Prostephanustruncatus*, has been evaluated by Obeng-Ofori *et al.,* (1998) and found camphor to be effective when used in contact toxicity, grain treatment and repellency assays. According to their experimental findings, camphor applied either topically, impregnated of filter papers or whole wheat and maize grains was highly toxic to all the four species. Another study done, also reported that EOs from *O. kilimandscharicum*and *O. suave* have remarkable knockdown effects (30-50%) when used as repellent against *Anopheles arabiensis, A. gambiae* and *Culex quinquefasciatus*(Seyoum *et al.,* 2006).

This discoveryof insecticidal synergists initiated the use of insecticide synergist, Piperonylbutoxide(PBO), which is obtained from Safrole a main component of sassafras. *Ocimumkilimandscharicum*of the family Lamiaceae, commonly known as African Blue Basil and 'okita' in native luo language, is a perennial, under shrub with simple ovate-oblong leaves. Earlier findings showed that the plant consists of linalool, camphor and 1,8-cineole. Literature review revealed no report available on the chemical composition of the essential oil and its economic importance from Kenya. In light of the scanty data on insecticidal efficacy of the herbal medicine especially in the tropical regions where there are large forested land; the aim of this study was to understand the antimosquicidal efficacy of combination therapy using two plant species and determine possible synergistic relationships.

Pyrethrins have been used in many insecticide, fogging products and pet products for over 100 years but their formulation without synergists are ineffective and thus paralyzed insects normally recover after a while (Matthews, Bateman & Miller, 2014). Piperonylbutoxide (PBO) is often used with pyrethrins, making the mixture moreeffective by not allowing insect's system to detoxify pyrethrins. However, the latest information regarding toxicityofPBOhasdeterminedthat it canposeadistincthealthriskwhenitbecomesairborne(Kumar*etal.,* 2002).Pregnant women when exposed to this substance during the third trimester leads to delayed mental development in young children(Romero*etal.,* 2009).Astudyfound asignificantassociationbetweenpiperonylbutoxide(PBO),acommon additive in pyrethroid formulations, measured in personal air collected during the third trimester of pregnancy, and delayed mental development at 36 months was observed (Horton*et al.*, 2011). Piperonylbutoxide (PBO) is also suspected to be carcinogenic, mutagenic, and teratogenic (Mahadevan*et al.*, 2009). PBO also attributes to chronic toxicity where it changes in liver and kidney in test animals at high doses (Wang*et al.*, 2012).

MATERIALSANDMETHODS

CollectionofPlantSpecimens

The plant specimens were obtained from Kipkaren in Nandi County, which is situated in the western part of Rift Valley Province. It borders Kakamega to the north-west, Uasin Gishu district to the north-east, Kericho district tothe south-east, Kisumu district to the south-east, and Vihiga district to the west. The plant was identified by a plant taxonomist in the Botany Department, University of Eldoret.

Preparationofextracts

The fresh plant leaves of the *O. kilimandscharicum*(1000 g) was subjected to hydro-distillation using Clevengertype apparatus for 4 hours. This produced oil yield of 0.4% v/w. The oil was collected and dried over anhydrous sodium sulphate and stored in sealed vials at -4°C until analysis.

Bioassayonadult mosquitoes

The WHO cones were used to evaluate the synergistic activityof the compounds. Twenty adult *A. gambiae*3-4 days old, were introduced to the test chamber its surfaces (filter paper) having been smeared with pretreated solutions.The test compounds and 0.2% dry weight of pyrethrins, in the ratios of 1:1, 1:2, 1:3, 1:4 and1:5 ratios of pyrethrins to potentially synergistic compounds were used until a suitable concentration was obtained. The knockdown and mortality rates were monitored for three minutes then 24hours.

The knockdown mosquitoes were removed, provided with food and water while the percentage kill was determined after 24 hours. A solution of pyrethrins and pyperonylbutoxide with same concentration as test solutions were used as standards in each case. Results showed *ocimum*is very effective as it had 100% and 90% mortality and knockdown respectively between 1 hr and 24 hrs.

