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Larvicidal Assessment of Fractions of *Aristolochia albida* Rhizome on *Culex quinquefasciatus*

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ABSTRACT

Aristolochia albida is a plant, native of Africa that has found various applications including anti-parasite and anti-snake venom. In the present study, *Aristolochia albida* rhizome was extracted successively with chloroform and methanol by maceration technique. The extracts were tested for their larvicidal activity against 3rd and 4th instar larvae of *Culex quinquefasciatus* mosquito, according to WHO guidelines for larvicidal bioassay, with slight modifications. Fractionation of the chloroform extract was carried out using column chromatography. Larvicidal bioassay was carried out on the fractions obtained and the most potent fraction (fraction S) was further analyzed using preparative Thin Layer Chromatography (TLC) and characterized using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infra-Red (FTIR) spectroscopic analyses. Chloroform extract showed the highest mortality (100%) at 25 ppm, with LC_{50} and LC_{90} values of 4.24 ppm and 7.59 ppm. Fractionation of the chloroform extract of *Aristolochia albida* rhizome yielded 180 fractions. Fraction S was the most potent fraction having the lowest LC_{50} value (1.12 ppm). Preparative TLC profile of fraction S yielded five distinct spots (S1-S1a, S2-S2a and S3). Assessment of the larvicidal activity of the fractions confirmed that they act in a synergistic manner. Likely compounds identified using GC-MS were columbin, benzene acetaldehyde, oleic acid, naphthalene and fucosterol. FTIR analysis identified some functionalities, such as, alcohols, aromatics and carboxylic acids. It was concluded from the present study that chloroform extract and fractions of *Aristolochia albida* possess larvicidal activity against *Culex quinquefasciatus.*

Keywords: Plant extracts, Larvicidal activity, *Aristolochia albida*, *Culex quinquefasciatus.*

Introduction

Presently, the risk of contracting arthropod-borne diseases has increased due to change in climactic conditions and intensifying globalization.[1] Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. Mosquito-borne diseases remain a major health problem in human and animals. Diseases transmitted by mosquitoes include malaria, dengue hemorrhagic fever, Japanese encephalitis, yellow fever and filariasis. These are mostly in tropical and subtropical countries of the world.[2]

A major tool in mosquito control is the application of synthetic insecticides such as organochlorine and organophosphate compounds, but this has not been very successful due to human, technical, operational, ecological and economic factors. Thus, these have prompted researchers to look for alternative approaches ranging from promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friendly alternatives such as biological control of vectors has become the central focus of the control programme in lieu of the chemical insecticides.[3]

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Culex quinquefasciatus is a cosmopolitan mosquito with worldwide distribution, especially in the tropical and subtropical areas and is associated with human dwellings. The adult females lay eggs preferentially in relatively large, permanent aquatic habitats with high concentrations of decomposing organic matter, such as sewage effluents and septic tanks. However, immature stages of this species can be found in artificial containers often filled with polluted or organic-rich water but rarely coexist in the same container with the dengue vector *Aedes aegypti*.[4]

Culex quinquefasciatus causes tremendous nocturnal discomfort and allergic responses due to its nuisance biting.[4] The nuisance biting usually affects a greater proportion of the population than the proportion harboring diseases transmitted by the mosquito species. Sometimes the nuisance level could be extremely high and intolerable. This situation is compounded by the fact that the susceptibility of *Culex quinquefasciatus* to pyrethroids such as permethrin and deltamethrin is relatively lower than those of the anophelines such as *Anopheles gambiae* s.l. and *Anopheles funestus* s.l.[5]

Aristolochia albida is a native plant of West Africa. It belongs to the family Aristolochiaceae, comprising of seven genera of herbs, undershrubs, shrubs and lianas, which are distributed throughout the world in different climatic areas, chiefly in the tropics, subtropics and some in the North Temperate Zone.[6] Various species of *Aristolochia have* been used as medicaments and tonics.[7] Previous research findings reported that a furanoid diterpene lactone belonging to the clerodane series was isolated from the rhizomes of *Aristolochia albida*[8] and identified as columbin.[9] This diterpene was found to significantly reduce the toxic symptoms and protect mice against the lethal doses of venoms of snakes; *Naja nigricolis* (spitting cobra) and *Bitis arietans* (puff-adder), commonly found in Northern Nigeria.[10] This diterpenoid furanolactone (Columbin) from *Aristolochia albida* have also been reported to inhibit growth of culture forms of *Trypanosoma brucei*.[11]

