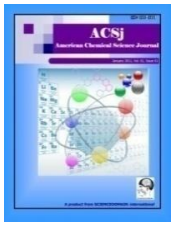

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Chemistry of Trail Pheromones from Cubitermes Termites (*Amitermes dentatus*): An Innovation in Pest Management

O. U. Igwe[1*] and P. N. Eze[1]

[1]Department of Chemistry, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria.

Authors' contributions

This work is a collective contribution of two authors. Author OUI designed and supervised the research, interpreted the GC-MS spectra and drew the structures with Chemsketch software and also wrote the manuscript. Author PNE carried out the analyses and supplied some references. Both authors read and approved the final manuscript.

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ABSTRACT

Trail pheromones were extracted with petroleum ether from the sternal gland near the anterior portion of the fifth abdominal sternit of cubitermes termites (*Amitermes dentatus*) and fourteen pheromonal compounds were characterised using Gas Chromatography-Mass Spectrometry (GC/MS) Technique in combination with Fourier Transform-Infrared Spectroscopy (FT-IR). The compounds analysed include ethylbenzene (6.70%), 3,4-dimethylheptane (6.32%), decane (6.87%), 2,7-dimethyloctane (12.28%), 2-methylundecane (19.00%), 3-methylnonane (5.14 %), 2,6-dimethyloctane (4.23%), undecane (11.44 %), 2-methyldecane (3.94%), methyl decanoate (2.35%), 14-octadecenoic acid methyl ester (10.71%), heptacosanoic acid methyl ester (1.88%), 2-butyloctan-1-ol (3.45%) and E-2-octadecadecen-1-ol (5.70%). FT-IR analysis of the extract showed peaks at 1460.16 (CH_3 bending), 1549.86 (C=C), 1636.65 (C=C), 2866.32 (C−H), 2934.79 (C−H) and 3426.66 (O − H) cm^{-1} indicating the presence of alkane, aromatic, alkene and alcoholic compounds in the extract. These compounds consist of 69.22% hydrocarbon, 14.94% ester, 9.15% alcohol and 6.70% aromatic compound. The highest component was 2-methylundecane followed by

**Corresponding author: E-mail: okescoty@yahoo.com; okenwauigwe@gmail.com;*

2,7-dimethyloctane. This examination has revealed that the trail pheromone in termites is not just a single compound but a mixture of compounds. The synthetic forms of these semiochemicals might be applied in luring and mass trapping termites for possible extermination. This method of pest control ensures environmental friendliness as the problems posed by dangerous chemicals are eradicated.

Keywords: Cubitermes termites; Amitermes dentatus; Pheromones; GC/MS analysis; Pest management.

1. INTRODUCTION

Pheromones are a class of semiochemicals that insects and other animals release to communicate with other individuals of the same species. In insects, these pheromones are detected by the antennae on the head and the signals can be effective in attracting faraway mates, and in some cases, can be very persistent, remaining in place and active for days [1]. Pheromones are very active biologically in insects where they trigger many of the activities necessary for the survival of the insects. Pheromone molecules are light and very volatile and can float for miles. Insect pheromones are used in pest control by government agencies and private agriculture [2]. These pheromones are non-toxic and biodegradable chemicals that can be used to lure insects into traps or foiled into wasting energy that they normally need for locating food and mates [1].

The *Cubitermes* termites are a type of termite that is endemic to Africa and its epigeic, mushroom-shaped nests are typical of certain landscapes and they inhabit diverse environments ranging from rainforest to Sudanese savannas [3]. Semiochemicals in termite colonies are employed in many activities such as foraging, defense, attraction to and induction of feeding on food sources, colony segregation, mate location, and even caste regulation. Several studies on termite pheromones have been published but little is known on the identity of these semiochemicals [4]. Trail pheromones are secreted by the sternal glands and are related to foraging behaviour. The pheromone is deposited when the insect presses its abdomen against the substrate, releasing the pheromonal secretion from the sternal gland. Termites lay exploratory trails during the search for a food source for latter nest-mate recruitment to the food source [4,5]. Behavioural observations suggest that the trail pheromone of termites is a multi-component blend, with an active compound acting as a common orientation signal for different termite species and specific secondary compounds that determine the specificity of trails among species [4,6].

