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Phytochemistry and GC-MS screening and biocidal potentiality of clove (*Syzygium aromaticum L.*) pods against mosquitos' larvae

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Abstract

Many measures have been used for mosquito control, including the elimination of breeding places, exclusion via window screens and mosquito nets in addition to natural products including clove (*Syzygium aromaticum*). This study was run at University of Gezira, Sudan, to run phytochemical and GC-MS screening for clove pods before used it as mosquito control agent. The standard methods, materials and devices were used to screen the phytochemical components and the chemical constituents (GC-MS). The WHO protocol for testing the susceptibility of mosquito's larvae to insecticides was followed in bioassay. The aqueous and the ethanol extracts from clove pods were prepared and used against *Anopheles, Culex* and *Aedes* larvae. The results showed that, *Aedes* mosquito was relatively more susceptible (LC50= 498 mg/L) to clove aqueous extract than *Anopheles* (LC50= 561 mg/L) and *Culex* (LC50= 615 mg/L), and similar findings were observed for clove pods ethanol extract, which is relatively more potent than the aqueous extract. The biocidal activity can be attributed to the presence of the detected saponins, flavonoids, tannins and alkaloids. The GC-MS for the ethanol extract showed that, the principal compounds were Eugenol (81%) and caryophyllene (4.65%). Further studies should be run to improve knowledge about how to use this natural product in more economic trends.

Keywords: Clove pods; Phytochemistry; GC-MS; Mosquitoes; Natural products

1. Introduction

In order for a mosquito to transmit a disease to the host there must be favorable conditions, referred to as transmission seasonality [1]. Seasonal factors that impact the prevalence of mosquitoes and mosquito-borne diseases are primarily humidity, temperature, and precipitation. A positive correlation between malaria outbreaks and these climatic variables has been demonstrated in China [2] and El Niño has been shown to impact the location and number of outbreaks of mosquito-borne diseases observed in East Africa, Latin America, Southeast Asia and India [3]. Climate change impacts each of these seasonal factors and in turn impacts the dispersal of mosquitoes.

Mosquito-borne diseases are currently most prevalent in East Africa, Latin America, Southeast Asia, and India; however, emergence of vector-borne diseases has recently been observed. One statistical model predicts by 2030, the climate of southern Great Britain will be climatically suitable for malaria transmission *Plasmodium vivax* for 2 months of the year. By 2080 it is predicted that the same will be true for southern Scotland [4].

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Cloves (*S. aromaticum*) are the aromatic flower buds of a tree in the family Myrtaceae. They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice [5]. Cloves are available throughout the year owing to different harvest seasons in different countries [6].

In traditional medicine, there is evidence that clove oil containing eugenol is effective for toothache pain and other types of pain for fever reduction, as a mosquito repellent, and to prevent premature ejaculation have been inconclusive [7,8]. Use of clove for any medicinal purpose has not been approved by the US Food and Drug Administration, and its use may cause adverse effects if taken orally by people with liver disease, blood clotting and immune system disorders, or food allergies [7].

Eugenol (which responsible for clove aroma) comprises 72–90% of the essential oil extracted from cloves [9, 10]. Other important essential oil constituents include acetyl eugenol, beta-caryophyllene, vanillin, crategolic acid, tannins [9, 11], flavonoids, triterpenoids and several sesquiterpenes [12]. Eugenol has not been classified for its potential toxicity [10].

In this study, the phytochemical and GC-MS screening in addition to the insecticidal activity of clove (*S. aromaticum*) pods against *Anopheles, Culex* and *Aedes* larvae were studied in Gezira State, Sudan.

2. Material and methods

2.1. Materials

The samples of Clove (*S. aromaticum*) pods were brought from the local market of Wad Medani city, whereas the mosquito larvae were collected from Tayba village, Gezira State, Sudan.

