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Phytochemistry and GC-MS Screening and Biocidal Potentiality of Ginger (*Zingiber officinale*) Rhizome against Mosquito's Larvae

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Abstract

Mosquitoes can act as vectors for many viruses and parasites through carrying these organisms from person to person. Although great number of natural products are lethal to mosquitoes, but few researches are conducted to understand the magnitude of the behavioral response to these plant parts. The objectives of this study were to screen the phytochemical and other chemical components from ginger rhizome in addition to test their aqueous and ethanol extracts on mosquito's larvae. The standard methods, materials and devices were used to screen the phytochemical components and the chemical constituents (GC-MS). The aqueous and the ethanol extracts from clove pods were prepared and used against *Anopheles, Culex* and *Aedes* larvae. The results of the aqueous extract showed that, the LC_{50} was 153.77 mg/L for *Anopheles* larvae, 179.05 mg/L for *Culex* larvae, and 208.37 mg/L for *Aedes* larvae, also the ethanol extract of ginger rhizome was more potent than the aqueous extract. The biocidal activity can be attributed to the presence of saponins, flavonoids, glycosides, alkaloids and steroids. The GC-MS for the hexane extract showed the presence of alkaloid: gingerol (18%) and eugenyl acetate- (19%) that gives the sweet aromatic, spicy taste, but the ethanol extract detected gingerol (43%) and zingiberene (14%). The obtained data will be useful to understand the mechanisms and reasons of biocidal activity of ginger rhizome against the tested mosquito's larvae.

Keywords: Ginger rhizome; Phytochemistry; GC-MS; Mosquitoes; Natural products

1. Introduction

Mosquitoes (Insecta: Diptera) transmit many vector-borne diseases and cause millions of deaths and Health problems every year [1]. Mosquitoes remains the most important vector-borne diseases to most countries of the tropical and subtropical zones. *Anopheles* is a genus of mosquito involves 460 species, 100 of which can transmit human malaria [2]. *Aedes aegypti* is the primary vector of dengue fever and is highly anthropophilic. It is found in association with humans and lives where human is found [3]. *Culex* as an important vector have a broad geographic distribution in the world [4]. *Culex* involved in transmission of West Nile virus, Sindbis arboviruses, Dirofilariaimmitis and Rift Valley fever virus [5].

Ginger (*Zingiber officinale*) family Zingiberaceae is an herbaceous perennial flowering plant whose rhizome is widely used as a spice and a folk medicine [6]. It is grows about one meter tall bearing flowers having pale yellow petals with purple edges [7].

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Ginger is fragrant kitchen spice [8] and it used worldwide; whether it be used to spice up meals, or as a medicine, the history of needs for ginger has been recorded [9]. Ginger also used as vegetables, candy, soda and alcoholic beverages [10].

The volatile oils compose 1-3% of the weight of fresh ginger, and it consist of zingerone, shogaols, and gingerols as the major pungent compound. Zingerone have lower pungency and a spicy-sweet aroma. Shogaols are more pungent and higher antioxidant activity [11].

The lethal activity of some natural products against insects has been tested in different parts of world specially against mosquito's larvae and the other important vectors that transmit infectious disease agents. Prevention of some disease remains almost entirely dependent on vector control, which is considered to be the most effective method for reducing disease transmission in all areas of the world [12].

The present study aims to evaluate the biocidal activity of *Z, officinale* (ginger) aqueous and ethanol extract against *An. arabiensis*, *Cx. quinquefasciatus*, and *Ae. aegypti* larvae under laboratory conditions. The study also evaluate the phytochemical and GC-MS output for the used ginger rhizome part.

2. Material and methods

The samples of ginger (*Z. officinale*) rhizome, were brought from the local market of Wad Medani City, whereas the *An. arabiensis*, *Cx. quinquefasciatus*, and *Ae. aegypti* larvae were collected from Tayba village, Gezira State, by an expert technician.

2.1. Preparation of extracts

The ginger rhizome was brought from the local market and was cleaned manually, then cut into small parts and let to dry at room temperature away from direct sunlight, then crushed after several days to fine granules. A well calculated amount of the granules was extracted with water, 99% ethanol and hexane. The water and the ethanol extracts were used for the biocidal tests, while ethanol and hexane extracts were used in GC-MS tests.

2.2. Phytochemical screening

The crushed granules of ginger rhizome was used as a raw materials for the qualitative phytochemical screening tests following Uraku [13], so as to determine the presence (+) or absence (-) of some of the main classes, *viz*, alkaloids, glycosides, tannins, flavonoids, saponins and steroids.