Although it has been known for some time that a number of termite species use trail pheromones for orientation toward food or sources of disturbance, the detailed properties and functioning of these pheromone systems have not been well worked out, despite reports of the isolation of several of the chemicals responsible [7]. The trail substance is produced, at least in *Kalotermes*, *Zootermopsis* and *Nasutitermes*, in the sterna gland, and the ubiquity of this gland among termites has led to the postulate that most, if not all, termites secrete a trail pheromone in this gland [7,8]. n-caproic acid was reported to be the trail pheromone of *Zootermopsis nevadensis*, a kind of termites [9]. However, another report stressed that termites follow trails of many different substances and the demonstration of trail following in response to some pure chemical is far from proving that the natural pheromone has been found, adding that, caproic acid was almost certainly not the trail pheromone of *T. trinervoides* [7].

Although termites play, based on their feeding habits, a key role in organic matter disturbance and in the improvement of the physical and chemical characteristics of tropical soils [10,11], they can also cause economic loss by damaging structures such as buildings, bridges, dams, and even roads; or by damaging crops, forest trees, or rangelands. It must be recognized at the outset that termites are a part of the natural ecosystem in much of the world, and that the presence of termites is not, by itself, evidence of a termite pest problem. Termites become a pest when economic damage is caused by termite activity [12]. Termites becomes a problem when they damage structural timber and other materials in structures. Damage may extend to household furniture, paper products, many synthetic materials and food items. Each year hundreds of thousands of structures (bridges, dams, decks, homes, retaining walls, roads, utility poles, and underground cables and pipes) require treatment for the management of termites

[12]. In this research, the chemistry of trail pheromones from cubitermes termites (*Amitermes dentatus*) is studied.

2. MATERIALS AND METHODS

2.1 Insect Collection

Colonies of *A. dentatus* were collected from behind Limah Hall inside Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, and housed in circular glass nests. The organism was identified and authenticated in the Zoology Department of Michael Okpara University of Agriculture, Umudike. Only about 300 adult workers were used for the investigation. Hereafter, the word termite refers only to adult workers of *A. dentatus* unless otherwise stated.

2.2 Extraction of Trail Pheromones

The trail pheromone of the family termitidae (which contains the genus, *Amitermes*) is produced in a sternal gland near the anterior portion of the fifth abdominal sternite [7]. These glands were excised with fine brand new razor blade after anaesthetising the organism by cleaning with chloroform which also removes 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 ℃ 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 ℃ held at 80 ℃ for 1 min, and at 200 ℃ for 4 min (rate 10 ℃/min), and finally at 280 ℃ for 5 min (rate 10 ℃/min). The injection temperature was 250 ℃ . GC/MS analysis was conducted using GCMS-QP 2010 Plus Shimazu Japan with column oven temperature of 80 ℃. 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 ℃ , interface temperature was 250 ℃, 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 spec., 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 [13].

2.5 FT-IR Analysis

FT-IR measurement of the extract was performed using FTIR-8400S Fourier Transform Infrared Spectrophotometer, 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 DISCUSSION

The chemistry of trail pheromones in termites was studied by using GC/MS technique which showed the presence of fourteen pheromonal compounds as shown by the chromatogram in Fig. 1. Out of these fourteen compounds, eight were hydrocarbons (69.22%), three were esters (14.94%) and two were alcohols (9.15%) while one was an aromatic compound (6.70%). The highest component of the fourteen compounds was 2-methylundecane (19.00%) followed by 2,7-dimethyloctane (12.28%). 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 from termite are shown in Fig. 2. FT-IR analysis of the pheromonal extract showed peaks at 1460.16 cm^{-1} indicative of a CH_3 bending vibration. The peak at 1549.86 cm^{-1} was due to C=C of aromatic compound while that of 1636.65 cm^{-1} was for C=C of alkenes. Peaks at 2866.32 and 2934.79 cm^{-1} were due to the presence of C−H vibration of alkanes. The broad peak at 3426.66 cm^{-1} indicated the presence O−H vibration from alcoholic compounds in the pheromonal extract. Table 1 shows the interpretation of FT-IR absorption of the extract. The mass spectra of the three most abundant compounds in the pheromonal extract are shown in Figs. 3, 4 and 5 while the structures of the fourteen compounds analysed are shown in Fig. 6. It has been reported that termites produce a mixture of chemical substances, mainly

hydrocarbons from the cuticle [4,14], which were believed to be involved in nest mate recognition [15,16] based on the fact that hydrocarbon cuticular composition differs among colonies of the same termite species [17,18], and that these differences might be correlated with intercolonial aggressions [4]. It has also been reported that recent studies in this area suggest a genetic mechanism for cuticular hydrocarbons, but current research has not ruled out environmental effects on the composition of these substances in termites [4,19]. The trail pheromone reported in *Cubitermes* spp. was (*Z*,*Z*,*E*)-dodeca-3,6,8-trien-1-ol [20]. However, we hereby report the existence of 2-butyloctan-1-ol (3.45%) and E-2-octadecadecen-1-ol (5.70%) in the trail pheromone of *A. dentatus*.

