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Gut Microbiome Composition and GC-MS Analysis of the Oil Extract from Termite species in Uyo, Akwa Ibom State, Nigeria

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Abstract

Termites are eusocial insects of the order Isopter, with members gut harbouring certain bacteria and fungi. This study analysed the micro-organisms in the gut of termite species. Ten (10) termitaria were excavated, and termites collected were identified into two species Macrotermes sp. and Odontotermes sp. Fifteen (15) Workers, 25 mandibulated soldiers, and one (1) Queen for Macrotermes sp., and fifteen (15) workers, 25 soldiers, and one Queen for Odontotermes sp were subjected to isolation and characterization of micro-organisms' symbionts from their gut using microbial techniques, grams staining, biochemical, and molecular techniques. The microbial communities harboured in the termites' gut of the workers, soldiers, queen, and king castes of the Macrotermes sp. and Odontotermes sp. were identified as bacteria of the genera Bacillus sp., Salmonella sp., Shigella sp. and Escherichia coli, and fungi of the genera, Fusarium sp, Termitomyces sp, and Chaetomium sp. The GC-MS analysis of the oil extract from the termite queen demonstrated the presence of thirty-one (31) chemical components. The most prevailing constituents were thymol (23.31 %), seconded by gamma-Terpenene (17.31 %), and followed by p-Cymene (16.40 %), Phenol-2-methyl-5-(1-methyl ethyl)- (7.19%), Lupeol (5.94 %), Terpinen-4-ol (3.19), and Caryophyllene oxide (2.47 %). The results indicated that the termite species host diverse groups of symbiotic bacteria carrying out different enzymatic functions in the gut. This work was the first to report the GC-MS component of the oil extract from termite queen in south-south Nigeria. Nevertheless, further study on the interaction of microbiomes in the gut of termite species is recommended.

Keywords: Macrotermes sp, Odontotermes sp, Bacillus sp, Salmonella sp, Shigella sp.

INTRODUCTION

Termites are eusocial insects and members of the order Isoptera (Auer *et al.*, 2017; Akpan *et al.*, 2020; Akpan *et al.*, 2022). Several castes, including reproductive (Queen) and non-reproductive (worker and soldier), make up the termitarium (Devaraji and Kesti, 2019).

In tropical and subtropical regions, termites are the most common invertebrate decomposers of decomposing organic matter (Brune, 2014). The combination of a sophisticated social structure and a unique ability to absorb plant materials, especially wood, is often credited with their ecological success (Akpan *et al.*, 2020; Akpan *et al.*, 2022). Subterranean, dry-wood, and damp-wood termites are the three main categories of termites according to their environment (Tai *et al.*, 2015; Auer *et al.*, 2017). Globally distributed, termites are effective wood-degrading insects that play a critical role in the environment's carbon cycle and may serve as sources of biochemical catalysts (Brune, 2014, Scharf, 2015). In order to break down the lignocelluloses in their feeds, termites have evolved and modified to form symbiotic interactions with a variety of microorganisms in their intestines (Brune, 2014; Scharf, 2015). With a significant impact on soil's chemical and physical composition, plant deterioration, nitrogen and carbon cycles, and microbial activity, termites play an essential role in the ecosystems (Brune and Dietrich, 2015).