The inhibitory effects of Columbin were also reported on partially purified acidic phospholipase A_2, from *Naja nigricolis in* a dose-dependent pattern.[12]

One of the most effective alternative approaches to the biological control programme is to explore the floral biodiversity with a view of finding safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Unlike conventional insecticides which are based on a single active ingredient, plant-derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavourial and physiological processes. Thus, there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito-borne diseases.[3]

Botanicals are basically secondary metabolites that serve as a means of defense mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. These secondary metabolites have non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components.[13] This in turn, affects insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system; such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway .[13]

Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities.[14] Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical variation and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees can be used for extraction of mosquito toxins. Phytochemicals can be extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, *etc*., of larger plants or trees. In all cases where the most toxic substances were concentrated upon, they can be found and extracted for mosquito control.[3]

More than 2000 plant species have been known to produce chemical factors and metabolites significant in pest control programmes. Members of the plant families; Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various types of larvicidal, adulticidal or repellent activities against different species of mosquitoes.[3]

Previous studies have shown that mosquitoes prefer to bite humans in order to obtain blood meal than any other available host; thus, causing allergic response and increasing the chances of disease transmission. It is easier and more efficient to control the delicate larvae that are relatively immobile and more concentrated, having not yet left their aquatic breeding sites.[15]

Some current biological control methods used in mosquito control include the use of: mosquito predators (fish, amphibians, copepods, including larvae of other mosquito species), Bti (*Bacillus thuringiensis* var. *israelensis*) and entomopathogenic fungi, Wolbachia Endosymbiotic Bacteria, through IIT (incompatible insect technique), sterile insect technique (SIT) and genetically modified mosquitoes; using a lethal self-limiting gene.[16]

However, use of mosquito predators poses a threat to native aquatic fauna, including amphibians, and is frequently not suitable in the majority of urban environments exploited by the larvae of some species, hence, require further research highlighting the need to carefully consider the ecological cost of introducing predatory species in mosquito control. A potential concern for a Wolbachia-replacement approach is the future development of resistance to Wolbachia's inhibitory effects. Although no studies to date have demonstrated that this is likely to happen. The use of SIT for mosquitoes that transmit human disease has been limited due to the reduced performance of sterilized males caused by sterilization. An additional problem for SIT programmes (and any other mosquito suppression strategy that aims for eradication) is the difficulty of the initial need to reduce the wild population densities, prior to the release of sterile males. In lieu of genetically modified mosquitoes, Culicine mosquitoes however contain homomorphic sex chromosomes (containing only a small nonrecombining region) which may limit this approach for major Culicine mosquito vectors.[16]

Culex quinquefasciatus was used as a model for this research due the results of its genome project, that revealed a repertoire of 18,883 protein-coding genes; which is 22% larger than that of *Aedes aegypti* and 52% larger than that of *Anopheles gambiae*. It was discovered that the increased gene numbers in *Culex quinquefasciatus* are partly explained by the presence of expanded gene families of olfactory (smell) and gustatory (taste) receptors, several types of immune-related genes, as well as genes with possible roles in drug metabolism that could influence resistance to insecticides. The wide range of smell and taste receptors may explain the opportunistic blood-feeding behaviour of *Culex quinquefasciatus* females, which are able to detect and feed upon birds, humans and livestock.[17]

According to previous researches, *Aristolochia albida have* been reported to possess some medicinal properties which includes anti-microbial,[18] anti-trypanocidal [11] as well as anti-snake activity,[12] however studies on its larvicidal effect against *Culex quinquefasciatus* mosquito has not yet been ascertained, hence the need for this research.

Materials and Methods

Plant sample collection and identification

Aristolochia albida rhizomes were purchased on 5th April 2016, from a herbalist in Amaru market, Zaria city, Kaduna State. The plant part was identified and authenticated in the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria and a voucher specimen (1700) was deposited.

Preparation and extraction of plant sample

Rhizomes of *Aristolochia albida* were washed and air dried at room temperature. Dried samples were ground into a powdered form, using pestle and mortar. The powdered plant material (500 g) was successively extracted with chloroform (1.3 L), methanol (1.7 L) and distilled water (1 L) by maceration. The extracts were concentrated over a water bath at 50ºC (chloroform and methanol extracts) and at 70ºC (water extract). The crude extracts were stored in glass containers and kept in a dessicator prior to use.[19]

Qualitative phytochemical screening of Aristolochia albida

The methods described by Trease and Evans[20] were used to test for the presence of phytochemicals in the crude extract.