2.3. GC-MS test

This test was done in the Central Lab., University of Gezira, Gezira State, Sudan. The ethanol and hexane extracts were tested using GC-MS methods. The chromatograph, retention time, peak, % area, the chemical formula and the molecular weight of the identified components were provided by the NIST library database,

2.4. Biocidal tests

The susceptibility *An. arabiensis*, *Cx. quinquefasciatus* and *Ae. aegypti* larvae were tested following the instructions of WHO [14]. The test period was 24 hours and based on three replicates. Control batch was also designed. The field collected larvae of the three species were separated in three small dishes and were immediately tested for their susceptibilities. The accidentally collected aquatic organisms were immediately eliminated.

2.5. Data analysis

Following Abbott [15] and Finney [16], the log-concentrations and the corresponding tested mortalities were used to run the Probit analysis. The diagnostic doses (LC_{50} and LC_{95}) of the aqueous and the ethanol extracts of ginger rhizome against the tested mosquito larvae were calculated from the regression equation.

3. Results and discussion

3.1. Phytochemical screening

Table (1) showed the presence of saponins, flavonoids, glycosides, alkaloids and steroids in ginger rhizome dried powder, but tannins were not detected. Geographical differences were appeared from the obtained results when compared to the results of other research areas of the world.

Rhizome of *Z. officinale* obtained from University of Calabar Botanical Garden, Nigeria, showed the presence of alkaloids, saponins, flavonoids, polyphenols, cardiac glycosides and reducing sugars were present in both the aqueous extract and petroleum ether extract. While tannins, phlobatannins, anthranoids, hydroxyl anthroquinones and anthraquinones were absent in both aqueous and petroleum ether extracts [17]. Phytochemical screening of ginger from Punjab, Pakistan, showed presence of alkaloid, phlobotannins, flavanoids, glycosides, saponins, tannin and terpenoids and absence of steroids [18].

Table 1 Phytochemical screening of the main classes in ginger rhizome

Main class	Status
Saponins	+
Flavonoids	+
Tannins	-
Glycosides	+
Alkaloids	+
Steroids	+

(-) Means absence of the main class;(+) mean presence of the main class

3.2. GC-MS test

Table 2 Compounds identified by GC-MS from the hexane extract of ginger rhizome

Peak	R. time	Area%	Name	Mol. form.	Mol. wt
1	4.264	1.71	Hexane,2-Nitro	$C_6H_{13}O_2$	131
2	5.704	1.43	Octanal	$C_8H_{16}O$	128
3	9.196	4.27	Decanal	$C_{16}H_{20}O$	156
4	11.586	1.58	Phenol,2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164
5	13.948	1.47	Phenol,2-methoxy-4-(2-propenyl)-acetate	$C_{12}H_{14}O_3$	206
6	15.705	18.07	Gingerol	$C_{17}H_{26}O_4$	244
7	18.075	2.56	Hexacosane	$C_{26}H_{54}$	366
8	19.265	1.42	Hexacosane	$C_{26}H_{54}$	366
9	19.566	2.20	1,2-Benzenedicarboxylic acid,butyl 8-methyl	$C_{22}H_{34}O_4$	362
10	19.812	2.82	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652
11	20.163	1.84	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
12	20.405	1.82	Hexacosane	$C_{26}H_5$	366
13	22.423	4.62	Furan, 2,5-dibutyl-	$C_{12}H_{20}O$	180
14	23.149	13.74	Eugenyl acetate	$C_{12}H_{14}O_3$	206
15	24.006	1.35	Undecane, 1-bromo-	$C_{11}H_{23}Br$	235

16	25.050	2.99	Naphthalene- decahydro-1-pentadecyl-	$C_{25}H_{48}$	348
17	225.470	4.54	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390
18	25.997	5.24	Eugenyl acetate	$C_{12}H_{14}O_3$	206
19	26.830	4.44	2,6,6-trimethylcyclohex-1-enylmethanesulfo	$C_{16}H_{22}O_2S$	278
20	27.321	3.61	13-Docosamide, (Z)	$C_{22}H_{43}NO$	337

Table (2) showed that, about 20 different compounds were identified through GC-MS from the hexane extraction of ginger rhizome. The principal compound was the alkaloid: gingerol (18%) and eugenyl acetate- (19%) which gives the sweet aromatic, spicy taste. Table (3) showed that, about 20 different compounds were identified from the ethanol extract, within which gingerol (43%) is the main principle. Terpenes (isoborneol and alpha-terpineol) were detected in small amount (1.6% and 0.57%, respectively). Zingiberene (14%) and other sesquiterpenes (beta-bisabolene (3.44%); Cyclohexaene, 3-1,5-dimethyl-4-hexenyl (6.69%) and Dihydrocarvyl acetate (which act as flavor ingredient because it has a minty taste and usually used as one of the food additives) were also detected.