Factorof synergism

Relative potency for the synergists based on the FOS and lethal concentrations, showed that the synergist *O. kilimandscharicum*had relative potency(R.P) of 2.2. Earlier studies had shown that *Tagetes minuta*had R.P of 1.04 and 0.894 for larvae mortalities and adult knockdown respectively. Meaning that the higher the R.P, the more toxic (better)thesynergist. Similarstudiesalsoshowedthatsteamdistillationofleavesof *Menthaaruensis*(Labiatae)had a Factor of synergism of 0.94. Other plant families like Burseraceae, P.E extract of resin of *Commiphoramukul*had FOS of 1.43 and Ether extract of resin had 0.95 (Pyrethrum post, 1973).

Gaschromatography-massspectrometry*:*

The composition of the essential oils was determined using an Agilent 7890A Gas Chromatography – Mass Spectrometry instrument from Government chemist- Nairobi.Helium at 25cm/sec (0.73ml/min) was used as a carrier gas, and hydrogen was used for the flame. The GC conditions used were as follows: capillary column; fused silica(polydimethylsiloxane, 0.25μmfilmthickness);temperatureprogram:70°Cfor8 min,75 –230°Cfor3 min, 230 – 240 °C for 5 min, 270 °C 5 min; carrier gas, Helium at 5 bar, linear velocity of 25cm/sec (0.73ml/min) ; injection port splitless at 250 °C; injection volume, 0.1 μL. The MS conditions were as follows: ionization EI at 70 eV; m/z range, 30-300 °C; scan rate 1 sec-1; ionization chamber at 180 °C; and transfer line at 280°C.

Identificationofthecomponents:

Identification of the essential oil constituents was based on a comparison of their retention times, and these constituents were further identified and authenticated using their MS library search (NIST and WILEY), and by comparison with MS literature data (Adams, 2007).

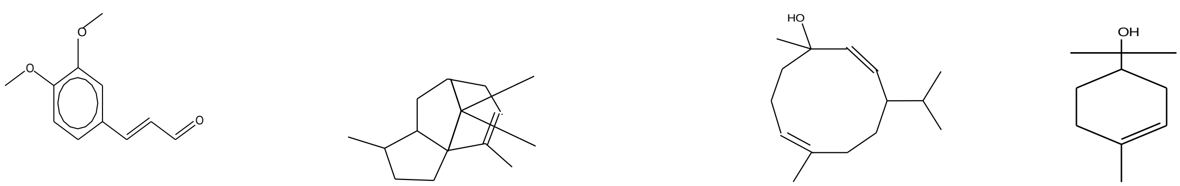
RESULTSANDDISCUSSION

Thechemicalcompositionandretentiontimesoftheessentialoilofleavesof *O.kilimandscharicum*arepresentedin Table 1. The constituents listed in order of their elution decreasing order on the column. The constituents of *O. kilimandscharicum*were listed in order of their elution on the column according to their retention time. The separation and identification of the compounds in *O. Kilimandscharicum*resulted into seventeen compounds.Among them with the functional group *methylenedioxyphenyl* were; 2-propenal, 3-(3,4-dimetho xyphenyl) (**6)**, Methyl Eugenol (**12**), Eugenol (**13**) and Estragol (**21**).

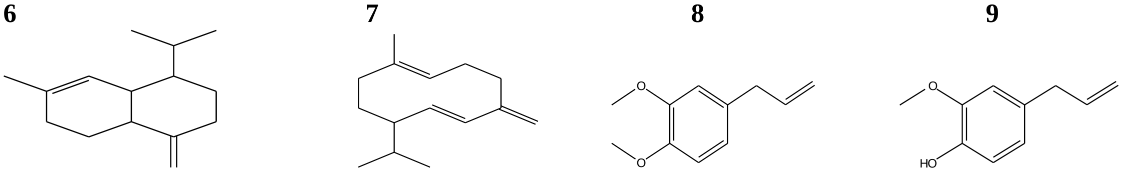
Another differentstudybyIsman(2006) andTripathi *etal*.,(2009)alsoindicated thatsomecompoundsfound inthe essential oil, included anethole (**23**), elemicine(**24**), estragole (**17**), methyl eugenols (**12**), methylene dioxy Compounds in (Figure 1), apiole (**25**), and myristicine (**26**). A similar study by Bhasin, M., (2012) showed that the essentialoilobtainedbyhydro-distillationoftheleavesof *Ocimumkilimandscharicum*Guerke, wasanalyzedbygas chromatography coupled with mass spectrometry (GC/MS). Forty-one constituents were identified. Chemical composition of the essential oil of *O. kilimandscharicum*were reported from India where the major constituentswere linalool (**16**), (41.94-58.85%), methyleugenol (**12**) (17.0-15.82%) and apiole (**29**) (10.18-6.38%).

