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Chemical Analyses of Sex Pheromones from the Abdominal Glands of Bitter Leaf Weevil (*Lixus camerunus*)

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Abstract

Sex pheromones from the abdominal glands of bitter leaf weevil (*Lixus camerunus*) were extracted with petroleum ether and fifteen pheromonal compounds were characterized using gas chromatography-mass spectrometry (GC/MS) and Fourier transform-infrared (FT-IR) spectroscopy techniques. The compounds identified were 2,4-dimethylpentane (1.90 %), 2,7-dimethyloctane (3.73 %), 1,6-dimethylpiperidin-3-one (5.62 %), decane (3.51 %), hexadecanoic acid (6.79 %), [S-(Z)]3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (17.18 %), cyclopropanepentanoic acid-2-undecyl-methyl ester (7.86 %), methyl decanoate (1.35 %), (5Z)-tetradec-5-ene (10.76 %), 2-butyloctan-1-ol (3.64 %), (11E)-tridec-11-en-1-ol (3.15 %), (2E)-tridec-2-enal (10.46 %), (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (13.18%), (2Z,5Z)-pentadeca-2,5-dien-1-ol (7.25%) and 5-(2-methylidenecyclopropyl)pentan-1-ol(3.61%). The FT-IR analysis of the extract showed peaks at 1464.02, 1647.26, 2925.15 and 3423.76 cm^{-1} indicating the presence of alkane, alkene, alcohol and carboxylic acid compounds, respectively. These compounds consisted 48.01 % alcohol, 19.90 % hydrocarbon, 10.46 % aldehyde, 9.21 % ester, 6.79 % fatty acid and 5.62 % ketone. The highest component was 11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol) followed by 11-trimethyldodeca-2,6,10-trien-1-ol (farnesol). *L. camerunus* is a major pest of the bitter leaf plant (*Vernonia amygdalina*) which is cultivated and consumed voraciously as vegetable in Nigeria. These pheromone compounds might artificially be used to lure and mass trap the weevils in pest control management thereby increasing the yield and quality of bitter leaf vegetable grown in Nigeria. This investigation has shown that the sex pheromones derived from the abdominal glands of *L. camerunus* are mostly alcohols, hydrocarbons, aldehydes, esters and fatty acids.

Keywords: Bitter leaf weevil (*Lixus camerunus*), Pheromones, GC/MS analysis, FT-IR analysis

1. Introduction

Pheromones are species-specific chemicals that affect insect behaviour, but are not toxic to insects. They are active in extremely low doses and are used to bait trap or confuse a mating population of insects. Pheromones can play an important role in integrated pest management for structural, landscape, agricultural or forest pest problems [1]. Effective pest control management assumes that there is no one method or chemical that is able to solve the damage caused by insects, therefore the use of several methods that do not have damaging side effects to the environment is needed [2]. Sex pheromones are critical factors for reproductive success in almost all species of insects. Since the emission of sex pheromone is necessary to

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attract a mate, failure to produce and emit pheromone can be utilized as a potential strategy for manipulating adult bitter leaf weevil (*L. camerunus*) behaviour. Information obtained from different studies suggests that pheromone biosynthesis activating neuropeptide (PBAN) may be useful in controlling pest insects since it regulates a key physiological role in pheromone production [3]. In pest control, sex pheromones are the main type of pheromone used. Both male and female insects will secret pheromones [2].

L. camerunus is a major pest of bitter leaf plant (*V. amygdalina*). The leaf is consumed as vegetable in South East, Nigeria. *L. camerunus* has also been reported to be the major vector of brown rot and die-back disease in amaranth *(Amaranthus hybridus* L.) in Nigeria [4]. The transmission and infection were found to be related to oviposition damage on healthy *A. hybridus* stem by *L. camerunus* [4]. Although management of insect pests in vegetables has been achieved successfully with the use of conventional pesticides, the development of pest resistance, negative impact on beneficial non-target insects, environmental pollution and problems associated with mammalian toxicity, coupled with the fact that most farmers cannot afford their use has resulted in the shift in pest control from the chemical era to the environmental era [5]. This has necessitated the development of alternative strategies for sustainable pests' management in vegetable production [5, 6].