The trail pheromone of the family termitidae (which contains the genus, *Amitermes*) has been reported to be produced in a sternal gland near the anterior portion of the fifth abdominal sternite [7]. Since the trail pheromone acts through some distance, it was presumed that it was detected by means of the antennae. Amputation of the antennae rendered the termites incapable of trail following [7]. The chemical parsimony of termites is functional, i.e., the same compound is secreted by different glands, different species and for different functions [21]. This therefore suggests that the fourteen pheromonal compounds could be secreted by any of the glands and that these compounds may not only be involved in trailing but could also be used by the organism as alarm, aggregating, defence and sex pheromones. In some species such as *C. bequarti*, a report stated that the same compound, dodecatrien-1-ol, is used both as a sex and a trail pheromone. However, despite the ecological and economic role of termites, little is known on their semiochemicals as compared to what is already known for other social insects. Further information on these chemical mediators is essential for the development of alternative methods for termite control [4].

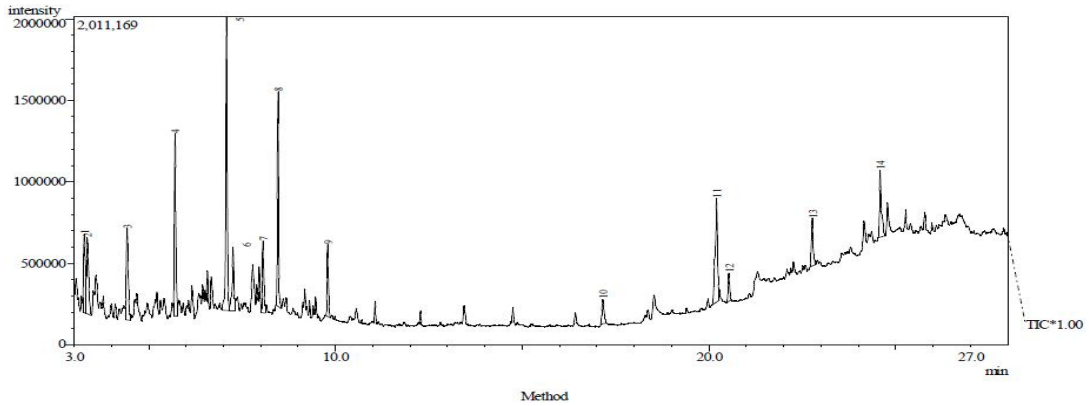

Fig. 1. GC-MS chromatogram of *Ametermes dentatus* pheromone extract

