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GC-MS Analysis of Cuticular Hydrocarbon Profiling on *Anopheles Gambiae* Mosquitoes Exposed to Melon Leaf Extract

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Abstract

Anopheles mosquitoes are vector of malaria, a deadly disease that affects millions of people worldwide. Developing effective and sustainable methods for mosquito control is crucial in malaria prevention efforts. This study investigates the potential of melon leaf extract as natural alternative for disrupting the cuticular hydrocarbon (CHCs) profile of mosquitoes, which may play a vital role in the behavior of mosquitoes including desiccation resistance, communication, and species recognition. Altering these hydrocarbon can affect mosquito survivability and vector competence, making it a significant area of research in vector control. Melon leaf extract, known for its diverse bioactive compounds, was hypothesized to modify CHCs profile of anopheles mosquitoes. Adult's mosquitoes were exposed to sub lethal dose under controlled conditions, and their cuticles were subsequently analyzed using GC-MS. The results of these study demonstrated that exposure to melon leaf extract significantly altered the CHCs profile of the mosquitoes. Notable changes were observed in the abundance and presence of specific hydrocarbon chains, including alkanes, alkenes and methyl-branched hydrocarbons. The control group of this study revealed a total number of 78 compounds in mosquitoes reared in 100 % distilled water while 97 compounds were en-countered in mosquitoes reared 1 ml methanol solvent + 99 ml of distilled water respectively. The mosquitoes reared in sub lethal concentration of methanol melon leaf extract revealed 20 com-pounds. These alteration suggest potential in the mosquito physiological processes, which could influence their ability to thrive and transmit diseases.

Introduction

Mosquito-borne diseases continue to pose a significant challenge to public health worldwide, with malaria remaining one of the most lethal infectious diseases, particularly in tropical and subtropical regions. Mosquitoes belonging to the genus Anopheles serve as the principal vectors of malaria parasites and are responsible for

transmitting the disease to millions of people annually (WHO, 2022). Although considerable progress has been achieved in reducing mosquito populations and controlling malaria transmission, the increasing occurrence of insecticide resistance, coupled with environmental concerns arising from the extensive use of synthetic insecticides, has highlighted the need for more sustainable and environmentally friendly vector control strategies (Wang et al., 2021).

The insect cuticle is covered by an outer protective layer known as the epicuticle, which contains a complex mixture of lipid compounds. These lipids include both branched and straight-chain hydrocarbons, saturated and unsaturated hydrocarbons, free fatty acids, sterols, and aldehydes (Blomquist et al., 1987). Hydrocarbons are not restricted to the cuticular surface; they are also found within the inner layers of the cuticle, epidermal tissues, fat bodies, various internal organs, and are particularly abundant in lipophorin, the major lipid-transport protein present in the haemolymph (Blomquist et al., 2010). The synthesis of these hydrocarbons occurs primarily in specialized cells called oenocytes. Following synthesis, they are transported through the haemolymph by high-density lipoproteins and subsequently deposited onto the cuticle as cuticular hydrocarbons through specialized pore canals (Haruhito & Haruo, 1982; Schal et al., 2001; Holze et al., 2021).

The search for environmentally sustainable mosquito control measures has increasingly focused on plant-derived products, which are rich sources of bioactive compounds with insecticidal and repel-lent activities. One plant of interest is *Cucumeropsis mannii* (melon), a widely cultivated crop recognized for its nutritional, medicinal, and therapeutic benefits. Beyond its traditional uses, melon has shown potential as a natural insecticidal agent against mosquitoes. Melon leaves, in particular, are believed to possess antifeedant properties, as they are generally avoided by grazing ruminants. In many communities, the leaves have also been employed in traditional medicine and local pest management practices because of their insect repellent effects (Okwundu et al., 2021). Despite these promising attributes, scientific investigations into the mosquito control potential of melon leaf extracts remain relatively limited.

Cuticular hydrocarbons (CHCs) are important lipid constituents located on the surface of the in-sect cuticle. They play critical roles in maintaining water balance, facilitating chemical communication, enabling mate recognition, and protecting insects from environmental stress. Changes in the composition or abundance of these hydrocarbons can affect insect behaviour, physiology, and eco-logical interactions. Although numerous studies have highlighted the insecticidal potential of plant extracts, little information is available regarding their influence on the cuticular hydrocarbon pro-files of mosquitoes. Therefore, this study seeks to examine the effects of melon leaf extract on the cuticular hydrocarbons of Anopheles mosquitoes using Gas Chromatography–Mass Spectrometry (GC–MS). The findings are expected to enhance understanding of plant-based mosquito control mechanisms and support the development of sustainable and environmentally friendly approaches for reducing the transmission of mosquito-borne diseases (Holze et al., 2021).