Mosquito larvae collection and breeding

Adult (blood fed) mosquitoes were collected around Amina hostel during the early hours of the day, around 7:30-8:00 am. The larvae were identified and authenticated in the Entomology Research laboratory of the Department of Zoology, Faculty of Biological Sciences, Ahmadu Bello University, Zaria. Adult blood-fed mosquitoes were collected in plastic bottles and introduced to cages containing water sprinkled with larval feed (Brewer's yeast and crackers biscuits), at a temperature of about 25-29℃, and a relative humidity of 80-90% in the laboratory. Adult mosquitoes laid eggs in rafts which later hatch to hundreds of miniature larvae. Larvae develop to 3rd and 4th instar larvae which were used for the bioassay; according to WHO guidelines for larvicidal bioassay, with slight modification. [21]

Preparation of stock solution of Aristolochia albida extracts

Twenty millilitres (20 mL) of each crude extract (chloroform, methanol and aqueous) of *Aristolochia albida* rhizome were prepared separately by weighing 200 mg into a beaker, which was dissolved in 5 mL DMSO (dimethyl sulfoxide). Distilled water (15 mL) was added, to make it up to 20 mL. This was stored in a screw-cap vial, prior to use.[21]

Larvicidal activity screening of the crude extract of Aristolochia albida

The larvicidal activity was performed according to the standard WHO method.[21] Replicates (three) of 25 late 3rd and early 4th instar larvae of Culex mosquito strains were used for the bioassay. Five concentrations (25, 50, 100, 200 and 400 ppm) of the chloroform, methanol and aqueous extracts were prepared in 100 mL boiled and cooled water. Controls containing DMSO and water were run simultaneously. The numbers of dead and live larvae in the replicates were recorded after 6 h, 12 h, 24 h and 48 h. The results were expressed as percentage

mortality. The dose mortality response of the respective extracts was subjected to log-probit regression analysis to determine lethal concentrations (LC) at LC_{50} and LC_{90}.[21]

Larvicidal activity screening of fractions of chloroform extract of Aristolochia albida
Stock solution of each fraction was prepared by weighing 150 mg of the fraction dissolved in 5 mL DMSO. Distilled water was added to make it up to 20 mL. Concentrations of 25, 50 and 100 ppm were used for the bioassay. Mortality was recorded after 6, 12, 24, and 48 h.[21]

Thin layer chromatography (TLC)
TLC analyses were performed on precoated silica gel 60 F_{254} plates. The plates were visualized under UV (254, and 366 nm), and by spraying with p-anisaldehyde reagent and oven-dried at about 60°C to reveal the spots.[22]

Column chromatography
The crude chloroform extract of *Aristolochia albida* rhizome was subjected to silica gel (60-120 mesh,120 g) column chromatography (25 cm x 3.1 mm). The column was eluted with hexane:ethyl acetate in a step wise gradient of increasing polarity (n-Hexane:Ethylacetate; 100:0; 90:10; 80:20; 60:40; 40:60; 20:80; 0:100). Fractions (30 mL each) were collected. The solvent was allowed to evaporate, and the fractions were analysed for purity on TLC plate. Fractions with similar TLC profiles were pooled together; after which larvicidal assay was carried out with the various pooled fractions at different concentrations.[23]

Preparative Thin Layer Chromatography (PTLC)
Preparative thin layer chromatography was carried out on fraction S (most potent fraction) obtained from the column chromatographic separation of *Aristolochia albida* rhizome. Fraction S (0.5 g/3 mL of distilled water) was applied as a band on PTLC plate. The plate was developed using hexane:ethyl acetate (1:1) and air-dried. A small portion (1 cm) of the developed plate was cut off, sprayed with P-anisaldehyde and oven-dried at 60°C. The cut off portion of the TLC plate was matched with the developed TLC plate, to ascertain the position of the bands on the developed TLC plate. The developed plate was carefully scraped into different beakers and transferred into centrifuge tubes; methanol (5 mL) was then added to the tubes and centrifuged for 30 minutes. The supernatant layer was decanted into beakers and then air-dried to recover the separated portions.[22]