Integrated insect pest management involves the use of more than one and usually several methods designed to reduce damage to plants [7]. The use of pheromones to control phases of the lives of pest species is one method of pest management. Certain pheromone traps have been developed and are in common usage by homeowners [8]. Hundreds of pheromones are known with which one sex (usually the female) of an insect species attracts its mates. Many of these sex attractants or their close chemical relatives - are available commercially. They have proved useful weapons against insect pests. Distributing a sex attractant throughout an area masks the insect's own attractant and thus may prevent the sexes getting together [9]. Insect sex attractants are also valuable in monitoring pest populations. By baiting trap with the appropriate pheromone, a build-up of the pest population can be spotted early. Early detection of pest build-up is a key ingredient in the integrated pest management system [9].

There is paucity of information on pheromones extracted from *L. camerunus*. However, as aforementioned, few literatures have shown that the organism is an insect pest that destroys leafy vegetables especially bitter leaf in South East, Nigeria. Herein is therefore reported the chemical analyses of sex pheromones from the abdominal glands of *L. camerunus* in the hope it would afford alternative approach to bitter leaf pest management.

2. Materials and Methods

2.1. Insect Collection

Colonies of *L. camerunus* were collected from Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, and were housed in circular glass nests. The organism was identified and authenticated in the Department of Zoology and Environmental Biology of the university. About 90 adult *L. camerunus* were used for the investigation. Hereafter, the words 'bitter leaf weevil' refers only to the adults of *L. camerunus* unless otherwise stated.

2.2. Extraction of Pheromone Compounds

Abdominal glands of *L. camerunus* were excised with fine brand new razor blade after anaesthetising the organisms by cleaning with chloroform which also removed cuticular surface contaminants. The tissue was extracted in petroleum ether for 20 min at room temperature. Extract was placed in screw cap vials and stored at -15^{o}C until analysis.

2.3. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

GC analysis was carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 80-280^{o}C held at 80^{o}C for 1 min, and at 200^{o}C for 4 min (rate 10^{o}C/min), and finally at 280^{o}C for 5 min (rate 10^{o}C/min). The injection temperature was 250^{o}C. GC/MS analysis was conducted using GCMS-QP 2010 Plus (Shimazu Japan) with column oven temperature of 80^{o}C. The carrier gas was helium with a pressure of 108.2 Kpa and linear velocity of 46.3 cm/s. Total flow was 6.2 mL/min, column flow was 1.58 mL/min, injection mode was split, flow control mode was linear velocity, purge flow was 3.0 mL/min and split ratio was 1.0. Also, ion source temperature was 230^{o}C, interface temperature was 250^{o}C, solvent cut time was 2.5 min., detector gain was 0.00 KV, detector gain mode was relative and the threshold was 1000. For the mass spectrometry, start time was 3.0 min, end time was 28.0 min, event time was 0.5 s, scan speed was 1250, and start m/z was 40 while end m/z was 600.The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermle Z 233 M-Z centrifuge, Germany, was used. All solvents used were of analytical grade and were procured from Merck, Germany.

2.4. Components Identification

The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature [10].

2.5. FT-IR Analysis

FT-IR measurement of the extract was performed using FTIR-8400S, Shimadzu, Japan, in a diffused reflectance mode at a resolution of 4 cm^{-1} in sodium chloride (NaCl) pellets in the range 4500-400 cm^{-1}.

3. Results and Discussions

From the chromatogram of the extract shown in Figure 1, peaks 1 to 15 represented the pheromone compounds. These compounds consisted 48.01 % alcohol, 19.90 % hydrocarbon, 10.46 % aldehyde, 9.21 % ester, 6.79 % fatty acid and 5.62 % ketone. Table 1 shows the summary of the FT-IR absorption of the extract from *L. camerunus*. Table 2 shows the nomenclatures, molecular formulae, molecular weights, retention times, peak areas and the nature of these compounds. The FT-IR spectra of the extract are shown in Figure 2. A peak at 1464.02 cm^{-1} indicated the presence of C-H bending from alkanes. Another peak at 1647.26 cm^{-1} was due to C=C from alkenes while that of 3423.76 $cm^{-1}$was characteristic of O-H functional group from alcohols. The four most abundant components of the extract were 11-trimethyl-1,6,10-dodecatrien-3-ol (17.18 %), 11-trimethyldodeca-2,6,10-trien-1-ol (13.18 %), (5Z)-tetradec-5-ene (10.76 %) and (2E)-tridec-2-enal (10.46 %). The mass spectra of these four compounds are shown in Figures 3, 4, 5 and 6 while the structures of the identified fifteen pheromone compounds are shown in Figure 7.