Materials and methods

Study Area

The mosquitoes were collected from their breeding habitat in Bosso dam and the study was carried out in Minna, the capital of Niger State, Nigeria. Minna is located within Longitude 6° 33'E and Latitude 9° 37'N, covering a land area of 88km2 with an estimated human population of 1.2 million (The Nigeria Congress, 2007). The area has a tropical climate with mean annual temperature, relative humidity and rainfall of 30.2°C, 61% and 1334mm, respectively. The climate presents two distinct seasons; a rainy season between April and October, with highest mean monthly rain-fall in September, and a dry

season (November-March) completely devoid of rains. Its vegetation is typically grassing dominated savannah with scattered short trees. Minna has three main ethic groups namely Gwari's, Nupe's and Hausa's; and their major occupation is farming (Olayemi & Ande, 2009).

Collection and processing of plant material

Fresh plant of *Cucummeros manni* (melon leave) were collected from the field in Bosso area of Niger State, and authenticated by a Senior Botanist in the Department of plant Biology, FUT, Minna. The fresh leaves were washed clean with running water and shade dried at room temperature to avoid environmental factor like sun for 14 days. Thereafter, pulverized using a blender (Aina et al., 2021).

Preparation of plant extracts

The dried pulverised plant was extracted using Soxhlet's apparatus and solvents used was methanol. Each of the milled plant, 50g was weighted with electric weighing balance and wrapped in a filter paper using a stapler to hold it. The weighed powdered plant material was then placed in the extracting flask of Soxhlet's apparatus, after which 250 mL of methanol was added to the plant material in a round bottom flask of the Soxhlet set up and set at 60 ˚C, after which the crude ex-tracts were preserved in the refrigerator at 4 ˚C until needed for use (Aina et al., 2021). Following the clinical examination, the method described in (World Health Organisation, 1991) was adopted for collecting blood samples. Five millilitres (5 mL) of blood sample were collected through the cephalic vein using a 5 mL disposable syringe and a 23-gauge needle into a sample vial containing 1mg ethylene di-amine tetra acetate-K (EDTA-K) as an anticoagulant. The samples were refrigerated before being analyzed later that day.

Collection of Anopheles mosquito

Early first instar larva anopheles mosquito were collected from rice farm water bodies in Bosso area using a scoop and a bowl. After collection, the mosquito were brought to the Laboratory of the Department of Animal Biology, Federal University of Technology Minna. The mosquitoes were transferred to a one litre capacity bowl with 500 ml of distilled water.

Preparation of stock and working solutions

Analytical grade of methanol was used and 1g of the extracts was weighed with an electric weighing balance and 10ml of the solvent (methanol) was added to it. To prepare the stock solution, the extract was dissolved in the solvent that was used in extracting it, and the working solution was prepared by adding 90 mL of distilled water to 10 mL of the stock solution. A positive control containing (distilled water +1 ml solvents) and Negative control containing 100 % dis-tilled water; Subsequently, test concentrations was 0.1 mg/L obtained from the working solution (Aina et al., 2021).

Mosquito Exposure to Plant Extract, Extraction and Analysis of Cuticular hydrocarbon

The mosquito larvae obtained from the wild were exposed to plant extract and fed with fish feed. The feeding and changing of their rearing continued until the larvae develops into the 4th instars (L4) larva stage, pupal stage and adult emergence which was further used for cuticular hydrocarbon profiling using GC-SM analysis. This is in line with the techniques of (Ukubuiwe et al., 2016).

Hydrocarbon was extracted with high-performance liquid chromatography grade hexane. All glassware was pre-rinsed three times with hexane to remove possible contaminants and allowed to dry in a fume hood for 5–10 min before beginning extractions. Between each extraction, forceps were rinsed in three separate containers of hexane to avoid cross-

contamination of samples. Each mosquito was wiped with a wet Kimwipe® to remove any external debris and then submerged in 1 ml hexane for 5 min in a glass vial with a Teflon-lined cap. Extracts were concentrated to ~200 µl under a stream of nitrogen, and 1 µl aliquots were analyzed by GC-MS with an Agilent 6890 GC interfaced to an Agilent 5973 mass selective detector operated in electron impact ionization mode (70 eV). The GC was fitted with a 30 m×0.25 mm I.D. DB-5MS column (J &W Scientific, Folsom, CA, USA), programmed from 100°C for 1 min, 10°C/min to 280°C and held for 20 min. Helium was the carrier gas. The injector and transfer line temperature were 280°C, the ion source temperature was 250°C, and the quadrupole temperature 200°C. Injections were made in splitless mode, with purge on at 0.5 min. Straight-chain saturated alkanes were identified by their molecular ions and from comparisons of retention times and mass spectra with those of authentic standards. Methyl branched compounds were identified from their Kovat's retention indices relative to straight chain hydrocarbons, in combination with diagnostic ions from enhanced fragmentations at methyl branch points (Nelson, 1993; Nelson & Blomquist, 1995; Carlson et al., 1998) . An analysis of quantitative variation in peak volumes was then done by principal component analyses and cluster analyses. All the multivariate analysis were performed with the "BlackBox package" for data analysis in Aoki (Carlson et al., 1998).