2.2. Preparation of extracts

The selected plant product was first cleaned manually and then let to dry at room temperature away from direct sunlight, then crushed to fine powder. A well calculated amount of the powder was extracted with water, 99% ethanol and hexane. The water and the ethanol extracts were used to run the biocidal tests, while ethanol and hexane extracts were used for GC-MS tests.

2.3. Phytochemical screening

The crushed powder of clove pods was used to run the phytochemical screening tests following Uraku [13], to determine the main classes: alkaloids, glycosides, tannins, flavonoids, saponins and steroids. A qualitative test was only done using (+ and -), respectively for the presence or absence of the main class.

2.4. GC-MS test

The ethanol and hexane extracts were examined using GC-MS protocols. The test was done in the Central Lab., University of Gezira, Gezira State, Sudan. The NIST library was automatically named, calculated the molecular weight and structured of the identified components.

2.5. Biocidal tests

Following the instructions of WHO [14], the susceptibility *Anopheles*, *Culex* and *Aedes* larvae were tested. The field collected larvae were immediately tested and never reared. The test period was 24 hours and based on three replicates. Control batch was also designed.

2.6. Data analysis

The Probit analysis was used to calculate the diagnostic doses (LC_{50} and LC_{95}) of clove pods aqueous and ethanol extracts against the tested mosquito larvae.

3. Results and discussion

3.1. Phytochemical screening

Table (1) showed the presence of saponins, flavonoids (in relatively more quantity), tannins and alkaloids in clove pods dried powder, while glycosides and steroids were not detected. Same finding was proved by Khatri *et al.*, [15] who found: clove buds contain 15–20% essential oil, which is dominated by eugenol (70–85%), eugenyl acetate (15%) and β-caryophyllene (5–12%). Other essential oil ingredients of clove oil are vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin and triterpenoids like oleanolic acid.

Table 1 Phytochemical screening of the main classes in clove pods dried powder

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

(-) Means absence of the main class; (+) mean presence of the main class
(++) mean presence of the main class in relatively more quantity

3.2. GC-MS test

Table (2) showed that, about 18 different compounds were identified through GC-MS from the hexane extraction of clove pods. The principal compounds found in clove pods were the sesqueterpene Eugenol (26%); caryophyllene (5.45%) and caryophyllene oxide (2.08%). The phytochemical screening for clove pods did not detected Steroids, but GC-MS detected the phytosterol gamma-sitosterol in small amout (1.85%). Table (3) showed that, about 11 different compounds were identified from the ethanol extract of clove pods. The principal compounds were Eugenol (81%); caryophyllene (4.65%) but caryophyllene oxide (0.76%) and the sesqueterpene: humulene (0.65%) were found in small quantities. Also traces of the tannin compound: Benzenetriol (0.72%) and the flavonoid: trimethoxyacetophenone (0.7%).

Table 2 Compounds identified by GC-MS from the hexane extract of clove pods

Peak	R. time	Area%	Name	Mol. form.	Mol. wt
1	4.268	1.34	Hexane,2-Nitro	$C_6H_{13}O_2$	131
2	4.530	0.90	2-Pentanone, 3-methyl-	$C_6H_{12}O$	100
3	5.180	1.65	Cyclopentane,1-acetyl-1,2-epoxy	$C_7H_{10}O_2$	126
4	11.688	26.18	Eugenol	$C_{10}H_{12}O_2$	164
5	12.999	5.45	Caryophyllene	$C_{15}H_{24}$	204
6	14.069	27.62	Phenol,2-methoxy-4-(2-propenyl)-acetate	$C_{12}H_{14}O_3$	206
7	14.402	1.58	Naphthalene,1,2,3,5,6,8a-hexahydro-4,	$C_{15}H_{24}O_2$	204
8	15.244	2.08	Caryophyllene oxide	$C_{15}H_{24}O$	220
9	16.324	16.25	2,3,4,-trimethoxyacetophenone	$C_{11}H_{14}O_4$	210
10	16.811	1.48	Nonadecane	$C_{19}H_{40}O_2$	268
11	18.067	0.99	Hexacosane	$C_{26}H_{54}$	366
12	19.261	2.07	Hexacosane	$C_{26}H_{54}$	366