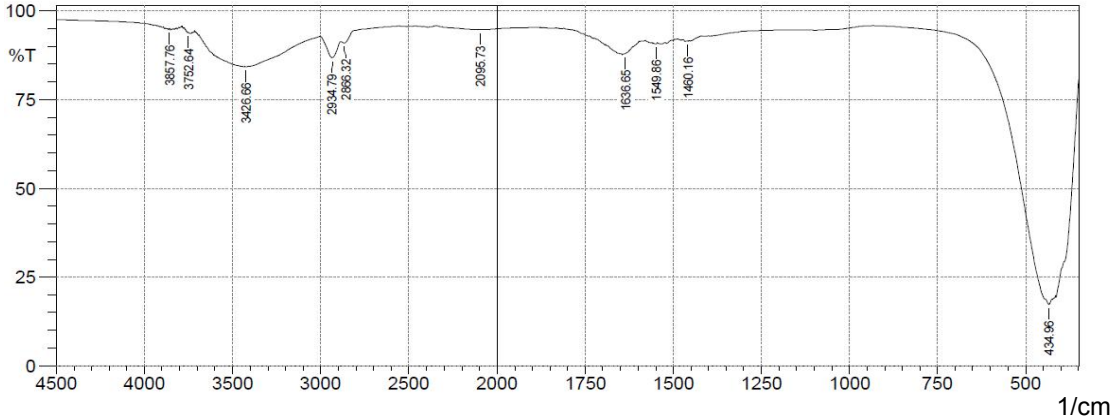

Fig. 2. FT-IR spectra of *Ametermes dentatus* pheromone extract

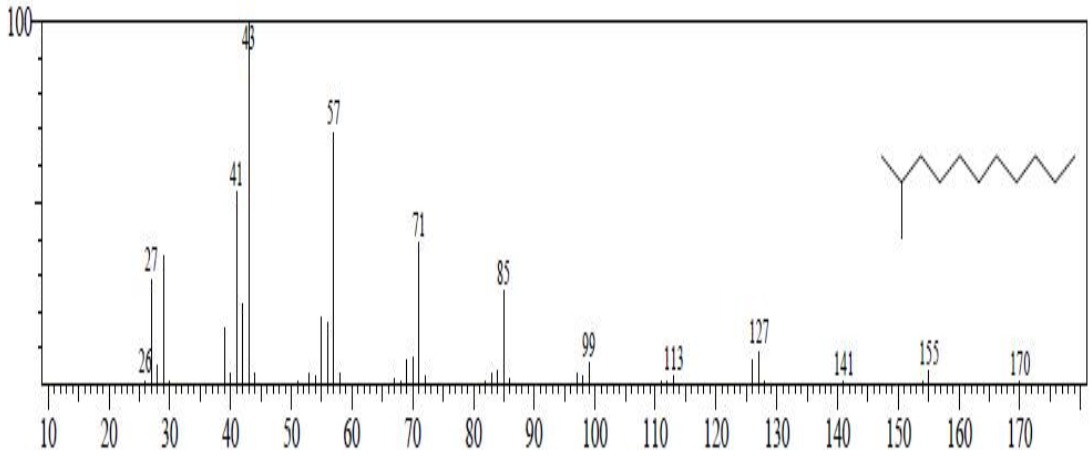

Fig. 3. Mass spectra of 2-methylundecane

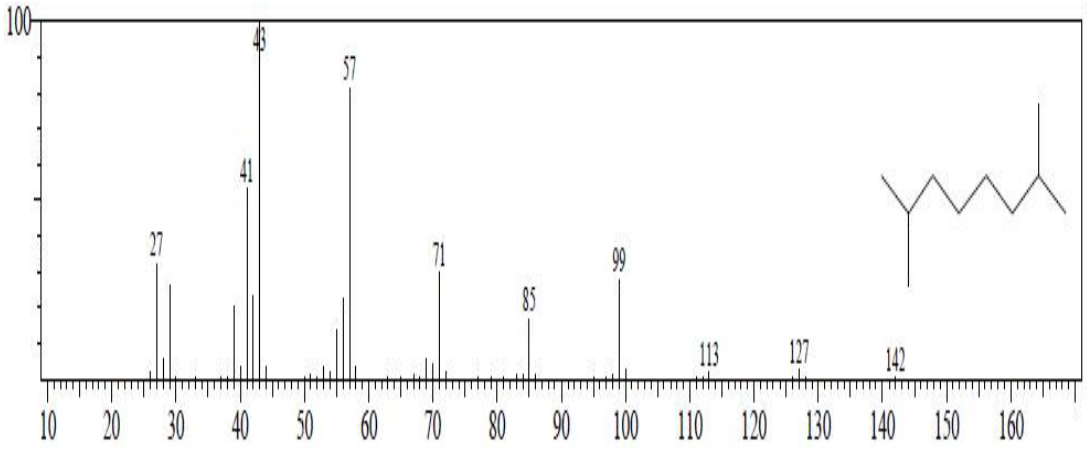

Fig. 4. Mass spectra of 2, 7-dimethyloctane

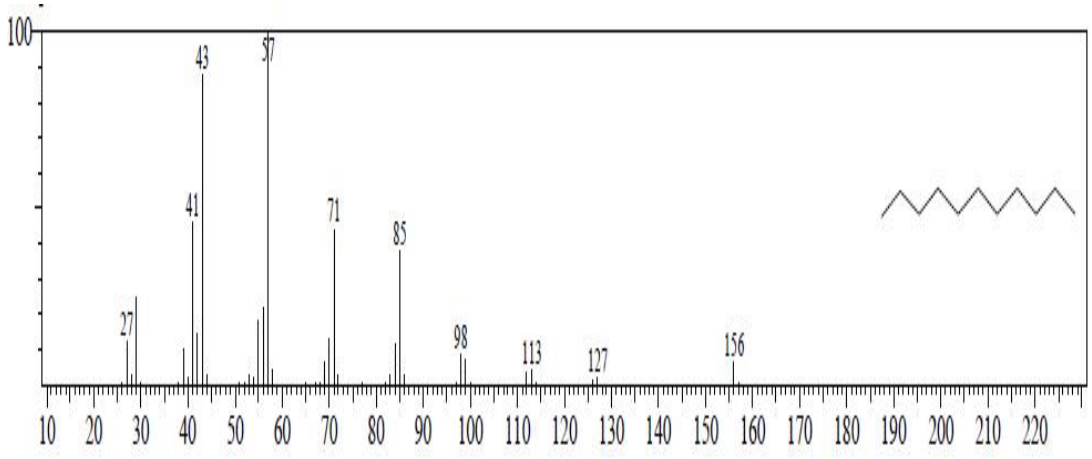

Fig. 5. Mass spectra of undecane

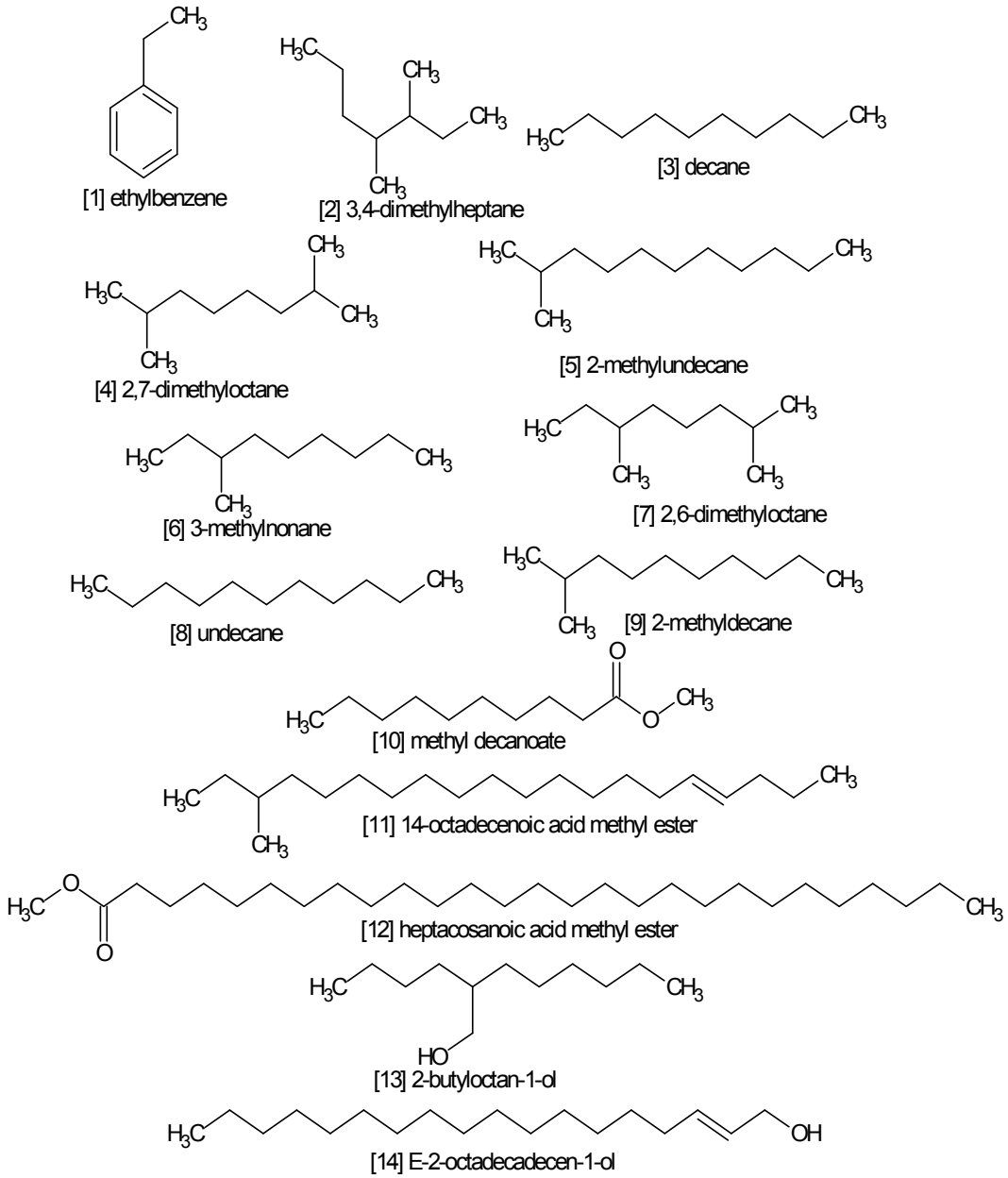

Fig. 6: Structures of semiochemicals identified from the GC/MS result of *Ametermes dentatus* extract

Termites can attack plants at any stage of development from the seed to the mature plant. Termite damage to stored products generally results in invasion by *Aspergillus*. The fungus causes indirect yield losses and contaminates production with aflatoxins [22]. Pheromone is strategically becoming a promising agent for the control of insect pests. One of the methods would be to apply insecticides to a sandy area treated with pheromone, thus killing both the adults and the egg nymphs. Unfortunately, insecticides are often repellent to insects, so this may be a drawback, in addition to the other problems with toxic substances [23]. Another method would be to spread the pheromone over a wide area to disperse the egg laying ones so that no groups would form [23]. The pheromone could also be used in traps for monitoring populations. Isolation, identification