Phytochemical screening of melon leaves

Qualitative and quantitative phytochemical screenings of the crude extracts were carried out using the standard procedure described by (Harborne, 1998; Trease & Evans, 1989).

Results and Discussions

Results

Qualitative and Quantitative Analysis of Methanol Leaf Extract of Melon

All metabolite components were present in the methanolic extracts, flavonoids, tannins, alkaloids phenol, terpenoids and saponins (table1).

Table 1: Qualitative Phytochemical Constituents of the Plant Extracts of Methanol Melon Leaf Extracts

Metabolite	leaf extracts
Phenol	+
Flavonoid	+
Tannins	+
Alkaloids	+
Saponins	+
Steroids	+
Terpenoids	+
Glycoside	+

Table 2 shows the quantitative constituents present in melon leaf. Phenol had the highest content of 958 while alkaloids had the lowest content.

Table 2: Quantitative Phytochemical Constituents of melon leaves Plant Extracts

Phytochemicals	Methanol
Flavonoid	51.28 ± 0.02^{a}
Phenol	958.69 ± 0.30^{b}
Tannins	113.08 ± 0.70^{d}
Alkaloids	33.28 ± 0.01^{f}
Saponins	223.71 ± 0.13^{h}

Table 3, shows the mosquitoes reared or exposed to 100% distilled water (negative control). This revealed the presence of 78 hydrocarbons. This profiling provides insight into the cuticular composition of the mosquitoes. The compound contained carbon chain ranging from C2- C38. They consist of alkanes, alkenes, alkynes, acid, methyl groups and esters. The most abundant compounds were 2-Pentanone, 4-hydroxy-4-methyl (24.48%), 7-Octylidenebicyclo [4.1.0] heptane (15.99%), Dibutyl phthalate (12.59%) and Bis (2-ethylhexyl) phthalate (5.259%).

Table 3: Cuticular Hydrocarbon Profiling of Anopheles Mosquitoes (Negative Control} using GC-MS Analysis

Pk	RT	Area Pct%	Library/ID	MW	MF	Qual
1	5.1472	24.4751	2-Pentanone, 4-hydroxy-4-methyl-	116	C6H12O2	64
2	5.4652	1.6988	Dimethyl Sulfoxide	78	C2H60S	87
3	5.6358	0.3297	Dimethyl Sulfoxide	78	C2H60S	87
4	8.031	0.2868	Decane, 2-methyl-	156	C11H24	49
5	8.1131	0.7916	Pentanoic acid, 2-hydroxy-4-methyl-, methyl ester	146	C8H16O3	23
6	8.9396	0.4847	1,3-Propanediamine, N-methyl-	88	C4H12N2	43
7	8.9918	0.4042	N-Methylallylamine	85	C5H11N	49
8	9.8612	0.6072	Piperidine, 1,2-dimethyl-	113	C7H15N	50
9	10.8259	0.1574	Octane, 3,3-dimethyl-	142	C10H22	35
10	10.9452	0.2611	5-. alpha. -Aminoethyltetrazole	128	C3H7N5	43
11	10.9614	0.4121	Cyanoacetylurea	142	C4H5N3O2	47
12	11.1918	0.1473	1-Undecene, 4-methyl-	168	C12H24	38
13	11.4653	0.5756	n-Tridecan-1-ol	200	C13H28O	50
14	11.552	0.5616	Octane, 4-methyl-	114	C9H20	43
15	11.6434	0.6842	Hexane, 2,3,4-trimethyl-	128	C9H20	43
16	12.4201	0.2271	Cyclododecane	168	C12H24	89
17	12.5122	0.2141	Decane, 2,3,6-trimethyl-	184	C13H28	47
18	13.6572	0.0401	Tenamfetamine	163	C10H13NO2	46
19	14.302	0.3126	2-Propenamide	71	C3H5N0	46
20	14.4303	0.2693	2-Butenediamide, 2-methyl-, (Z)-	114	C5H10N20	53
21	14.5126	0.5256	Dodecane	170	C12H26	58
22	14.6621	0.4145	Ether, 6-methylheptyl vinyl	184	C11H220	46
23	14.7962	0.2624	1,3-Propanediamine, N-methyl-	88	C4H12N2	59
24	15.1594	0.2686	Trichloroacetic acid, 2-tetradecyl ester	375	C16H29C13O2	53
25	15.2392	0.2015	Tridecane	184	C13H28	52
26	15.3352	0.2569	Diethyl Phthalate	194	C10H1004	91
27	15.9877	0.272	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	212	C12H2003	70
28	16.1598	0.6406	7-(1,3-Dimethylbuta-1,3-dienyl)-	80	C14H24	12
29	16.4473	0.1118	Hexadecane, 7,9-dimethyl-	254	C18H38	46
30	16.7488	0.4346	6-Tridecene, 7-methyl-	196	C14H28	58
31	16.8363	0.2874	Octacosyl trifluoroacetate	508	C30H59F302	49
32	16.8768	0.4692	Tridecane, 7-propyl-	184	C16H34	83
33	16.9138	0.2841	1-Dodecanol, 2-octyl-	186	C20H42O	58
34	16.9642	0.2684	2-Propenamide	71	C3H5N0	52
35	17.0183	0.8375	Octacosyl trifluoroacetate	508	C30H59F302	30
36	17.0981	0.4834	1-Decanol, 2-hexyl-	158	C16H34O	53
37	17.2397	0.6897	9-Octadecene, (E)-	252	C18H36	96
38	17.3225	0.471	Cyclohexane, 1,2,4-trimethyl-	84	C9H18	60
39	17.9454	0.5802	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	248	C18H26O	98