13	19.555	1.20	1,2-Benzenedicarboxylic acid, butyl 8-methyl	$C_{22}H_{34}O_4$	362
14	19.781	1.50	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
15	20.156	1.06	Hexadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270
16	20.398	0.75	Hexacosane	$C_{26}H_{54}$	366
17	22.308	1.10	Docosanoic acid, ethyl ester	$C_{24}H_{34}O_2$	356
18	23.894	1.85	Gamma-Sitosterol	$C_{29}H_{50}O_2$	414
19	24.272	0.70	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238
20	25.463	4.23	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390

GC-MS analysis of clove (*S. aromaticum*) bud essential oil from Java and Manado, Indonesia, showed that, Java sample contained eugenol (55.60 %), eugenyl acetate (20.54 %), caryophyllene (14.84 %), and α-humulene (2.75 %). While, in Manado sample, the composition were eugenol (74.64 %), caryophyllene (12.79 %), eugenyl acetate (8.70 %), and α-humulene (1.53 %) [16].

GC-MS analysis of clove essential from India identified eugenol (58.29%) and eugenyl acetate (19.10%) as major components [17].

Table 3 Compounds identified by GC-MS from the ethanol extract of clove pods

Peak	R. time	Area%	Name	Mol. form.	Mol. wt
1	7.894	0.41	Phenol,4-(2-propenyl)-	$C_9H_{10}O$	134
2	8.029	0.21	2-Propenal, 3-phenyl-	C_9H_8O	132
3	9.088	81.21	Eugenol	$C_{10}H_{12}O_2$	164
4	9.166	0.72	1,2,3-Benzeneetriol	C_6H_6O	126
5	9.566	0.08	Docosanoic acid, ethyl ester	$C_{24}H_{34}O_2$	356
6	9.599	0.10	Naphtho[2,3-b] furan-2-one,3- [[2-(4-methoxy	$C_{15}H_{20}O_3$	248
7	10.050	4.65	Caryophyllene	$C_{15}H_{24}$	204
8	10.385	0.65	Humulene	$C_{15}H_{24}$	204
9	10.702	10.60	Phenol, 2-methoxy-4-(2-propenyl)- acetate	$C_{12}H_{14}O_3$	206
10	11.584	0.76	Caryophyllene oxide	$C_{15}H_{24}O$	220
11	12.337	0.70	2,3,4-trimethoxyacetophenone	$C_{11}H_{14}O_4$	201

3.3. Biocidal tests

Table (4) showed that, the aqueous extract of clove pods was tested at concentrations of 300.17 – 700.39 mg/L for a period of 24 hours against *Anopheles, Culex* and *Aedes* larvae. The results showed that, at the minimum concentration (300.17 mg/L), 15% of *Anopheles* larvae were killed, while only 5% (one larva) of *Culex* was killed, whereas, 20% of *Aedes* larvae were died. At the higher concentration (700.39 mg/L), the mortality of mosquito's larvae ranged between 75% (in *Anopheles* and *Culex*) - 80% (in *Aedes*). The regression analysis revealed that, the mortalities of *Aedes* and *Anopheles* larvae reflected higher correlation respective to concentrations ($R^2 \geq 0.94$). Also, the LC50 was 498 mg/L for *Aedes* larvae, 561 mg/L for *Anopheles* larvae, and 615 mg/L for *Culex* larvae, hence, *Aedes* larvae was relatively more susceptible to the aqueous extract of clove pods than the other two species of mosquitoes (has the least value of LC_{50}), while *Culex* larvae showed respectively more resistance to clove pods aqueous extract. The LC_{95} confirm the same conclusion. It was noticed that, the control mortality was 0. The polar content of clove pods was 49.26% (almost about half the dry weight of the pods).