Despite being important species in ecosystems because they recycle a lot of nutrients, termites are also pests that have a significant negative economic
impact (Claybourne, 2013; Rossmasssler *et al.*, 2015; Bourguignon *et al.*, 2016; Devaraj and Kesti, 2019).
Termites eat a diet low in nitrogen sources, thus, the gut symbionts' ability to fix nitrogen is a crucial part of the termite symbiotic system. Additionally, the gut symbionts play a part in the breakdown of nitrogen waste products that are released, like uric acid, which is produced during termite metabolism (Ohkuma, 2003; Devaraj and Kesti, 2019).
The gut of termites, according to Schauer *et al.* (2012), Kohler *et al.* (2012), Lima *et al.* (2014), and Kumar *et al.* (2020), the midgut is a major site of microbial colonization due to the high concentration of small-chain fatty acids that are accessible there. Biotic and abiotic factors and the gut physico-chemical circumstances affect the activity and composition of termites' gut microbiota (Tokuda *et al.*, 2014; Brune and Dietrich, 2015). According to Ali *et al.* (2019), termites are known to dissimilate a significant amount of cellulose (74–99%) and hemicellulose (65-87%) from the lignocellulose they consume. They are also crucial for the turnover and mineralization of complex biopolymers, including wood and other materials that contain cellulose and hemicellulose (Akpan *et al.*, 2020). Various termites' guts have yielded several cellulolytic bacterial strains (Ramin *et al.*, 2008; Brune, 2014; Brune and Dietrich, 2015; Sharma *et al.*, 2015). Termites consume a variety of foods, although their primary dietary source is cellulose (Sharma *et al.*, 2015). Termites lack the enzymes necessary to break down cellulose and lignin, which provide them access to additional carbohydrates; thus, there is a need to investigate the guts of the termite species found at the University of Uyo in Akwa Ibom state, Nigeria. The analysis of GC-MS of the oil of termites is also the aim of this study.

MATERIALS AND METHODS

Study area

The University of Uyo Main Campus in Uyo, Akwa Ibom State, served as the research site. The University's Main Campus is 1,443 hectares and is located along Nwaniba Road. It is situated in the southern part of the country, between latitudes 5.0408ºN and 7.9198ºE (The University of Uyo, 2016; Akpan *et al.*, 2020).
Four (4) sampling sites were identified from the study area for the study. At the University of Uyo Main Campus, Nwaniba Road, Uyo, all of the sampling locations were situated next to the University of Uyo Water Factory and Mini Market. In these locations, termite mounds can grow up to 1.4 to 1.9 meters in height. Bushes having Elephant grass (*Pennistrum purpureum*), Lemon grass (*Cymbopogon citratus*), and Siam weed (*Chromolaena odorata*) bordered these areas.
Ten (10) termite mounds were excavated from the four sampling sites, and thirty (30) soldiers and workers termites were collected. After being stored in sterile plastic containers, the termite samples were taken to the Department of Animal and Environmental Laboratory to be identified using Constantino's (1999) identification guidelines.

Isolation of microorganisms from the termite gut

According to Devaraj and Kesti's (2019) description, the termite samples were processed. Sterile water and 95% ethanol were used to surface sterilize them. Each termite group's gut was removed with forceps and scissors and then submerged in 0.7% sterile saline water. After vortexing the tube containing the intestines and one millilitre of 0.9% saline, it was let to stand for twenty minutes. The supernatant was used as a microbiological inoculum once the gut tissue had calmed down.
According to the manufacturer's instructions, several media were manufactured to analyze, isolate, and characterize bacteria and fungi from the termite species' guts. Using the supernatant as a microbial inoculum, the entire 100 and 50µl inoculum—which included 0.5g peptone, 0.3g beef extract, 0.5g NaCl, 1.5g agar, and 1g carboxymethylcellulose—was added to Nutrient Agar medium. For 24 to 48 hours, the Petri plates were incubated at 37°C (Devaraj and Kesti, 2019). Potato dextrose agar was incubated at room temperature for three to five days. A list of the newly formed colonies was made.
In contrast to *Shigella* and *Salmonella* agar, which were sterilized by boiling them, MacConkey, Nutrient, Eosin Methylene Blue, and Potato Dextrose agar were autoclaved at 121°C for 15 minutes at 15 pounds per square inch.