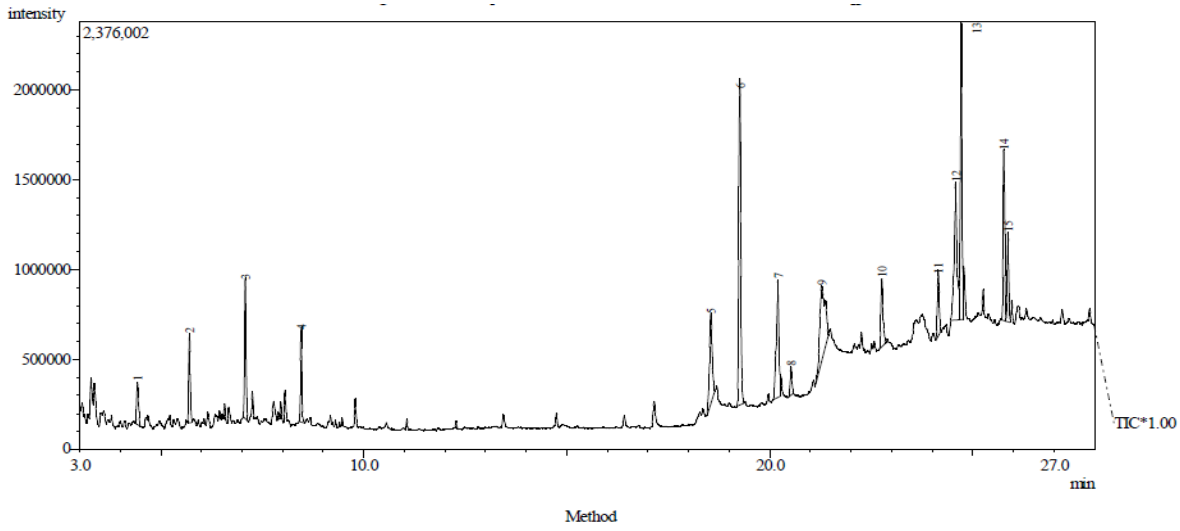

Figure 1. GC-MS Chromatogram of Bitter Leaf Weevil (*L. camerunus*) Pheromone Extract

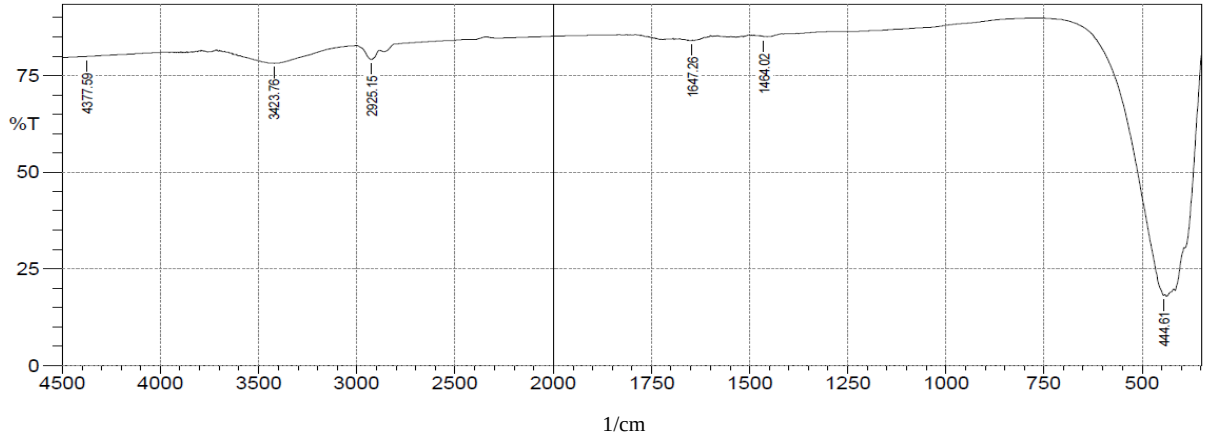

Figure 2. FT-IR Spectra of Bitter Leaf Weevil (*L. camerunus*) Pheromone Extract

Table 1. FT-IR Absorption of the Pheromone Extract from Bitter Leaf Weevil *(L. camerunus*)