Ginger rhizome from Eastern part of Nigeria, showed the presence of Gingerol at Peak 12 and Ricinoleic acid at the last [19]. GC-MS analysis of methanol extract of *Z. officinale* rhizomes from India, detected Zingiberene, AR-curcumene, α-Bergamotene, Gingerol, Zingerone, Caryophyllene and ç-Elemene [20].

Table 3 Compounds identified by GC-MS from the ethanol extract of ginger rhizome

Peak	R. time	Area%	Name	Mol. form.	Mol. wt
1	4.399	0.69	Dihydrocarvyl acetate	$C_{12}H_{20}O_2$	196
2	5.421	2.39	Exo-2,7,7-trimethylbicyclo [2,2,1] heptan-2-ol	$C_{10}H_{18}O$	154
3	7.079	1.62	isoborneol	$C_{10}H_{18}O$	154
4	7.335	0.57	Alpha-terpineol	$C_{10}H_{18}O$	154
5	7.516	1.41	Butanedioic acid, 2,3-bis(acetyloxy)-[R-(R	$C_8H_{10}O_8$	178
6	8.237	0.76	Benzeneethanamine, 2,5-dimethoxy-alpha	$C_{11}H_{17}NO_2$	195
7	9.075	1.74	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164
8	10.546	4.65	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-meth	$C_{15}H_{22}$	202
9	10.711	14.04	Zingiberene	$C_{15}H_{24}$	204
10	10.860	3.44	Beta-bisabolene	$C_{15}H_{24}$	202
11	11.001	6.69	Cyclohexaene, 3-(1,5-dimethyl-4-hexenyl)-	$C_{15}H_{24}$	204
12	11.704	7.96	Beta-D-glucopyranose, 4-O-beta-D-galactose	$C_{12}H_{22}O_{11}$	342
13	11.973	1.44	Pregan-20-one, 2-hydroxy-5,6-epoxy-15-met	$C_{22}H_{34}O_3$	346
14	12.554	1.51	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-	$C_{15}H_{24}O$	220
15	13.503	1.87	Farnesyl acetone	$C_{18}H_{20}O$	262
16	14.894	1.02	2,4-bis(hydroxyamino)-5-nitropyrimidine	$C_6H_6N_2O_2S_2$	348
17	15.201	2.07	Docosanoic acid, ethyl ester	$C_{24}H_{34}O_2$	356
18	16.584	2.22	E-11-hexadecenoic acid, ethyl ester	$C_{18}H_{34}O_2$	282
19	16.892	0.57	1-(5-bicyclo [2,2,1] heptyl)ethylamine	$C_9H_{17}N$	139
20	17.309	43.02	Gingerol	$C_{17}H_{26}O_4$	244

3.3. Biocidal tests

Table (4) showed that, the aqueous extract of ginger rhizome was tested at concentrations of 80.54 – 177.19 mg/L for a period of 24 hours against *Anopheles, Culex* and *Aedes* larvae. The results showed that, at the minimum concentration (80.54 mg/L), 10% of *Anopheles* larvae were killed, while 20% of *Culex* and *Aedes* were killed. At the higher concentration (177.19 mg/L), the mortality of mosquito's larvae ranged between 50% (in *Aedes*) to 65% (in *Anopheles*). The LC_{50} was 153.77 mg/L for *Anopheles* larvae, 179.05 mg/L for *Culex* larvae, and 208.37 mg/L for *Aedes* larvae, hence, *Anopheles* larvae was relatively more susceptible to the aqueous extract of ginger rhizome than the other two species of mosquitoes, while *Aedes* larvae showed respectively more resistance to the same aqueous extract. The LC_{95} confirm the same conclusion. It was noticed that, the control mortality was 0. The polar content of ginger rhizome was 12.45%.