Determination of possible synergy in the effect of fractions of chloroform extract of Aristolochia albida on mortality of Culex quinquefasciatus larvae
Stock solution of fraction S1-S1a, S2-S2a, S3 and oleic acid were prepared by weighing 100 mg of the fraction and dissolving in 5 mL DMSO. Distilled water was added to make it up to 20 mL. Concentrations of 1, 5, 10, 25, 50 and 100 ppm were used for the fraction bioassay in the combinations S1/S2, S1/S3, S2/S3, oleic/S1, oleic/S2 and oleic/S1/S2 of fraction S. Mortality was recorded after 6, 12 and 24 h.[24]

GC-MS analysis
GC-MS analysis was carried out on fraction S, using helium as the carrier gas, at a flow rate of 1.2 mL/min. The injection volume was 1 µL. The inlet temperature was maintained at 230ºC. The oven temperature was programmed initially at 50ºC for 5 min, then programmed to increase to 300ºC at a rate of 10ºC every 25 min. Total run time was 45 min. The MS transfer line was maintained at a temperature of 300ºC. The source temperature was maintained at 230ºC and the MS Quad at 150ºC. The ionization mode used was electron ionization mode at 70eV. Total Ion Count (TIC) was used to evaluate for compound identification and quantitation. The Spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST02 Reference Spectra Library. Data analysis and peak area measurement was carried out using Agilent Chemstation Software.[25]

FTIR analysis
The FTIR spectra of fraction S were recorded on KBr disc on an FTIR instrument (Model/Make: IFS 25, Bruker, Germany), with PC based software-controlled instrument operation and data processing. The spectral data were compared with a reference, to identify the functional groups existing in the sample.[26]

Statistical analysis
Statistical analysis was done using SPSS software (Version 20). The lethal concentration was calculated using Probit analysis, mortality data was analysed by ANOVA while differences between means was considered significant at $P \leq 0.05$.

Results and Discussion

The larvicidal potential of plant metabolites have been documented by several researchers.[23,27-30] None has reported the larvicidal potential of extracts and fractions of the rhizomes of *Aristolochia albida*. Therefore, to the best of our knowledge, this study represents the first report of the larvicidal potential of the chloroform, methanol and aqueous extract of *Aristolochia albida* on *Culex quinquefasciatus*.
Table 1 shows the percentage mortality and lethal effect of chloroform and methanol extracts of *Aristolochia albida* rhizome on larvae of *Culex quinquefasciatus* mosquito. Two of the extracts (chloroform and methanol) used in this investigation had notable larvicidal effect on larvae of *Culex quinquefasciatus* mosquito. After 48 h exposure, 25 ppm, 50 ppm, 100 ppm, 200 ppm and 400 ppm of the chloroform extract of *Aristolochia albida* resulted in 100% mortality. The same concentration of methanol extract produced 93%, 93%, 73%, 100% and 100% mortality, respectively. However, no mortality was recorded for the aqueous extract.
The LC_{50} and LC_{90} values for the chloroform extract were 4.24 ppm and 7.59 ppm, respectively while the LC_{50} and LC_{90} values for the methanol extract were 6.00 ppm and 10.17 ppm, respectively. From the LC_{50} values the chloroform extract was found to have the most potent larvicidal activity. Thus, potency of the extracts was in the order: Chloroform > Methanol > Aqueous. A gradient of increasing mortality with increase in extract concentration and increase in exposure time was observed in all the treatments. No mortality was observed in the controls, rather larvae in the control experiments were agile and actively wriggling, throughout the duration of the experiment.
Plant metabolites detected in the crude extract of *Aristolochia albida* rhizomes may have exerted single, additive or synergistic effect on the larvae. However, the biological activity of an extract is known to be dependent on some factors; one of which is the solvent used for extraction.[3] Based on the 24-hour LC_{50} values obtained, the chloroform extract was found to be more potent than the other extracts used in this study, suggesting that the organic solvent (chloroform) might have enhanced the extraction of constituents in the rhizomes of *Aristolochia albida* with potent larvicidal activity against *Culex quinquefasciatus* larvae.
Ghosh *et al*., reported that chloroform is a moderately polar solvent which mainly extracts phytochemicals such as steroids, alkaloids, sterols, terpenoids, saponins, flavonoids and tannins. Therefore, the presence of these phytochemicals distributed in each fraction might have influenced the larvicidal property of the plant.[31]
Results from this research indicated that organic extract exhibit a greater larvicidal effect than aqueous extract, a trend also noted in a previous larvicidal study.[32] It was also observed in this study that the crude chloroform and methanol extracts induced mortality within 24 h, suggesting a very high toxic effect which may be attributed to the complex mixture of active compounds.[33]
Effective larvicide can reduce the number of adult mosquitoes available to spread diseases; create nuisance and lay eggs.[34] Review papers have documented the toxic effect of plant extracts on mosquito eggs, larvae and pupae.[35,36] Larvicidal activity of *Plectranthus glandulosus* leaves against three major vector mosquitoes, viz. *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus* was reported with LC_{50} values of 167.85, 201.50 and 76.21 ppm, respectively.[37]
From the results obtained, it is evident that larval mortality increased with increase in concentration of both extracts and fractions of *Aristolochia albida* rhizome. This confirms the report of, Shadia *et al*., which states that there is a positive correlation between concentration and the percentage of larval mortality.[39]