S/N	FT-IR absorption (cm^{-1})	Functional group	Nature of compound
1	1464.02	C – H bending	Alkane
2	1647.26	C=C	Alkene
3	2925.15	C – H	Alkane
4	3423.76	O – H	Alcohol

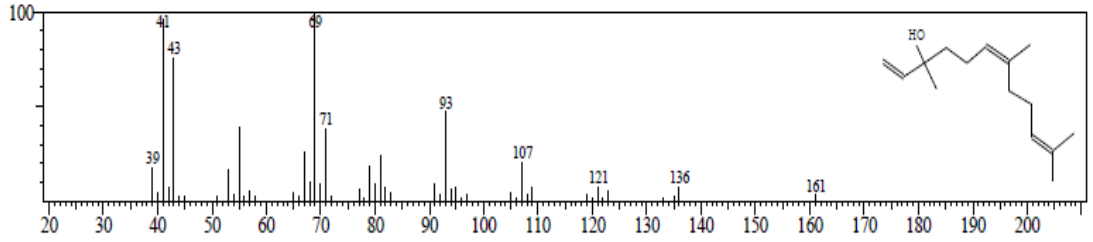

Figure 3. Mass Spectra of 11-trimethyl-1,6,10-dodecatrien-3-ol

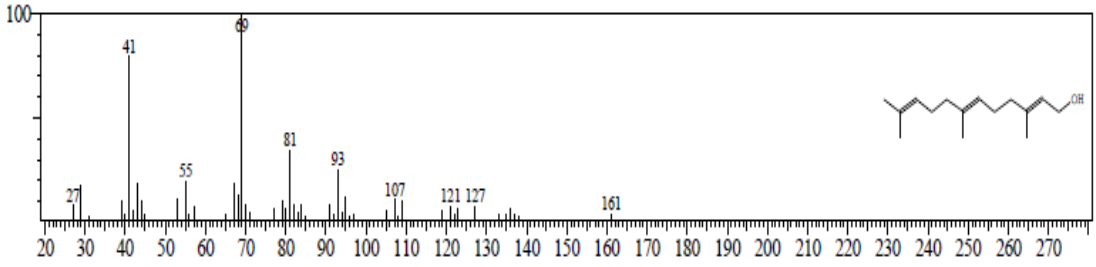

Figure 4. Mass Spectra of 11-trimethyldodeca-2,6,10-trien-1-ol

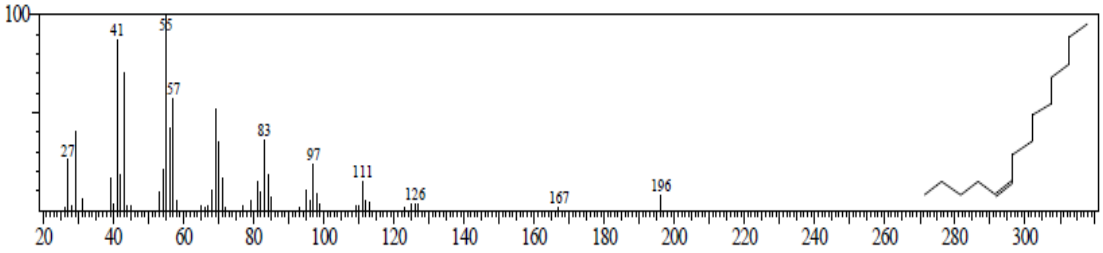

Figure 5. Mass Spectra of (5Z)-tetradec-5-ene

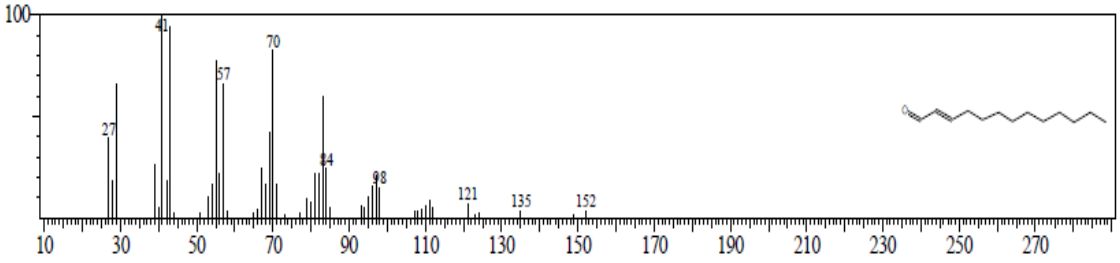

Figure 6. Mass Spectra of (2E)-Tridec-2-enal

The alcoholic sex pheromone compounds of the extract from *L. camerunus* consisted of [S-(Z)]3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol) (compound **6**), 2-butyloctan-1-ol (compound **10**), (11E)-tridec-11-en-1-ol (compound **11**), (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (farnesol) (compound **13**), (2Z,5Z)-pentadeca-2,5-dien-1-ol (compound **14**) and 5-(2-methylidenecyclopropyl)pentan-1-ol (compound **15**).Compounds similar to these alcoholic compounds have been reported as sex and trail pheromones of insects. For instance, (*E*)-2,6,10-trimethyl-5,9-undecadien-1-ol, (*Z*,*E*,*E*)-dodeca-3,6,8-trien-1-ol, (*Z*,*Z*)-dodeca-3,6-dien-1-ol, (*Z*)-dodec-3-en-l-ol, nonanol, decanol, undecanol and dodecanol have been reported as trail pheromones in so many species of termites and some ants [11, 12]. Dodecatrien-1-ol is the main sex pheromone identified in termites [13].The two isomers of (*Z*,*E*)-α-Farnesene have been reported as insect semiochemicals; they act as alarm pheromones in termites [14] or food attractants for the apple tree pest, the codling moth [15]. β-Farnesene has one naturally occurring isomer. It is also released by

aphids as an alarm pheromone upon death to warn away other aphids [16]. Several plants, including potato species, have been shown to synthesize this pheromone as a natural insect repellent [17, 18]. What was identified in the pheromone extract of *L. camerunus* in this research was farnesol (compound **13**). This compound might be used by the insect not only as sex