and circumventing the known pheromones possibly could be used in further experiments investigating termite responses and ultimately in direct control and monitoring of the termite population levels.

Table 1. FT-IR absorption of the extract from *Ametermes dentatus*

S/N	FT-IR absorption (cm^{-1})	Functional group	Nature of compound
1	1460.16	C−H bending	Alkane
2	1549.86	C=C	Aromatic
3	1636.65	C=C	Alkene
4	2866.32	C−H	Alkane
5	2934.79	C−H	Alkane
6	3426.66	O−H	Alcohol

Table 2. Pheromones identified from the GC-MS analysis of the cuticular extract from *Ametermes dentatus*

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)	Nature of compound
1	Ethylbenzene	C_8H_{10}	106	3.275	6.70	Aromatic
2	3,4-Dimethylheptane	C_9H_{20}	128	3.358	6.32	Hydrocarbon
3	Decane	$C_{10}H_{22}$	142	4.420	6.87	Hydrocarbon
4	2,7-Dimethyloctane	$C_{10}H_{22}$	142	5.704	12.28	Hydrocarbon
5	2-Methylundecane	$C_{12}H_{26}$	170	7.081	19.00	Hydrocarbon
6	3-Methylnonane	$C_{10}H_{22}$	142	7.257	5.14	Hydrocarbon
7	2,6-Dimethyloctane	$C_{10}H_{22}$	142	8.059	4.23	Hydrocarbon
8	Undecane	$C_{11}H_{24}$	156	8.458	11.44	Hydrocarbon
9	2-Methyldecane	$C_{11}H_{24}$	156	9.791	3.94	Hydrocarbon
10	Methyl decanoate	$C_{11}H_{22}O_2$	186	17.160	2.35	Ester
11	14-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	20.202	10.71	Ester
12	Heptacosanoic acid methyl ester	$C_{28}H_{56}O_2$	424	20.530	1.88	Ester
13	2-Butyloctan-1-ol	$C_{12}H_{26}O$	186	22.762	3.45	Alcohol
14	E-2-Octadecadecen-1-ol	$C_{18}H_{36}O$	268	24.581	5.70	Alcohol

4. CONCLUSION

The pheromonal extract of *A. dentatus* otherwise known as cubitermes termites mound was analysed with GC/MS and FT-IR techniques which revealed the presence of fourteen compounds. These compounds consisted 69.22% hydrocarbon, 14.94% ester, 9.15% alcohol and 6.70% aromatic compound. The highest component was 2-methylundecane followed by 2,7-dimethyloctane and undecane. This examination has revealed that the trail pheromone in termites is not just a single compound but a mixture of compounds. We are not sure how much sensitivity will be recorded since insect behavioural bio-assays were not carried out, hence, the idea of applying the synthetic forms of these semiochemicals in luring and mass trapping termites for possible extermination remains to be probed by other researchers. There is therefore the need to stretch this investigation to authenticate if a mixture or any of these compounds can trigger response in termites. This method of pest control ensures environmental friendliness as the problems posed by dangerous chemicals are eliminated.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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