Identification and characterization of microbial species

Morphological characteristics, Gram staining reactions for bacteria, and biochemical assays for both colonies were used to identify bacterial and fungal colonies, according to Peekate (2022), upon which Bergey's Manual of Determinative Bacteriology was used to confirm the identity of the bacterial isolates (Buchanan and Gibbons, 1994).
Furthermore, *Salmonella* and *Shigella* agar were used to measure the total heterotrophic fungi

count, while MacConkey agar was used to measure the total coliform count, Nutrient agar was used for the total heterotrophic bacterial count, and Eosin Methylene Blue agar was used for the faecal coliform count.
The following biochemical techniques were used to identify the isolates:

Gram staining reaction:

The purpose of this test was to distinguish between gram-positive and gram-negative microorganisms.
On a spotless, grease-free slide, a loopful of water was put. After being moved from the petri dish to a slide free of grease, a loopful of the test organism was spread out. Heat-fixing was used on the smear. After applying crystal violet dye and letting it sit for 30 to 60 seconds, the heat-fixed smear was rinsed with water and flooded with Lugol's Iodine, which was likewise left for 30 to 60 seconds before being rinsed with water. After being further saturated with 70% alcohol and left for ten to fifteen seconds, the slide was cleaned with water. Finally, the slide was immersed in Safrarin and left for 30 to 60 seconds before being drained and allowed to air dry. An ×100 objective was used to view the slide under a microscope.

Biochemical Tests:

Based on variations in the biochemical activity of the various bacteria isolates, biochemical tests were used to identify bacterial and fungi species, according to Peekate (2022). The different biochemical tests that were performed are listed as follows: the catalase test (Facklam and Elliott, 1995, Devaraj and Kesti, 2019), the Coagulase test (Holt *et al.*, 1994), the Indole test (MacFaddin, 2000), Oxidase examination (Win *et al.* 2006, Vashist et al., 2013), the citrate test, the Urease Test (Bailey and Scott, 1974), the Voges-Proskauer (V-P) test (Bachoon *et al.* 2008), the methyl red test (Crown and Gen, 1998).

Test for endospores

This test aimed to ascertain whether microorganisms could create a resilient structure that would allow them to endure for an extended amount of time in an adverse environment or situation. Using a sterile wire loop, the test organism was smeared onto a sterile, grease-free slide. After heat-fixing the smear, the slide was submerged in boiling water. On the slide was a paper towel that had been soaked in a malachite green solution. After 5 to 6 minutes of gentle heating, the Malachite green solution started to steam and was refilled as it evaporated. The slide was left to cool after using forceps to remove the paper towelling. After 30 seconds of water rinsing, the slide was counter-stained with Safrarin and left for another 30 to 60 seconds. After being cleaned with water, the Safranin was allowed to air dry before being examined under a microscope. Under a microscope, vegetative cells showed a green stain, whereas endospores showed a dark green stain.

Motility test

The purpose of this test was to evaluate an organism's mobility. After being distributed into test tubes, the motility test medium was autoclave sterilised for 15 minutes at 15 pounds and 12°C. Test tubes containing the medium were left upright to cool.
The medium was stabbed three-quarters of the way to the tube's bottom with a sterile inoculating needle to introduce the test organism. After 14 to 40 hours, the infected medium was tested after being incubated at 37°C. The diffused zone of development extending from the line of inoculation was a sign of motility. Motile organisms were defined as those that spread out from the inoculation line.

Test for the fermentation of carbohydrates

This test aimed to demonstrate the isolates' capacity to use various carbohydrates. In sterile glass test tubes, 1% methyl red indicator was added to sterile peptone water before the necessary amount of sugar (sterilised by Seitz filtering) was added.
After the test organism was added to the mix, sterile inverted Durham's tubes were placed into the media. After 48 hours of incubation at 37°C, they were checked for the generation of gas or acid. While the presence of air bubbles in Durham's tubes indicated gas production, a colour shift from red to yellow suggested acid production and a favourable outcome.

Fungal identification

The presence or absence of white and filamentous characteristics, foot cell vegetative structure, and conidiophore reproductive structure served as the basis for the fungi isolates' confirmatory tests.

Bacterial enumeration

The following culture media (Eosin Methylene Blue agar (EMB), Nutrient Agar (NA), and Mannitol Salt Agar (MSA)) were inoculated in triplicate and incubated at 37 °C for 24 hours in order to count and isolate bacteria using the pour plate method. Pure cultures were obtained by counting, characterising, and subculturing distinct bacterial colonies. The isolates were counted and the result recorded in CFU/ml (Matthew *et al.*, 2017).