pheromone but also as other means of communication in its habitat. Alcoholic compounds have also been reported as aggregation pheromones. Dickens [19] reported that there was a remarkable increase in catches of the boll weevil when traps were baited with trans-2-hexen-1-ol, cis-3-hexen-1-ol or 1-hexanol paired with the boll weevil aggregation pheromone. Moreover, trans-2-hexen-1-ol also extended the longevity of attractiveness of pheromone-baited traps [19].The hydrocarbon sex pheromones identified in this current study were 2,4-dimethylpentane, 2,7-dimethyloctane, decane and (5Z)-tetradec-5-ene while the only ketonic compound was 1,6-dimethylpiperidin-3-one. (Z)-9-tricosene and methylalkanes have been reported as sex pheromones in housefly [20] where it was stated that several oxidation products of (Z)-9-tricosene, such as (Z)-9,10 epoxytricosane and (Z)-14-tricosene-10-one and some methylalkanes found on female houseflies were shown to enhance male sexual activity in combination with (Z)-9-tricosene [21]. It is herein suspected that the combination of the hydrocarbon molecules from *L. camerunus* and the ketone as well as other molecules will enhance sexual activity in the males of this insect. Other classes of compounds present in the extract of *L. camerunus* were aldehyde and esters. The aldehyde found was (2E)-tridec-2-enal. Ethyl-4-methyloctanoate has been reported as aggregating pheromone in *Oryctes rhinoceros* [22] while (Z)-11-hexadecenal, (Z)-9-tetradecenal, hexadecanal and tetradecanal have been reported as the sex pheromones in *Heliothis virescens* [23]. The esters identified in the extract were methyl decanoate and cyclopropanepentanoic acid 2-undecyl-methyl ester. Hexadecanoic acid was also present in the sex pheromone extract of *L. camerunus*. These compounds may be used in synergy to bring about effective sexual communication and performance in *L. camerunus*. Again, these compounds may be used by the insect for other purposes like as alarm and trail pheromones. Behavioural responses are therefore required as evidence to validate these suspicions.