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Table 1: Percentage mortality and lethal concentration of chloroform, methanol and aqueous extract of *Aristolochia albida* against *Culex quinquefasciatus* mosquito.

	Percentage mortality						**Lethal concentration**	
Extract	**Conc (ppm)→ Time ↓**	25	50	100	200	400	LC_{50}	LC_{90}
CHIOROFORM	6 h	87	87	27	80	87	**4.24**	**7.59**
	12 h	100	93	87	87	100	3.089-5.38	5.89-13.57
	24 h	100	100	100	100	100		
	48 h	100	100	100	100	100		
METHANOL	6 h	33	53	-	20	80	**6.00**	**10.17**
	12 h	67	67	7	47	93	5.13-7.02	8.30-16.34
	24 h	93	93	27	87	100		
	48 h	93	93	73	100	100		

Table 2: Phytochemical constituents of chloroform, methanol and aqueous extract of *Aristolochia albida* rhizomes.

Phytochemical	**Extracts**		
	Chloroform	**Methanol**	**Aqueous**
Carbohydrates	+	_	+
Cardiac glycosides	+	+	_
Cardenolides	_	+	_
Sterols	+	+	+
Free anthraquinones	_	_	_
Combined anthracene	_	_	_
Saponins	_	+	+
Steroids	_	_	+
Triterpenes	+	+	_
Flavonoids	+	+	+
Tannins	_	_	+
Alkaloids	+	+	+

Key (+) = present; (-) = absent

Table 4: Lethal concentration (LC_{50} and LC_{90}) of chloroform-extract fractions of *Aristolochia albida* rhizome against *Culex quinquefasciatus mosquito*.

FRACTIONS	**Lethal Conc. (LC) in ppm**	
	LC_{50}	**LC_{90}**
B	2.79	30.12
P	2.71	34.15
Q	3.98	93.88
R	2.68	7.12
S	1.16	5.45
T	1.59	5.39
U	2.43	9.17

Table 3: Percentage mortality of chloroform-extract fractions of *Aristolochia albida* rhizome against *Culex quinquefasciatus* mosquito.

		PERCENTAGE MORTALITY (%)		
		CONCENTRATION (ppm)		
FRACTIONS	**Time**	**25**	**50**	**100**
Q	6 h	7	7	7
	12 h	20	40	33
	24 h	33	60	53
	48 h	47	73	73
B	6 h	13	13	27
	12 h	33	33	40
	24 h	33	53	60
	48 h	40	60	87
R	6 h	-	-	13
	12 h	7	7	47
	24 h	7	33	93
	48 h	27	47	100
U	6 h	-	13	13
	12 h	13	20	60
	24 h	33	40	87
	48 h	47	53	100
P	6 h	7	13	20
	12 h	27	13	33
	24 h	47	40	73
	48 h	53	80	100
T	6 h	-	13	13
	12 h	13	40	100
	24 h	53	100	100
	48 h	67	100	100
S	6 h	7	13	27
	12 h	33	40	100
	24 h	53	100	100
	48 h	100	100	100

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Table 5: Mean mortality (no. of dead larvae per treatment; n=15) due to synergistic application of fraction S sub-fractions of chloroform extract of *Aristolochia albida* on *Culex* larvae.