Molecular analysis of bacterial isolates using PCR and 16s r-RNA Sequence Analysis

The isolated cultures of the detected bacteria were prepared and sent for molecular identification. A single band of high-molecular-weight DNA was seen when the culture's DNA was

separated and tested on a 1.2% Agarose gel. The Veriti® 96-well Thermal Cycler (Model No. 9902) was used to amplify the isolated DNA using the 16S rRNA universal primers 8F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) (Fuks *et. al.*, 2018). There was only one distinct 1500 bp PCR amplicon band visible. After enzymatic purification, the PCR amplicon underwent Sanger sequencing. 704F and 907R primers were used in a bi-directional DNA sequencing reaction of the PCR amplicon using the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyser. Using aligner software, forward and reverse sequence data produced a consensus sequence of 1500 bp for the cultures' 16S rDNA. The Basic Local Alignment Search Tool (BLAST) from the NCBI website was utilized to do a BLAST alignment search on the 16S rDNA sequence of the National Centre for Biotechnology Information (NCBI) database. (Devaraj and Kesti, 2019; Ali *et al.*, 2019; Amadi *et al.*, 2024).

GC-MS analysis of the oil extract from termites

The oil used in the GC-MS was taken from the *Odontotermes* sp. termite queen. The components were separated using helium as a carrier gas at a steady flow rate of 1 millilitre per minute, and the GC-MS utilized in the research had a fused silica column loaded with Elite-1. Two microlitres of the termite queen's oil were employed for GC-MS analysis (Hervé *et al.*, 2020). After being injected into the device, the sample extracts were found. The oven was kept at 290 °C for two minutes during the twenty-third minute of the GC extraction procedure. With pieces ranging from 40 to 440 Da and a scanning interval of 0.5 s, mass spectra were acquired at 70 eV. The relative percentage amount of each component was determined by comparing each component's average peak area to the total areas (Hema *et al.*, 2010).

GC-MS identification from the termite queen oil's

The National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns, was used to interpret mass-spectrum GC-MS. The NIST library's collection of known components was compared to the spectrum of the unknown components. The test materials' percentage area, molecular weight, compound name, and retention duration were determined (Hema *et al.*, 2010).

RESULTS

This study identified two termite species; *Odontotermes* sp and *Macrotermes* sp. Microorganisms isolated from the *Odontotermes* sp. and *Macrotermes* sp. were fungi and bacteria. *Bacillus*, *Salmonella*, *Escherichia*, and *Shigella* were the bacteria that were identified and described from *Odontotermes* sp (Table 1). According to the findings presented in Table 2, *Bacillus* sp. *Termitomyces* sp. and *Chaetomium* sp. were the bacterial and fungal isolates separated from the gut of *Macrotermes* sp.

Table 1: Microorganisms isolated and characterised from *Odontotermes* sp.

Sample	Bacterial Isolates	Fungal Isolates
Old Worker	*Bacillus* sp., *Salmonella* sp., *Shigella* sp., *Ecscherichia coli.*	*Fusarium* sp, *Termitomyces* sp., *Chaetomium* sp
Young Worker	*Bacillus* sp.	*Termitomyces* sp, *Chaetomium* sp
Soldier	-	-
Queen	*Bacillus* sp.	*Termitomyces* sp
King	*Bacillus* sp.	*Termitomyces* sp

Table 2: Microbiomes isolated and characterized from the gut of *Macrotermes sp*.

Sample	Bacterial Isolates	Fungal Isolates
Worker	*Bacillus* sp.	*Termitomyces sp*, *Chaetomium* sp
Young Mandibulate Soldier	*Bacillus* sp.	*Termitomyces* sp
Mandibulate Soldier	*Bacillus* sp.	*Termitomyces* sp,
Queen	*Bacillus* sp.	*Termitomyces* sp,

Morphological and biochemical characterization of the Bacterial isolates

As seen in Table 3, most of the isolates were rod-shaped gram-negative bacteria. The majority of the isolates had negative reactions to the tests for urease, citrate, oxidase, spore, indole, and coagulase (Table 3).