40	18.0012	0.3715	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	472	C20H26O4	83
41	18.3505	0.2199	Hentriacontane	436	C31H64	74
42	18.3865	0.397	Tridecanoic acid, methyl ester	228	C14H2802	50
43	18.4583	0.2301	1,3-Propanediamine, N-methyl-	88	C4H12N2	64
44	18.5931	0.2401	Octatriacontyl pentafluoropropionate	656	C38H77F502	41
45	18.6802	0.544	Dodecane, 2,6,11-trimethyl-	212	C15H32	70
46	18.7132	0.1977	2-Butenediamide, 2-methyl-, (Z)-	114	C5H10N20	46
47	18.7623	12.5936	Dibutyl phthalate	278	C16H22O4	95
48	18.8715	1.0087	Dodecane, 2,6,11-trimethyl-	212	C15H32	64
49	18.9144	0.4962	Eicosane	228	C20H24	60
50	18.9642	0.5139	Carbonic acid, decyl tridecyl ester	440	C26H52O3	68
51	19.0307	0.5507	Carbonic acid, octadecyl vinyl ester	382	C22H44O3	49
52	19.1355	0.721	Octadecane, 1-iodo-	380	C18H37I	51
53	19.2237	0.3571	Hexadecane, 2,6,10,14-tetramethyl-	286	C22H46	42
54	19.372	0.2482	l-Alanine, N-(3-methyl-1-oxobutyl)-, methyl ester	189	C9H17NO3	35
55	19.6836	0.4515	cis-Vaccenic acid	282	C18H34O2	50
56	19.7424	15.9852	7-Octylidenebicyclo[4.1.0]heptane	207	C15H26	49
57	19.8674	5.2585	Bis(2-ethylhexyl) phthalate	386	C24H38O4	86
58	19.9419	1.8424	Disulfide, di-tert-dodecyl	402	C24H5OS2	90
59	20.0156	0.8153	2-Methoxy-N-methylethylamine	89	C9H11N0	43
60	20.0595	0.4158	Heptadecane, 2-methyl-	240	C18H38	44
61	20.1165	1.0435	1,3-Propanediamine, N-methyl-	88	C4H12N2	46
62	20.1736	0.8495	Octadecane, 1-iodo-	380	C18H37I	59
63	20.1933	1.1202	Eicosane	228	C20H24	55
64	20.2716	2.2814	Eicosane	228	C20H24	70
65	20.3248	1.7517	Pentadecane	212	C15H32	60
66	20.3658	1.0259	Ethanol, 2-(octadecyloxy)-	315	C20H42O2	70
67	20.4032	0.9297	Heptadecane, 2,6,10,15-tetramethyl-	300	C23H48	86
68	20.4322	1.2207	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	268	C12H9F3N2O	56
69	20.5048	1.3384	Docosane, 9-octyl-	423	C26H54	70
70	20.5779	1.6046	Dodecane, 2,6,11-trimethyl-	212	C15H32	52
71	20.6336	0.8672	Octadecane, 2-methyl-	269	C19H40	50
72	20.7315	0.7245	Phenylephrine	167	C9H13N02	38
73	20.8101	0.7342	Docosane	310	C22H46	45
74	21.1023	0.0775	Metaraminol	169	C9H13NO2	49
75	21.1527	0.0222	2-Amino-1-(o-methoxyphenyl)propane	151	C10H15N0	38
76	21.1816	0.0456	Atomoxetine	255	C17H21N0	45
77	21.2003	0.039	Propanamide, 3,3,3-trifluoro-2-(trifluoromethyl)-	235	C5H4F6N0	38
78	21.2815	0.2461	Propan-1-one, 2-amino-1-piperidin-1-yl-	158	C9H16N2O	35