Table1:ChemicalCompositionofEssentialoilasFoundin *Ocimumkilimandscharicum*

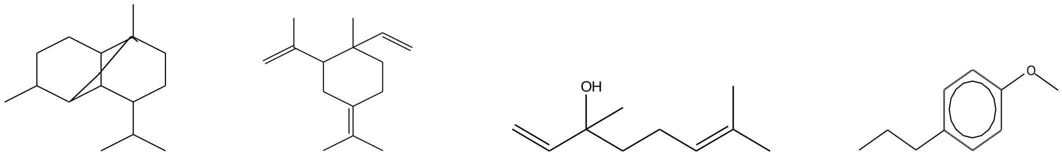
EssentialoilsComposition	**RTin min**
Propenal,3-(3,4-dimethoxyphenyl) (**6**)	16.518
α–Pathchoulene(**7**)	14.461
GermacreneD-4-ol(**8**)	14.365
αTerpineol(**9**)	14.149
Ɣ-Muurolene(**10**)	13.473
(-)-GermacreneD (**11**)	13.039
MethylEugenol(**12**)	12.604
Eugenol(**13**)	12.504
Alfa-Copaene (**14**)	11.805
o-Menth-8-ene,4-isopropylidene-1-vinyl (**15**)	11.253
Linalool(**16**)	11.055
Estragol(**17**)	9.265
Terpinenol-4-ol(**18**)	8.985
ß-Ocimene(**19**)	7.393
ß-pinene(**20**)	7.171
Cyclohexene,1-methyl-4-(1-methylidene) (**21**)	6.874
Cadinene δ (**22**)	6.678



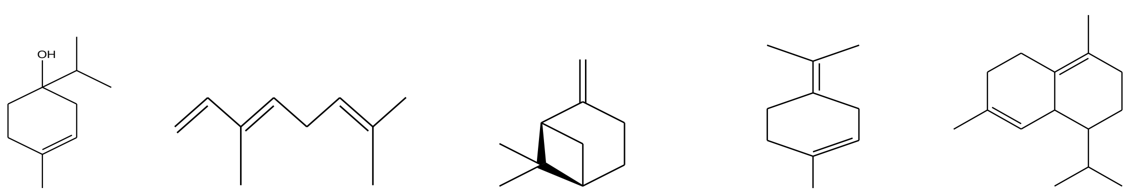
6 **7** **8** **9**



10 **11** **12** **13**

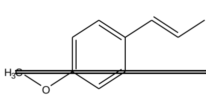


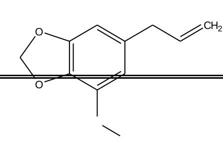
14 **15** **16** **17**

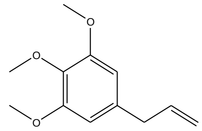


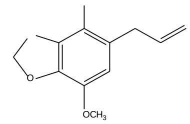
18 **19** **20** **21** **22**

Figure2.Structuresfortheoilconstituents of*O.kilimandscharicum*oil:6–22









O
CH$_3$

23 **24** **25** **26**

AcomparisonofasimilarstudybyJoshi,R.K.,(2013)showedthatabovecompoundswerethemostcommon constituents of *O. kilimandscharicum.*

GCMSSpectrumofoilisshowninFigures3and4 below.