Table 3: ***Morphological, biochemical characterisation of the Bacterial isolates***

	TESTS													
Gram/ Motility	**CAT**	**CHO**	**COA**	**IND**	**OXI**	**CIT**	**URE**	**UREA**	**MR**	**SPO**	**GLU**	**LAC**	**MAN**	**SB**
+R	+	-	-	+	+	-	+	-	+	+	AG	AG	AG	*Bacillus* spp
-R	+	-	-	-	-	-	-	+	-	+	AG	-	AG	*Salmonella* spp
-R	+	-	-	-	-	-	-	+	-	+	AG	-	AG	*Shigella* spp
-R	+	-	-	-	-	-	-	+	-	+	AG	AG	AG	*Escherichia coli.*

CAT = Catalase, CHO = Carbohydrate, COA = Coagulase, IND = Indole, CIT = Citrate, OXI = Oxidase, URE = Urease, MR = Methyl Red, SPO = Spore, GLU = Glucose, LAC = Latose, MAN = Mannitol R = Rod, + = Positive, - = Negative, A = Acid, AG = Acid and Gas
SB = Suspected Bacteria

Mean count of organisms isolated from *Odontotermes* sp. and *Macrotermes* sp.
The total bacteria count in *Odontotermes* sp gut was 85 cfu/ml, and the highest bacteria count 76 cfu/ml, was in the Old worker (Od./Ma.) gut. The results for the mean count of the other isolated microbiomes from the gut of the termite species are presented in Table 4.

Table 4: Total Mean Count of Isolates from *Odontotermes* sp.and *Macrotermes* sp gut

Sample	**TMHBC (cfu/ml)**		**TMCC (cfu/ml)**		**TMFCC (cfu/ml)**		**TMSSC (cfu/ml)**		**TMHFC (cfu/ml)**	
	Od.	*Ma.*	*Od.*	*Ma.*	*Od.*	*Ma.*	*Od.*	*Ma*	*Od.*	*Ma.*
Old Worker (Od./Ma.)	76	74	40	39	24	26	21	50	6	4
Young Worker (Od./Ma.)	1	1	-	-	-	-	-	-	1	1
Soldier (Od./Ma.)	-	-	-	-	-	-	-	-	-	-
Queen (Od./Ma)	1	2	-	-	-	-	-	-	1	1
King (Od./Ma)	7	5	-	-	-	-	-	-	-	-
Total	85	82	40	39	24	26	21	50	8	6

Keywords: TMHBC = Total Mean Heterotrophic Bacterial Count, TMCC = Total Mean Coliform Count, TMFCC = Total Mean Fecal Coliform Count, TMSSC = Total Mean Salmonella and Shigella Count, TMHFC = Total Mean Heterotrophic Fungal Count. Od. = *Odontotermes* sp, Ma. = *Macrotermes* sp

Blast analysis of 16SrRNA of Bacterial Isolates
The Blast data analysis results revealed 99% identification similarity to the bacterial isolates: *Bacillus* sp, *Shigella* sp, *Escherichia coli,* and *Salmonella* sp. (Table 5).

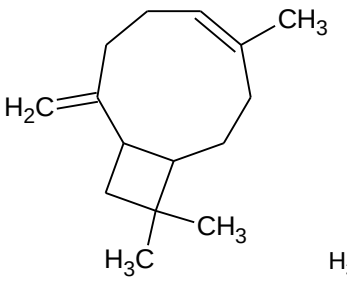
GC-MS analysis of the Termite Queen Oil
Thirty-one (31) significant chemical constituents were found in the Termite queen oil (*Odontotermes* sp.), according to the results of the GC-MS study (Table 6). Thymol (23.31 percent), gamma-terpenene (17.31 percent), p-cymene (16.40 percent), phenol-2-methyl-5-(1-methyl ethyl)- (7.19%), luteol (5.94 percent), terpinen-4-ol (3.19 percent), and caryophyllene oxide (2.47 percent) were the most prevalent constituents (Table 6).