Furthermore in (table 4) The cuticular hydrocarbons of methanol positive control reared in 99ml of distilled water and 1 ml of methanol solvent , the mosquitoes contains total number 97 compounds and consists of alkadienes, alkenes, n-alkanes, and methyl

alkanes groups and ester ranging from C to C_{21}. The most abundant compounds were Dodecanoic acid, methyl ester (6.867%), Hexadecanoic acid, 14-methyl-, methyl ester (5.559%), 2-Pentanamine (3.9142%) and Propanamide (2.9194%).

Table 4: Cuticular Hydrocarbon Profiling of *Anopheles* Mosquitoes (Methanol Positive Control) using GC-MS Analysis

PK	RT	Area Pct%	Library/ID	MW	MF	Qual
1	8.0472	0.7086	Benzeneethanamine	121	$C_8H_{11}N$	64
2	8.0654	0.9672	Amphetamine	135	$C_{19}H_{13}N$	59
3	8.1376	0.7409	Actinobolin	496	$C_{21}H_{36}0_4$	64
4	9.1872	0.6217	Cyclobutanol	74	C_4H_80	40
5	10.5569	1.1393	1-Methyldecylamine	171	$C_{11}H_{25}N$	59
6	10.5918	1.2205	dl-Phenylephrine	274	$C_9H_{13}N0_2$	59
7	10.6171	0.5861	dl-3-Aminoisobutyric acid, N-methyl-, methyl ester	195	$C_6H_{13}N0_2$	64
8	10.6544	1.5187	Actinobolin	496	$C_{21}H_{36}0_4$	64
9	10.6788	1.3362	R-(-)-Cyclohexylethylamine	127	$C_8H_{17}N$	64
10	10.7397	1.7373	R-(-)-Cyclohexylethylamine	127	$C_8H_{17}N$	64
11	10.7602	2.1112	2-Heptanamine, 5-methyl-	115	$C_8H_{19}N$	72
12	10.7992	1.6534	Benzenemethanol, .alpha.-[(methylamino)methyl]-	104	$C_8H_{11}N0$	59
13	10.8291	1.1116	2-Butanamine, (S)-	73	$C_4H_{11}N$	64
14	10.8528	1.5134	dl-Alanyl-dl-leucine	309	$C_{11}H_{22}N_20_4$	64
15	10.8945	1.7079	R-(-)-Cyclohexylethylamine	127	$C_8H_{17}N$	64
16	10.9274	2.7257	R-(-)-Cyclohexylethylamine	127	$C_8H_{17}N$	64
17	11.1943	3.9142	2-Pentanamine	87	$C_5H_{13}N$	59
18	11.2525	2.282	Propanamide	59	C_3H_7N0	64
19	11.8661	1.4936	dl-3-Aminoisobutyric acid, N-methyl-, methyl ester	195	$C_6H_{13}N0_2$	64
20	11.9841	1.5961	2-Heptanamine, 5-methyl-	115	$C_8H_{19}N$	72
21	12.0258	0.5839	Benzenemethanol, .alpha.-[(methylamino)methyl]-	104	$C_8H_{11}N0$	59
22	12.0464	0.5985	Cyclobutanol	74	C_4H_80	72
23	12.0875	2.1312	Benzenemethanol, .alpha.-[(methylamino)methyl]-	104	$C_8H_{11}N0$	64
24	12.1941	1.6484	Octodrine	159	$C8H_{19}N$	59
25	12.3818	2.6998	Amphetamine	135	$C_{19}H_{13}N$	58
26	13.3842	2.3211	Propanamide	59	C_3H_7N0	52
27	13.7691	1.136	Cyclobutanol	74	C_4H_80	59
28	14.196	6.8668	Dodecanoic acid, methyl ester	200	$C_{13}H_{26}O_2$	52
29	14.3053	0.3552	Benzeneethanamine	121	$C_8H_{11}N$	59
30	15.0251	1.1546	2-Amino-1-(o-methoxyphenyl)propane	226	$C_4H_{10}N0$	43
31	15.1767	0.5099	Benzeneethanamine	121	$C_8H_{11}N$	59
32	16.2039	0.231	Octodrine	159	$C_8H_{19}N$	59
33	16.3097	0.6433	Phenylephrine	167	$C_9H_{13}N0_2$	64