Table 2. Pheromones Identified from the GC-MS Analysis of the Abdominal Glands Extract of *L.camerunus*

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)	Nature of compound
1	2,4-Dimethylpentane	C_7H_{16}	100	4.423	1.90	Hydrocarbon
2	2,7-Dimethyloctane	$C_{10}H_{22}$	142	5.704	3.73	Hydrocarbon
3	1,6-Dimethylpiperidin-3-one	$C_7H_{13}NO$	127	7.080	5.62	Ketone
4	Decane	$C_{10}H_{22}$	142	8.456	3.51	Hydrocarbon
5	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	18.548	6.79	Fatty acid
6	[S-(Z)]3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (nerolidol)	$C_{15}H_{26}O$	222	19.261	17.18	Alcohol (terpenoid)
7	Cyclopropanepentanoic acid, 2-undecyl-methyl ester	$C_{20}H_{38}O_2$	310	20.197	7.86	Ester
8	Methyl decanoate	$C_{11}H_{22}O_2$	186	20.520	1.35	Ester
9	(5Z)-Tetradec-5-ene	$C_{14}H_{28}$	196	21.274	10.76	Hydrocarbon
10	2-Butyloctan-1-ol	$C_{12}H_{26}O$	186	22.755	3.64	Alcohol
11	(11E)-Tridec-11-en-1-ol	$C_{13}H_{26}O$	198	24.149	3.15	Alcohol
12	(2E)-Tridec-2-enal	$C_{13}H_{24}O$	196	24.573	10.46	Aldehyde
13	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-ol (farnesol)	$C_{15}H_{26}O$	222	24.712	13.18	Alcohol (terpenoid)
14	(2Z,5Z)-Pentadeca-2,5-dien-1-ol	$C_{15}H_{28}O$	224	25.763	7.25	Alcohol (terpenoid)
15	5-(2-Methylidenecyclopropyl)pentan-1-ol	$C_9H_{16}O$	140	25.868	3.61	Alcohol (terpenoid)

4. Conclusions

L. camerunus is a major pest of bitter leaf plant (*V. amygdalina*) which is voraciously used as vegetable in South East Nigeria, therefore there is the need to control this pest in such a way that the ecosystem is not disrupted. The use of pheromones, at this juncture, is the best bet. The sex pheromone compounds of the insect that have been studied might be used in luring and mass trapping the insect to remove large numbers from the breeding and feeding population. The compounds might also be applied in the disruption of mating in the insect population. All these will help in protecting the plant. However, there is the need to decipher which of these compounds has the highest attractiveness to the insect. Nevertheless, compound **6** i.e. 11-trimethyl-1,6,10-dodecatrien-3-ol is suspected to be the most active of the pheromone compounds discovered in *L. camerunus* (though not yet proved). The extract from the abdominal glands of *L. camerunus* was characterised with GC/MS and FT-IR techniques which afforded fifteen pheromone compounds comprising 48.01 % alcohol, 19.90 % hydrocarbon, 10.46 % aldehyde, 9.21 % ester, 6.79 % fatty acid and 5.62 % ketone. The highest component was 11-trimethyl-1,6,10-dodecatrien-3-ol followed by 11-trimethyldodeca-2,6,10-trien-1-ol.