Table 5: Blast analysis of 16SrRNA of the bacterial and fungal isolates from the two termite species gut used in this study

Scientific name	Max. Score	Total score	Query cover	E value	Accession number	Percentage identify	Reference
Bacillus sp	2966	2966	100%	0.0	FM180506.1	98	Blast 2 squences (Zhang et al., 2000)
Shigella sp	2782	2782	100%	0.0	JF833739.1	99	Blast 2 squences (Zhang et al., 2000)
Escheriachia coli	2527	2527	100%	0.0	HM209775.1	99	Blast 2 squences (Zhang et al., 2000)
Salmonella sp	2480	2480	75%	0.0	MR074910	99	Blast 2 squences (Zhang et al., 2000)

Table 6: Chemical composition of the Termite Queen Oil by GC-MS analysis

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	5.501	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methyl ethyl)-	$C_{10}H_{16}$	136	4.04
2	5.607	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	136	1.10
3	6.078	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methyl ethyl)-	$C_{10}H_{16}$	136	0.80
4	6.144	beta.-Pinene	$C_{10}H_{16}$	136	0.43
5	6.285	beta.-Myrcene	$C_{10}H_{16}$	136	2.12
6	6.495	alpha.-Phellandrene	$C_{10}H_{16}$	136	0.31
7	6.655	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	136	2.40
8	6.696	p-Cymene	$C_{10}H_{14}$	134	16.40
9	6.818	D-Limonene	$C_{10}H_{16}$	136	1.35
10	7.186	gamma.-Terpinene	$C_{10}H_{16}$	136	17.31
11	7.263	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl ethyl)-	$C_{10}H_{18}O$	154	1.20
12	7.660	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha.)-	$C_{10}H_{18}O$	154	0.49
13	8.489	Isoborneol	$C_{10}H_{18}O$	154	0.89
14	8.637	Terpinen-4-ol	$C_{10}H_{18}O$	154	3.16
15	9.867	Thymol	$C_{10}H_{14}O$	150	23.31
16	10.063	Phenol, 2-methyl-5-(1-methyl ethyl)-	$C_{10}H_{14}O$	150	7.22
17	11.030	alfa.-Copaene	$C_{10}H_{14}O$	204	0.45
18	11.139	gamma.-Muurolene	$C_{15}H_{24}$	204	0.26
19	11.476	Caryophyllene	$C_{15}H_{24}$	204	2.32
20	11.805	1,6-Cyclodecadiene,	$C_{15}H_{24}$	204	0.40
21	12.068	Humulene	$C_{15}H_{24}$	204	0.15
22	12.117	1H-Cycloprop[e]azulene	$C_{15}H_{24}$	204	2.78
23	12.206	Naphthalene	$C_{15}H_{24}$	204	0.95
24	12.269	Caryophyllene	$C_{15}H_{24}$	204	0.17
25	12.414	Selina-3,7(11)-diene $$ Naphthalene	$C_{15}H_{24}$	204	0.45
26	12.972	Caryophyllene oxide	$C_{15}H_{24}O$	220	2.47
27	16.325	1,2,5,5,8a-Pentamethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol	$C_{15}H_{26}O$	222	0.62
28	16.516	2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol	$C_{14}H_{24}O$	208	0.21
29	16.617	3-Adamantan-1-yl-butan-2-one	$C_{14}H_{22}O$	206	0.33
30	19.437	beta.-Sitosterol	$C_{29}H_{50}O$	414	1.23
31	21.220	Lupeol	$C_{30}H_{50}O$	426	5.94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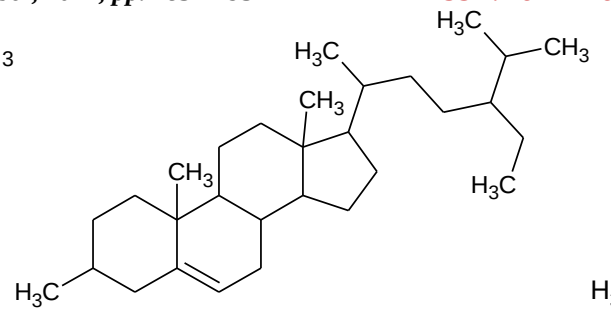
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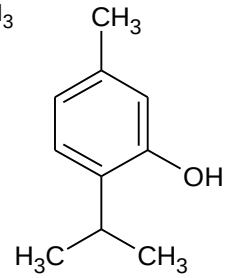
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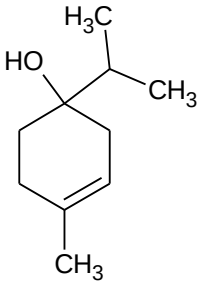
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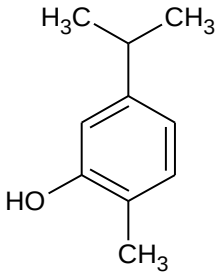
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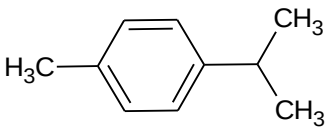
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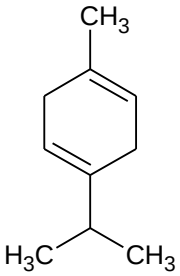
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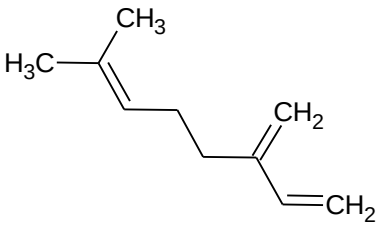
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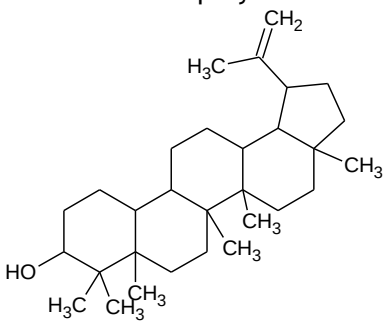
Lupeol