Table 4 Percentage mortality of mosquito's larvae subjected to aqueous extract of ginger rhizome after 24 hrs

Concentration		**Corrected Mortality**			**Probit**		
mg/L	**Log**	***Anopheles***	***Culex***	***Aedes***	***Anopheles***	***Culex***	***Aedes***
80.54	1.91	10	20	20	3.72	4.16	4.16
112.76	2.05	15	30	30	3.96	4.48	4.48
128.88	2.11	42.5	35	35	4.82	4.61	4.61
161.08	2.21	50	40	35	5.00	4.75	4.61
177.19	2.25	65	55	50	5.41	5.13	5.00
Probit analysis							
R^2					0.90	0.92	0.86
Intercept					-6.00	-0.70	0.20
Slope					5.03	2.53	2.07
LC_{50} (mg/L)					153.77	179.05	208.37
LC_{95} (mg/L)					325.78	796.50	1291.55

Control mortality= 0; Polar (aqueous) contents = 12.45%

Table (5) showed that, the ethanol extract of ginger rhizome was tested at concentrations of 55.32 – 183.34 mg/L for a period of 24 hours against *Anopheles, Culex* and *Aedes* larvae. At the minimum concentration (55.32 mg/L), 90% of *Anopheles*, 50% *Culex* larvae and 45% of *Aedes* larvae were died. At the higher concentration (183.34 mg/L), the mortality of mosquito's larvae ranged between 100% (in *Anopheles*) to 75% (in *Aedes*). The LC_{50} was not detected for *Anopheles* larvae (because there were only two values of mortality bellow 100% which were not sufficient to perform Probit analysis), but it was 56.11 mg/L for *Culex* larvae and 63.97 mg/L for *Aedes* larvae, hence, *Anopheles* larvae was relatively more susceptible to the ethanol extract of ginger rhizome than the other two species of mosquitoes. while *Aedes* larvae showed respectively more resistance to ginger rhizome ethanol extract. LC_{95} values also confirm the same conclusion. According to the LC's values, it was clear that, the ethanol extract of ginger rhizome was more potent than the aqueous extract. It was noticed that, the control mortality was 0. The polar content of ginger rhizome was 14.90%.

Insecticidal activity of ginger and Eucalyptus essential oils against adult form of *Culex theileri* mosquitoes, collected from small pools located near the Zayande-Rood River, Iran, were examined via direct exposure method. Results show considerable values of insecticidal activity against mosquitoes. *Eucalyptus globulus* (66% insecticidal activity) was more potent than *Z. officinale* (45% insecticidal activity). This study shows that these essential oils can be considered as good replaces for chemical pesticides [5].

Ginger among other plants e.g. *Aloe vera*, garlic and datura can be recommended for use in mosquito management programs as potential alternatives to synthetic insecticides [21]. Ginger essential oils possess good larvicidal, repellant, and antimicrobial activity [22].

Table 5 Percentage mortality of mosquito's larvae subjected to ethanol extract of ginger rhizome after 24 hrs

Concentration		**Corrected Mortality**			**Probit**		
mg/L	**Log**	***Anopheles***	***Culex***	***Aedes***	***Anopheles***	***Culex***	***Aedes***
55.32	1.74	90	50	45	6.28	5.00	4.87
69.16	1.84	97.5	60	50	7.05	5.25	5.13
83.004	1.92	100	65	55	-	5.39	5.13
110.67	2.04	100	75	70	-	5.67	5.52
183.34	2.14	100	85	75	-	6.04	5.67
Probit analysis							
R^2						0.99	0.96
Intercept						0.61	1.37
Slope						2.51	2.01
LC_{50} (mg/L)						56.11	63.97
LC_{95} (mg/L)						252.58	418.69

Control mortality= 0; Polar (ethanol) contents = 14.90%

4. Conclusion

Ginger rhizome contains saponins, flavonoids, glycosides, alkaloids and steroids.
GC-MS for the hexane extraction of ginger rhizome, detected the alkaloid: gingerol (18%) and eugenyl acetate- (19%). Gingerol (43%), Zingiberene (14%) and other sesquiterpenes (beta-bisabolene (3.44%); Cyclohexaene, 3-1,5-dimethyl-4-hexenyl (6.69%) were detected through GC-MS from ginger rhizome ethanol extract.
The LC_{50} for ginger rhizome aqueous extract was 153.77 mg/L for *Anopheles* larvae, 179.05 mg/L for *Culex* larvae, and 208.37 mg/L for *Aedes* larvae.
Anopheles larvae were relatively more susceptible to the aqueous extract of ginger rhizome than the other two species of mosquitoes.
The ethanol extract of ginger rhizome was more potent than the aqueous extract.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors (Dalia, Mutaman, Abdalla and Abdelmonem) declare no conflicts of interest regarding the publication of this paper.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

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