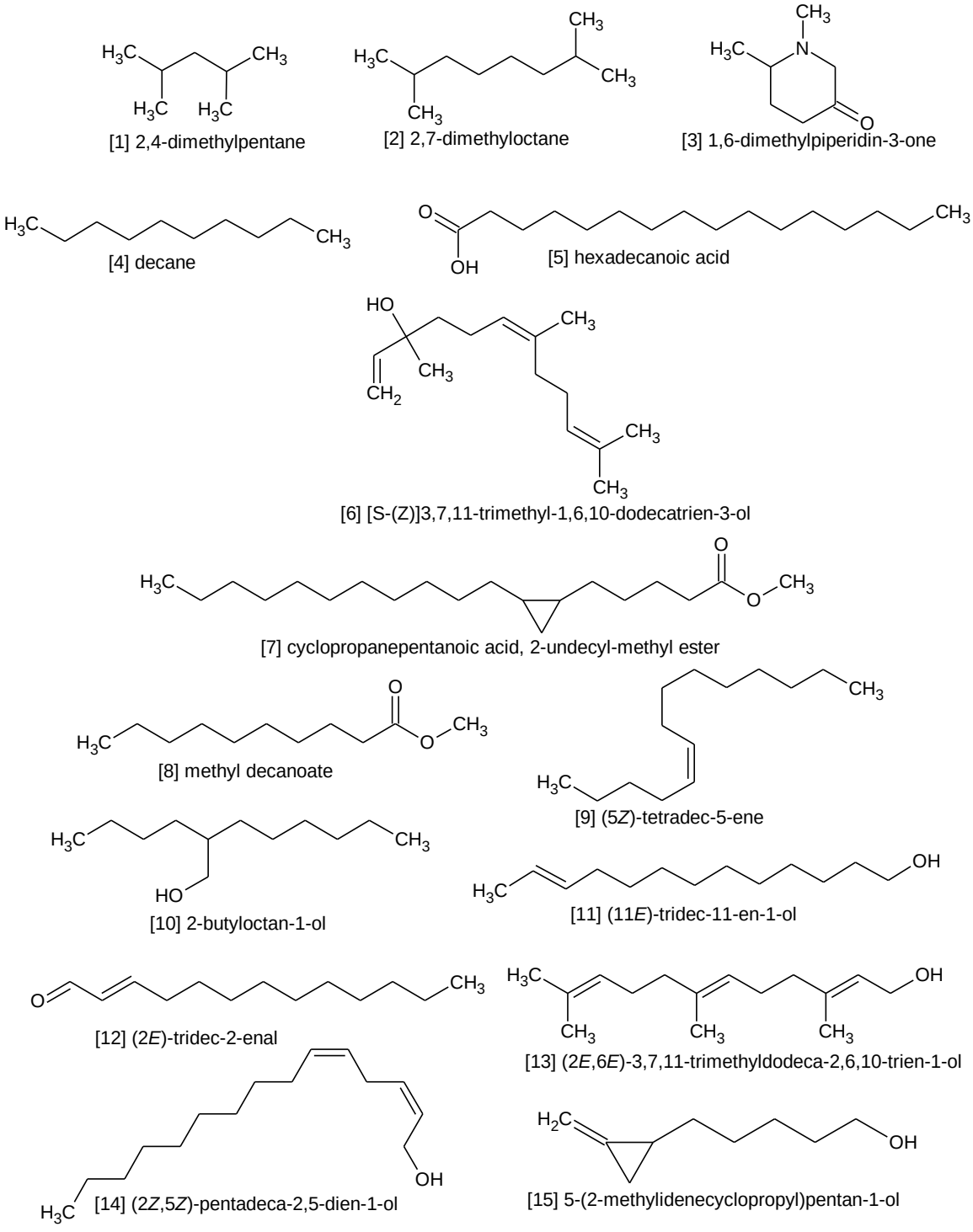

Figure 7. Structures of Pheromonal Compounds Identified from the GC/MS Result of Bitter Leaf Weevil (*L. camerunus*) Extract

The highest component is suspected as the main sex pheromone of *L. camerunus* while the other compounds act as enhancers. Although behavioural bio-assays were not carried out, these compounds might also be used for other communication and interactive purposes by the insect. These compounds might artificially be used to lure and mass trap the weevils in pest control management thereby increasing the yield and quality of bitter leaf vegetable grown in Nigeria. This investigation has shown that the sex pheromones derived from the abdominal glands of *L. camerunus* are mostly alcohols, hydrocarbons, aldehyde, esters and fatty acid and that the pheromones are mixture of these compounds rather than a single compound. This research serves as a springboard to validate and authenticate the efficacy of these compounds in luring *L. camerunus* by studying the behavioural responses of the organism to them.

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REFERENCES

[1] Seybold S.J., 1998. Donaldson S. Pheromones in insect pest management: In Cooperative Extension University of Nevada, Fact Sheet 98-41.

[2] Braun T. Pheromones. http://www.pcaapplicatorhours.com/pheromones.html, viewed 18/08/2010.

[3] AjithaV.S., Muraleedharan D., 2005. Tissue localization and partial characterization of pheromone biosynthesis activating neuropeptide in *Achaea janata*. J. Biosci., 30(2), 191-200.