Figure 1: Structures of some abundant chemical constituents in the GC-MS Analysis of the termite Queen Oil.

DISCUSSION

The results of the gut analysis of the termite species, *Odontotermes* sp, and *Macrotermes* sp, identified in this study, have revealed that bacteria Bacillus sp, Salmonella sp, Shigella sp., Escherichia coli, and fungi; Fusarium sp, Termitomyces sp, and Chaetomium sp, inhabit their guts.. According to Kumar *et al.* (2020), the termite species rely mostly on the bacterial and fungi communities to break down the cellulose-based dietary items. Majeed *et al.* (2012), Brune and Dietrich (2015), and Tai *et al.* (2015) reported that the eating habitat and habits of the termite species influenced the richness of the microorganism community.

In line with Costa *et al.* (2019) and Kumar *et al.* (2020), who discovered that *Odontotermes* sp gut harboured both aerobic (*Bacillus*) and anaerobic (*Escherichia* coli) bacteria, the previous worker of *Odontotermes* sp stomach harboured *Salmonella* sp, *Shigella* sp, *Bacillus* sp, and *Escherichia col*i. Although Costa *et al.* (2019) stated that the gut of *Macrotermete*s sp may only harbour coccoid lactic acid bacteria, every group of *Macrotermetes* sp in this study harboured exclusively aerobic bacteria, specifically Bacillus sp. The results on the bacterial isolates iedentified in the termite species revealed that all the categories of the two termite species, except for soldier s of *Odontotermes* sp have *Bacillus* sp. in their guts, which according to Zhou *et al.* (2018) and Adebajo *et al.* (2021) they are involved in the breaking down of lignocelluloses and it preserves the gut environment The predominant of *Bacillus* sp in *Odontotermes* sp and *Macrotermes* sp agreed with Wenzel *et al.*, (2002) are also involved in biphenyl degradation in termite gut (Bugg *et al.*, 2010) nd there is a possibility that the microorganisms identified in this study increase the nitrogen supply by recycling termite uric acid wastes (Brune, 2006; König, 2006). However, because of their relative abundance in the termite gut system and being isolated from termite gut, *Bacillus sp* by their function in the degradation of lignocellulose, may be regarded as mutualists (Matthew *et al.*, 2012).