GC-MSSpectrumofthe*Ocimum*oil

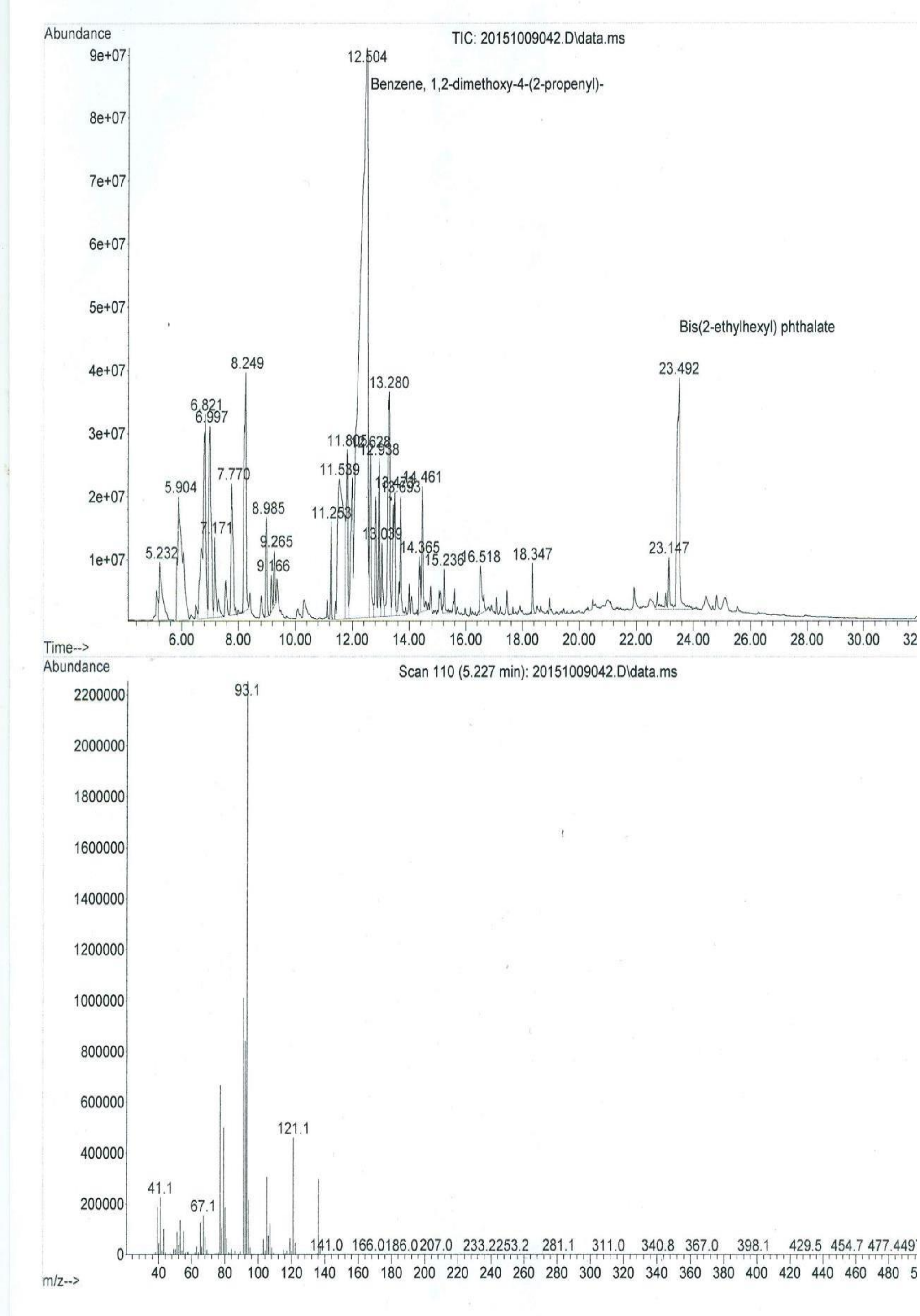


Figure3.GCMSSpectrumofthe*Ocimum*

ExpandedGCMSSpectrumofthe*Ocimum*oil

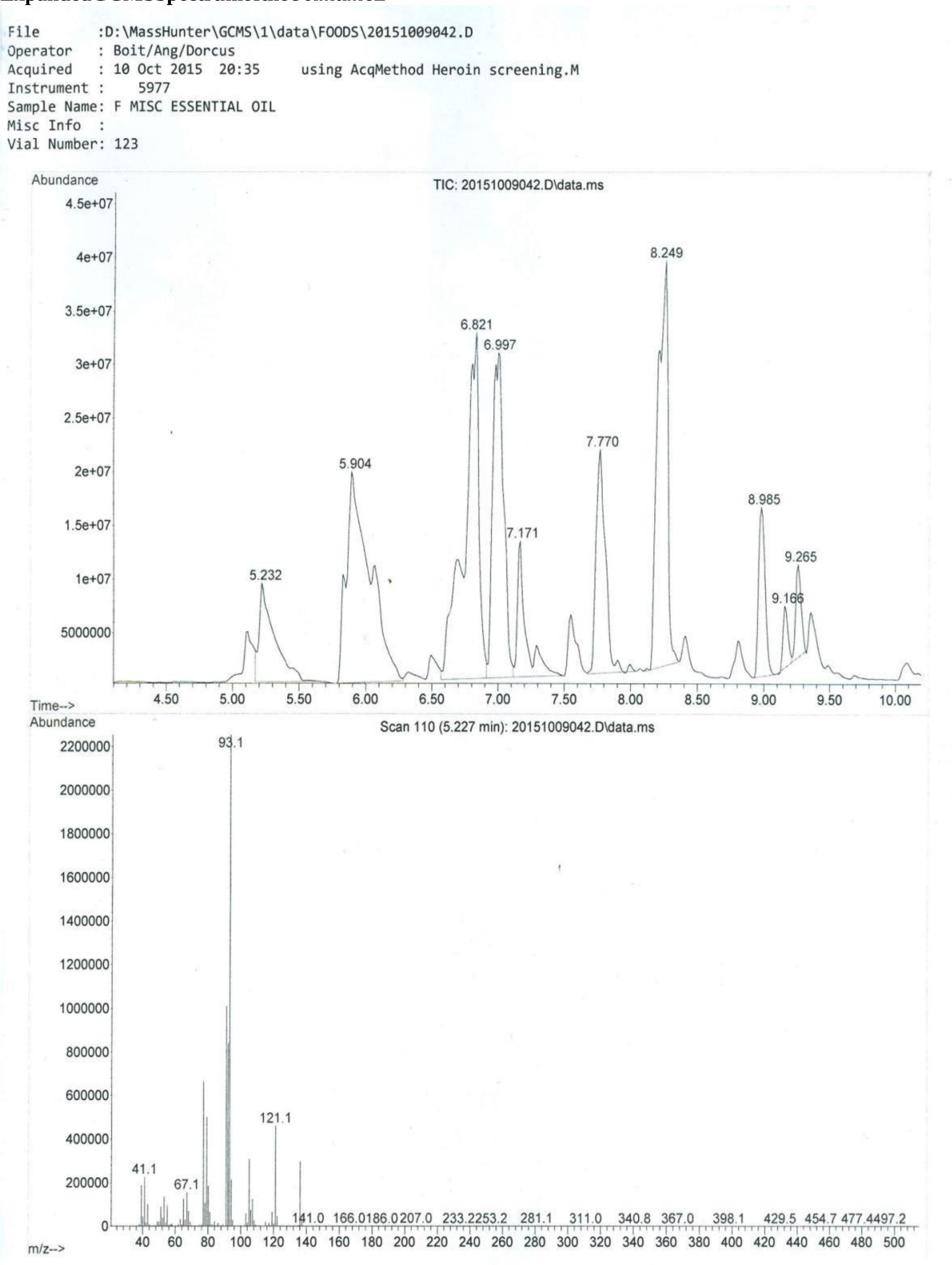


Figure4.ExpandedGCMSSpectrumofthe*Ocimum*oil

CONCLUSION

Characterization of the *O. kilimandscharicum*resulted into seventeen constituents (compounds) with some being monoterpenes (C10), besides, they are phenylpropanes, which are potential sources of synergists for pyrethrins. Structures that bear a functional group *methylenedioxyphenyl*, basic framework of chemical structure within which likelihood of the compound with synergistic activity is predicted.