34	16.3948	0.3229	12-Methylaminododecanoic acid, t-butyl ester	290	$C_{17}H_{36}N0_3$	59
35	16.4389	0.5225	Methylpent-4-enylamine	100	$C_6H_{13}N$	64
36	16.533	0.2899	Phenylephrine	167	$C_9H_{13}N0_2$	64
37	16.5506	0.2689	1-Propanamine, N,2-dimethyl-	100	$C_5H_{15}N$	59
38	16.5866	1.579	1-Octadecanamine, N-methyl-	284	$C_{19}H_{42}N$	50
39	16.7653	0.3437	3-Ethoxyamphetamine	193	$C_{11}H_{18}N0$	59
40	16.833	0.2627	Phenylephrine	167	$C_9H_{13}N0_2$	59
41	16.9461	0.2586	2-Ethoxyamphetamine	177	$C_{11}H_{18}N0$	59
42	17.0849	0.2136	2-Aminononadecane	177	$C_{19}H_{41}N$	59
43	17.2419	0.2351	Phenylephrine	167	$C_9H_{13}N0_2$	59
44	17.2901	0.617	Propanamide	59	C_3H_7N0	64
45	17.3226	0.2152	Benzeneethanamine	121	$C_8H_{11}N$	64
46	17.3663	0.6333	2-Butanamine, 3-methyl-	87	$C_5H_{14}N$	59
47	17.402	0.3219	Xanthine	152	$C_5H_4N_4O_2$	64
48	17.4753	0.2601	N-Methyl-2-phenyl-1-propylamine	163	$C_{10}H_{17}N$	64
49	17.4956	0.3007	3-Ethoxyamphetamine	193	$C_{11}H_{18}N0$	64
50	17.5238	0.1535	Xanthine	152	$C_5H_4N_4O_2$	64
51	17.5507	0.283	2-Butanamine, 3,3-dimethyl-	101	$C_6H_{17}N$	59
52	17.6984	0.2058	Brolamfetamine	NAB		59
53	17.7134	0.5923	3-Hydroxy-N-methylphenethylamine	165	$C_9H_{15}N0$	64
54	17.7812	0.2397	Cyclobutanol	74	C_4H_80	59
55	17.8371	0.5053	2-Pentanamine	87	$C_5H_{13}N$	59
56	17.9043	0.4919	3-Ethoxyamphetamine	193	$C_{11}H_{18}N0$	59
57	17.9538	0.1761	2-Aminononadecane	177	$C_{19}H_{41}N$	59
58	17.9891	0.717	Benzeneethanamine	121	$C_8H_{11}N$	59
59	18.0319	0.6158	Methylpent-4-enylamine	100	$C_6H_{13}N$	72
60	18.0592	0.5023	l-Guanidinosuccinimide	210	$C_5H1_0N_40_2$	59
61	18.0763	0.4285	Benzenemethanol, .alpha.-[(methylamino)methyl]-	152	$C_8H_{11}N0$	64
62	18.1431	1.3753	l-Guanidinosuccinimide	210	$C_5H_{10}N_40_2$	59
63	18.1746	0.3497	dl-Alanyl-dl-methionine	476	$C_{13}H_{29}N_5O_8S_2$	64
64	18.2163	0.661	Phenethylamine, p,.alpha.-dimethyl-	121	$C_{10}H_{17}N$	59
65	18.2314	0.268	dl-Phenylephrine	274	$C_9H_{13}N0_2$	59
66	18.2886	1.0091	l-Guanidinosuccinimide	210	$C_5H_{10}N_40_2$	64
67	18.3815	5.5588	Hexadecanoic acid, 14-methyl-, methyl ester	287	$C_{17}H_{35}O_2$	84
68	18.4446	1.2936	Phenylephrine	167	$C_9H_{13}N0_2$	64
69	18.4811	1.3577	p-Hydroxynorephedrine	183	$C_9H_{13}N0_2$	64
70	18.5973	1.2579	N-Desmethyltapentadol	221	$C_{13}H_{23}N0$	72
71	18.6331	0.4826	Acetamide, 2-fluoro-	61	C_2H_5NOF	64
72	18.6678	1.492	Imidazole-5-carboxylic acid, 2-amino-	127	$C_5H_8N_3O_2$	64
73	18.7103	0.6901	Acetamide, 2-fluoro-	61	C_2H_5NOF	59
74	18.7534	2.1013	Benzeneethanamine	121	$C_8H_{11}N$	50
75	18.8683	0.7875	2-Butanamine, 3-methyl-	87	$C_5H_{13}N$	59