Fungal species from the Agaricomycetes and

Sordariomycetes families exhibit symbiotic interactions in the stomachs of all termite species (Brune, 2014; Auer *et al.*, 2017). The most common fungus species found in all the termite groupings in this study was *Termitomyces* sp. This result was consistent with Zhou *et al.* (2018), who found that *Termitomyces* sp. is the predominant fungus in the gut of termite species. Termites' guts include a variety of fungi that aid in the digestion of cellulose, hemicelluloses, lignin, and lactase (Kumar *et al.*, 2020).

The results of the microorganisms isolated from the gut of the two termite species in this study comparatively revealed that the old workers of the colony of *Odontotermes* sp habour: *Bacillus*, *Salmonella* sp, *Shigella* sp., and *Echerichia coli* as bacterial isolates and *Fuscarium* sp, *Termitomyces* sp and *Chaetomium* sp, while other termite categories in the *Odontotermes* and *Macrotermes* colonies haboured only *Bacillus* sp for bacterial group and *Termitomyces* for fungal group. The old workers within the colony are primarily responsible for forging for food and they take over food acquisition role (Gordon, 2016). The presence of the classes of the species of the bacterial and fungi isolates in the gut of the old workers may further lead to an increase in per-capita food consumption of the termite colony because the isolated microorganisms enhance the old workers to take-in more cellulose materials for the growth of the colony. The young and old workers continuously bring in partly degraded plant materials substrates to their colony, and these substrates are fed upon by other categories in the colony (Um *et al.*, 2013).

Gas chromatography-mass spectrometry has recently been used as a very successful technique for identifying the chemical elements found in both plant and animal extract (Balamurugan *et al.*, 2012). Gamma-terpinene one of the chemicals found in the termite queen oil may have analgesic, anti-inflammatory, antioxidant, and antibacterial properties (Devi and Muthu, 2015). Terpinen-4-ol has been used as a flavouring agent, having anti-inflammatory and anti-cancer properties (Sarkar and Sawardekar, 2022). The terpinenes reported from the GC-MS extraction of the oil of the termite queen are three isomeric hydrocarbons that are classified as terpenes. Gamma-terpinene as one reported in this study, is natural and has been reported by several other researchers Forti and Ingold (2003) and Hamdan *et al.* (2013), is also isolated from a variety of plant and animal sources. It is a major component of essential oils made from Citrus Fruits and has strong antioxidant activity. It has a lemon odour and widely used in food, flavours, soaps, cosmetics, pharmaceutical, tabacco, confectionery and perfume industries (Hamdan *et al.*, 2013). Nevertheless, the essential oils reported from the GC-MS results of the termite queen oil strongly indicate that the essential oil from termite could serve several important health benefits to humans.

CONCLUSION

Termites' gut is a repository for diverse microorganism community composition. This study has firmly demonstrated that *Odontotermes* sp, and *Macrotermes* sp detected in University of Uyo harbour bacteria; *Bacillus* sp, *Salmonella* sp, *Shigella* sp., *Escherichia coli*, and fungi; *Fusarium* sp, *Termitomyces* sp, and *Chaetomium* sp. that are of major value to the food function of the termite species. The GC-MS of the oil of the queen of the termite species demonstrated how rich the oil of the queen of termite is, composed of various chemical elements that are of great helpful to man. Further study is proposed on the GC-MS of the oil of the termite queen. It is strongly advised that more investigations which could lead to the uncovering of novel species of microbiomes beyond bacteria and fungal groups, should be done on the gut of termites.

Conflict of interest: None.

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