REFERENCES

Adams,R.P.,(2007).Identificationofessentialoilcomponentsbygaschromatography/massspectrometry,4th ed. AlluredPubl.Corp.,CarolStream, IL.Pp87

Benard,B.C.&Philogerie,B.J.R.,(1993).Insecticidesynergists;Role,ImportanceandPerspectives.*J.Toxicol. Environ.Health*.**38**:199-223

Bhasin,M.K.,Kaul.B.L.,&Khosla.M.K.(2012).*Ocimum*-Taxonomy,medicinalpotentialitiesandeconomic value of essential oil. *J. Biosphe*., **1**: 48-50.

Casida,J.E.(1970).Biochemistryofinsecticides,*JournalofEntomology*,**12**,pp69

Casida,J.E.,&Quested,G.B.(1995).PyrethrumFlowers:Production,Chemistry,ToxicologyandUses.Oxford Univ. Press, Oxford, UK, 356 pp.

Dewick,P.M.(2002).Medicinalnaturalproducts:abiosysyntheticApproach.2nd ed.JohnWileyandSonsLtd. Chichester.66-78.

Isman, MB., (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Ann Rev Entomol.* **51**: 45–66.

Joshi, R.K. (2013). Chemical Composition of the Essential Oil of *O. kilimandscharicum. Global Journal of Medicinal Plant Research*,**1**(2):207-209.

Kimbaris, A. C., Koliopoulos, G., Michaelakis, A., & Konstantopoulou, M. A. (2012). Bioactivity of *Dianthus caryophyllus*, *Lepidiumsativum, Pimpinellaanisum, and Illiciumverum*essential oils and their major components against the West Nile vector Culexpipiens. *Parasitology Research***111**(6): 2403-2410.

Kokwaro,D.L(1976).MedicinalplantsofEastAfrica,*2nd edition*Macmillan.678-611

Kroes,R.,&Walker,R.(2004).Safetyissuesofbotanicalsandbotanicalpreparationsinfunctionalfoods. *Toxicology*.**198**(1):213-220.

Kumar, A., Valecha, N., Jain, T., & Dash, A. P. (2007). Burden of malaria in India: retrospective and prospectiveview. *The American journal of tropical medicine and hygiene*.**77**(6): 69-78.

Kumar,S.,Thomas,A.,Sahgal,A.,Verma,A.,Samuel,T.,&Pillai,M.K.K.(2002).Effectofthesynergist, piperonylbutoxide,onthedevelopmentofdeltamethrinresistanceinyellowfevermosquito,*Aedesaegypti* L.(Diptera:Culicidae).*Archivesofinsectbiochemistryandphysiology*.**50**(1):1-8.

Metcalf,R.L.,Weinheim,W.(2002).InsectControlinUllmann'sEncyclopediaofIndustrialChemistry. **4**,567-580

Murray,C.J.,Rosenfeld,L.C.,Lim,S.S.,Andrews,K.G.,Foreman,K.J.,Haring,D.,...&Lopez,A.D.(2012). Globalmalariamortalitybetween1980and2010:asystematicanalysis.*TheLancet*.**379**(9814):413-431.

Tripathi, A.K., Upadhyay, S., Bhuiyan, M., & Bhattacharya, P.R. (2009).A review on prospects of essential oils as biopesticide in insect-pest management. *J Pharmacognosy Phytother.* **1**(5): 052-063

Obeng-Ofori., Reichmuth, C.H., Bekele, A.J.& Hassanali, A. (1998). The efficacy of the volatile oils of three plants for controlling *Lucilla sericata*. *International Journal of Pest Management.***44**(4): 203-209.

Romero, A., Potter, M. F., & Haynes, K. F. (2009). Evaluation of piperonylbutoxide as a deltamethrin synergist for pyrethroid-resistant bed bugs. *Journal of economic entomology*, **102**(6): 2310-2315.

Seyoum,A., Passion, K., Kungas, S., Kabiru, E.W., Lwande, W., Killeen, G.F., Knols,B.G., (2006).Traditional use of mosquito repellant plants in Western Kenya. Macmillan, 876.

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