76	18.9142	0.8178	2-Aminononadecane	172	$C_{19}H_{41}N$	64
77	18.9592	0.5297	1-Octadecanamine, N-methyl-	284	$C_{19}H_{41}N$	58
78	19.0665	0.4747	N-Desmethyltapentadol	221	$C_{13}H_{23}N0$	59
79	19.1001	0.3223	l-Guanidinosuccinimide	210	$C_5H_{10}N_40_2$	64
80	19.1639	0.2973	Alanylglycine, TMS derivative	283	$C_9H_{21}N0_4Si$	64
81	19.2714	0.3876	Alanylglycine, TMS derivative	283	$C_9H_{21}N0_4Si$	59
82	19.2868	0.4355	3-Hydroxy-N-methylphenethylamine	151	$C_9H_{13}N0$	58
83	19.3276	0.4215	dl-Phenylephrine	274	$C_9H_{13}N0_2$	59
84	19.3576	0.3614	p-Hydroxynorephedrine	183	$C_9H_{13}N0_2$	59
85	19.4388	0.5162	2-Butanamine, 3-methyl-	87	$C_5H_{13}N$	59
86	19.4899	0.6144	Glycine	75	$C_2H_5N0_2$	58
87	19.5231	0.747	2-Aminononadecane	172	$C_{19}H_{41}N$	72
88	19.5825	1.0083	Alanylglycine, TMS derivative	283	$C_9H_{21}N0_4Si$	64
89	19.6063	0.6495	Chlorodifluoroacetamide	133	$CHCIF_2N0$	58
90	19.6495	0.8725	N-Desmethyltapentadol	221	$C_{13}H_{23}N0$	64
91	19.6823	2.0576	Benzeneethanamine	121	$C_8H_{11}N$	58
92	19.7309	2.9194	Propanamide	73	C_3H_7N0	52
93	19.8435	2.0351	Alanylglycine, TMS derivative	283	$C_9H_{21}N0_4Si$	59
94	19.8754	0.6704	2,2-Dichlorocyclopropanecarboxamide	170	$C_5H_6C_{I2}N0$	53
95	19.9113	0.7049	N-Methyl-2-phenyl-1-propylamine	163	$C_{11}H_{15}N$	52
96	19.9354	1.1095	N-Desmethyltapentadol	221	$C_{13}H_{23}N0$	59
97	20.099	0.2411	L-Alanine-4-nitroanilide	227	$C_9H_{13}N_3O_4$	50

Also in (table 5) The cuticular hydrocarbons mosquitoes reared or exposed to 0.2 mg/L of melon leaf plant extract, revealed that the mosquitoes contains total number 20 compounds and consists of alkadienes, alkenes, n-alkanes, and methyl alkanes groups and ester ranging from C_3 to C_{26}. The most abundant compounds were cis-9-hexadecenal (79.0062 %), 1-octadecanamine, N-methyl (10.0582 %), N-methyl-2-phenyl-1propylamine (6.7775 %) and benzeneethanamine (2.7534 %).

Discussion

The present study demonstrated that melon leaf extract had a noticeable effect on the cuticular hydrocarbon profile of Anopheles mosquitoes. Cuticular hydrocarbon are important for maintaining the integrity of an insect cuticle, particularly in preventing water loss and contributing to survival under environmental stress. In that any disruption in their composition can contribute or have a serious physiological consequences on mosquito. The hydrocarbon profiling of the mosquitoes cuticles revealed significant alterations in CHCs profile of the anopheles mosquitoes treated with methanol melon leaf extract compared with the control groups. Specifically there were observable changes in the relative abundance of certain hydrocarbons, and reduction or absence of others.