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

Concentration		**Corrected Mortality**			**Probit**		
mg/L	**Log**	***Anopheles***	***Culex***	***Aedes***	***Anopheles***	***Culex***	***Aedes***
300.17	2.477	15	5	20	3.69	3.36	4.16
400.22	2.602	25	12.5	30	4.33	3.87	4.48
500.28	2.699	27.5	25	45	4.42	4.33	4.87
600.32	2.778	55	35	60	5.13	4.61	5.25
700.39	2.845	75	75	80	5.67	5.67	5.84
Probit analysis							
R^2					0.94	0.91	0.95
Intercept					-9.02	-10.90	-6.87
Slope					5.10	5.70	4.40
LC_{50} (mg/L)					561.07	615.85	498.57
LC_{95} (mg/L)					1176.49	1194.52	1176.13

Control mortality= 0; Polar contents = 49.26%; Apolar = 35.32%

Table (5) showed that, the ethanol extract of clove pods was tested at concentrations of 269.48 – 628.80 mg/L for a period of 24 hours against *Anopheles, Culex* and *Aedes* larvae. The results showed that, at the minimum concentration (269.48 mg/L), 20% of *Anopheles* and *Culex* larvae were killed, while 35% of *Aedes* larvae were died. At the higher concentration (628.8 mg/L), the mortality of mosquito's larvae ranged between 97.5% (in *Anopheles* and *Aedes*) - 90% (in *Culex*). The LC_{50} was 303.89 mg/L for *Aedes* larvae, 340.97 mg/L for *Anopheles* larvae, and 347.29 mg/L for *Culex* larvae, hence, *Aedes* larvae was relatively more susceptible to the ethanol extract of clove pods than the other two species of mosquitoes. While *Culex* larvae showed respectively more resistance to clove pods ethanol extract. LC_{95} values also confirm the same conclusion. According to the LC's values, it was clear that, the ethanol extract of clove pods was more potent than the aqueous extract.

Cloves and clove oil are both classified as minimum risk pesticides [18]. Clove oil generally recognized as safe substances [19].

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

Concentration		**Corrected Mortality**			**Probit**		
mg/L	Log	*Anopheles*	*Culex*	*Aedes*	*Anopheles*	*Culex*	*Aedes*
269.48	2.43	20	20	35	4.16	4.16	4.61
359.32	2.55	52.5	50	67.5	5.08	5.00	5.44
449.16	2.65	78.5	85	87.5	5.81	6.04	6.18
538.96	2.73	97.5	90	97.5	7.05	6.28	7.05
628.80	2.80	100	95	100	-	6.64	-
Probit analysis							
R^2					0.97	0.97	0.98
Intercept					-16.68	-12.48	-12.95
Slope					8.56	6.88	7.23
LC_{50} (mg/L)					340.97	347.29	303.89
LC_{95} (mg/L)					530.03	601.27	512.32

Control mortality= 0

4. Conclusion

- Saponins, flavonoids, tannins and alkaloids were detected in clove pods, but glycosides and steroids were not detected.
- GC-MS from the hexane extract of clove pods detected the sesqueterpene Eugenol (26%) and caryophyllene (5.45%), while Eugenol (81%); caryophyllene (4.65%) were detected from their ethanol extract.
- For the aqueous extract of clove pods, the LC50 was 498 mg/L for *Aedes l*arvae, 561 mg/L for *Anopheles* larvae, and 615 mg/L for *Culex* larvae.
- For the ethanol extract of clove pods, the LC50 was 303.89 mg/L for *Aedes* larvae, 340.97 mg/L for *Anopheles* larvae, and 347.29 mg/L for *Culex* larvae.
- *Aedes* larvae were relatively more susceptible to the aqueous and ethanol extracts of clove pods than the other two species of mosquitoes.
- The ethanol extract of clove pods was more potent than the aqueous extract.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors (Dalia M. M. Elbashir, Mutaman A. A. Kehail, Yasir Mohamed Abdelrahim and Abdelmonem E. H. Ali) 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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