[4] Anno-nyAko F.O., Adebanjo A., Agunloye O., 1993.Brown rot and die-back disease induced by acucurlionid *(Lixus camerunus* Klobe) in amaranth*(Amaranthushybridus*L.) in Nigeria. Ghana J. Agric. Sci., 27, 87-91.

[5] Okunlola A.I.,Ofuya T.I., 2010.Farmers Perception of problems in the cultivation of selected leaf vegetables in South Western Nigeria. Sains Malaysiana 39(3), 513-518.

[6] Uvah I.I., 1992. Crop diversity and management. Nigeria J. Entomol., 5, 5-11.

[7] Byers J.A., 1991. Pheromones and chemical ecology of locusts. Riol. Rev., 66, 347-378.

[8] Pheromones in Insects. http://www.si.edu/Encyclopedia_SI/nmnh/buginfo/pheromones.htm, viewed 15/11/2014.

[9] Pheromones. http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/P/Pheromones.html, viewed 15/11/2014.

[10] Igwe O.U., Okwu D.E., 2013. GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the seeds of *Brachystegiaeurycoma* Harms. Asian J. Plant Sci. Res., 3(2), 47-45.

[11] Bordereau C., Pasteels J.M., 2011. Pheromones and chemical ecology of dispersal and foraging in termites.In:Bignell DE, Roisin Y, Lo N, Editors. Biology ofTermites: A Modern Synthesis, New York: Springer, USA.

[12] El-Sayed A.M., 2008. The pherobase: database of insect pheromones and semiochemicals. http://www.pherobase.net.

[13] Costa-leonardo A.M., Casarin F.A., Lima J.T., 2006. Chemical communication in Isoptera. Neotropical Entomol., 38(1), 001-006.

[14] Šobotník J., Hanus R., Kalinová B., Piskorski R., Cvačka J., Bourguignon T., Roisin Y., 2008. (E,E)-α-Farnesene, an Alarm Pheromone of the Termite Prorhinotermescanalifrons. J. Chem. Ecol., 34 (4), 478-486.

[15] Hern A., Dorn S., 1999. Sexual dimorphism in the olfactory orientation of adult Cydiapomonella in response to alpha-farnesene. Entomol. Exp. Appl., 92(1), 63-72.

[16] Gibson R.W., Pickett J.A., 1983. Wild potato repels aphids by release of aphid alarm pheromone. Nature 302 (5909), 608–609.

[17] Farnesene. http://en.wikipedia.org/wiki/Farnesene, viewed 12th August, 2014.

[18] Avé D.A., Gregory P., Tingey W.M., 1987. Aphid repellent sesquiterpenes in glandular trichomes of Solanumberthaultii and S. tuberosum. Entomol. Exp. Appl., 44, 131-138.

[19] Dickens J.C., 1989. Green leaf volatiles enhance aggregation pheromone of the boll weevil Anthonomusgrandis. Entomol. Exp. Appl., 52, 191-203.

[20] Uebel E.C., Schwarz M., Lusby B.R., Miller R.W., Sonnet P.E., 1978. Cuticular non-hydrocarbons of the female housefly and their evolution as mating stimulants. Lloydia, 41, 63-67.

[21] Rogoff W.M., Gretz, G.H., Sonnet P.F., Schwarz M., 1980. Responses of male house flies to muscalure and to combinations of hydrocarbons with and without muscalure. Environ. Entomol., 9, 605-606.

[22] Sudharto P.S., 2000. Synergy between empty oil palm fruit bunches and synthetic aggregation pheromone (ethyl 4-methyloctanoate) for mass trapping of Oryctes rhinoceros beetles in the oil palm plantations in Indonesia. In Cutting Edge Technologies for Sustained Competitiveness: Proceedings of the 2001 PIPOC International Palm Oil Congress, pp. 661–664, Malaysian Palm Oil Board, Kuala Lumpur, Malaysia

[23] Dickens J.C., Smith J.W., Light D.M., 1993. Green leaf volatiles enhance sex attractantpheromone of the tobacco budworm, Heliothisvirescens (Lepidoptera:Noctuidae). Chemoecol., 4, 175–177.