These changes observed in this study suggest that the plant extracts interfere with processes involved in hydrocarbon production or deposition on the mosquito cutile. This may be linked to the phytochemical constituents identified in the extract, which include alkaloids, flavonoids, saponins, tannins, and phenolic compounds. These compounds are known to exhibit biological activity against insects through mechanisms, often acting on multiple physiological targets at once. This findings is in

conformity with the work of Gonzalez et al., (2011) who revealed that, the bioactive compounds in methanol extract likely disrupt the enzymes and pathways involved in the biosynthesis of CHCs. i.e. phenolic and flavonoids can interfere the activity of cytochrome P450 and monooxygenases, which are for hydrocarbon synthesis. Additionally, alkaloids may disrupt the normal functioning of insect cuticle, leading to increased desiccation and vulnerability to environ-mental stressors (Akhtar et al., 2018). One likely reason for the reduction in cuticular hydrocarbon is disruption of lipid metabolism. Since hydrocarbons are synthesized from lipid precursors in the epidermal cells, any interference with lipid biosynthesis can affect their production. The flavonoids and phenolic compounds may contribute to this effect by inducing oxidative stress.

Table 5: Cuticular Hydrocarbon Profiling of *Anopheles* Mosquitoes (Methanol) using GC-MS Analysis

PK	RT	Area Pct%	COMPOUNDS	MW	M F	Qual
1	5.1593	79.0062	cis-9-Hexadecenal	238	$C_{16}H_{30}0$	64
2	5.5099	6.7775	N-Methyl-2-phenyl-1-propylamine	149	$C_{10}H_{17}N$	74
3	5.6938	2.7534	Benzeneethanamine	121	$C_8H_{11}N$	59
4	5.8749	10.0582	1-Octadecanamine, N-methyl-	141	$C_{19}H_{42}N$	64
5	9.1699	0.1378	Imidazole-5-carboxylic acid, 2-amino-	127	$C_5H_8N_3O_2$	46
6	13.3617	0.085	Benzamide, N-ethyl-N-(3-methylphenyl)-4-methoxy-	257	$C_{18}H_{23}N0_2$	43
7	14.6822	0.1141	1-hexadecanesulfonamide, N-(2-aminoethyl)-	305	$C_{18}H_{40}N_20_3S$	47
8	16.8539	0.0621	Propanamide	73	C_3H_7N0	47
9	18.2117	0.0656	2,5-Dimethoxy-4-propylamphetamine	237	$C_{26}H_{40}N_20_4$	45
10	18.3659	0.1175	Pentadecanoic acid	242	$C_{15}H_{31}C00H$	92
11	18.5555	0.0622	Propanamide	73	C_3H_7N0	50
12	18.6791	0.1297	Adipamide	144	$C_6H_{12}N_20_4$	58
13	18.7366	0.1142	4-Fluorohistamine	129	$C_5H_9N_3F$	47
14	18.8823	0.1145	Propanamide	73	C_3H_7N0	47
15	19.531	0.057	Acetamide, 2-cyano-	56	C_3H_5N0	43
16	19.6642	0.1226	12-Octadecenoic acid, methyl ester	282	$C_{19}H_{37}0_2$	95
17	20.8286	0.052	Propanamide	73	C_3H_7N0	38
18	20.9009	0.0677	Metaraminol	167	$C_9H_{13}N0_2$	43
19	21.024	0.0322	Norpseudoephedrine	151	$C_{10}H_{15}N0$	38
20	21.2186	0.0705	MDMA methylene	193	$C_{12}H_{17}N0_2$	52

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Furthermore, the results of this study revealed that melon leaves contain bioactive compounds such as phenol, flavonoids, saponins, tannins and alkaloids, which exhibits insecticidal properties. According to Akhtar et al., (2018), these compounds can disrupt normal insect

physiological pro-cesses, leading to altered behaviour, impaired development, and mortality. The phenolic com-pounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al., 2007). They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han et al., 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown et al., 1998; Krings et al., 2001). Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (Ali et al., 2008). Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are known for their antioxidant, anti-inflammatory and anticancer properties. They play vi-tal roles in the plant defence against pathogens and pests. (Salah et al., 1995; Del-Rio et al., 1997; Okwu, 2004). Exploring melon leaf extraction of these compound using methanol, a non-polar solvent allows analysis to these bioactive compound.

The results of this study suggest that melon leaf extract does not act through a single mechanism but rather through the combined action of its phytochemical constituents. This multi-target effect likely contributes to its ability to disrupt cuticular hydrocarbons and impair mosquito physiology.

Conclusion

Melon leaf extract could serve as an effective tool in mosquito control by altering the cuticular profile of *Anopheles gambiae*. Disruption of CHCs profile may impair the mosquitoes physiological processes, thereby reducing the population and transmission potential of malaria vectors. Further research is needed to optimize extraction and application methods and to assess the long -term effects of melon leaf extract on mosquito's population.

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Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

Author Contribution

O. A. Aina drafted the work, analyzed and interpreted the data I. K. Olayemi designed the work and A. C. Ukubiwe revised the work. All authors reviewed the results and approved the final version of the manuscript.

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