Fractions	Time	Concentration		
		10 ppm	25 ppm	50 ppm
S1-S1a/S2-S2a	6 h	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	12 h	0.67 ± 1.15[a]	1.00 ± 1.73[a]	2.00 ± 1.00[a]
	24 h	5.00 ± 4.00[ab]	6.67 ± 7.51[b]	13.00 ± 2.65[c]
S1-S1a/S3	6 h	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	12 h	0.00 ± 0.00[a]	1.67 ± 2.08[a]	1.00 ± 1.00[a]
	24 h	1.00 ± 1.00[a]	3.33 ± 2.08[ab]	4.67 ± 3.52[c]
S2-S2a/S3	6 h	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	12 h	0.33 ± 0.58[ab]	1.00 ± 1.00[ab]	1.33 ± 0.58[b]
	24 h	1.00 ± 1.00[a]	4.33 ± 1.53[b]	6.67 ± 3.05[b]
Oleic[y]+S1-S1a	6 h	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	12 h	0.33 ± 0.58[a]	1.00 ± 1.00[a]	0.67 ± 0.58[a]
	24 h	0.33 ± 0.58[a]	1.33 ± 1.53[a]	4.33 ± 2.52[b]
Oleic[y]+S2-S2a	6 h	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
	12 h	0.00 ± 0.00[a]	0.67 ± 0.58[a]	1.33 ± 1.53[a]
	24 h	0.33 ± 0.58[a]	1.67 ± 1.53[ab]	3.00 ± 1.73[b]
Oleic[y]+S1-S1a	6 h	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.67 ± 1.15[a]
	12 h	0.67 ± 1.15[a]	1.33 ± 1.53[a]	3.33 ± 1.53[b]
	24 h	2.00 ± 2.65[ab]	5.00 ± 2.00[b]	13.00 ± 2.65[c]

y-The oleic acid used is an analog of oleic acid
x-Values are expressed as mean ± SD of three replicates
z-Values with different superscript along rows are significantly (P≤0.005) different

Table 6: Compounds identified using GC-MS analysis from fraction S of *Aristolochia albida* rhizome.

Retention Time (mins)	Compound	Chemical Formula	Percentage composition
28.35	Oleic acid	$C_{18}H_{34}O_2$	99
34.11	Columbin	$C_{20}H_{22}O_6$	59
35.36	Benzeneacetaldehyde	$C_9H_{10}O$	15
35.83	Naphthalene	$C_{10}H_8$	25
37.20	Fucosterol	$C_{29}H_{48}O$	25

Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenols from different plants have been reported previously for their larvicidal activities.[40] Some plant metabolites such as alkaloids, cardenolides, cardiac glycosides, flavonoids, saponins, sterols, steroids, tannins and triterpenes were detected in the rhizomes of *Aristolochia albida*; however, studies on their larvicidal effect against *Culex quinquefasciatus* mosquito has not been evaluated. The chloroform extract was the most potent extract among the three solvents used; this however may be due to the presence of phytochemicals such as: cardiac glycosides, sterols, triterpenes, flavonoids and alkaloids in the extract (Table 2). Cardiac glycosides are known to inhibit the action of membrane-bound enzyme Na^+/K^+-ATPase.[41] Terpenoids are known to possess insecticidal properties,[42] neurotoxic and mediate their toxic action via acting on acetylcholinesterase and octopaminergic system.[43] Flavonoids are known to possess insecticidal properties and act as mitochondrial poisons for insect vectors.[42] The mode of action of alkaloids on insect vectors varies with the structure of their molecules, but many are reported to affect acetylcholinesterase or sodium channels.[44]

Fractionation of the chloroform extract of *Aristolochia albida* rhizome using silica gel column chromatography yielded 180 fractions which were pooled together based on similarities of their TLC profile to give 21 pooled fractions.

Table 3 shows the percentage mortality of *Culex quinquefasciatus* larvae upon treatment with fractions of chloroform extract of *Aristolochia albida* rhizome. Five of the fractions (T, U, R, B and S) used for the bioassay had notable larvicidal activity against larvae of *Culex quinquefasciatus* mosquito. The LC_{50} and LC_{90} values were 1.59 ppm and 5.45 ppm, 2.43 ppm and 9.17 ppm, 2.69 ppm and 7.12 ppm, 2.79 ppm and 30.13 ppm, 1.12 ppm and 5.45 ppm for fractions T, U, R, B and S, respectively (Table 4).

The TLC profile of fraction S gave three distinct spots. Further analysis was carried out using preparative TLC to separate and recover the various fractions from fraction S. However, after TLC of the three separated assumed-compounds, five spots were seen on the TLC plate (S1-S1a, S2-S2a and S3).

Synergy test was then carried out using the recovered three fractions (from PTLC) for comparison of toxicity. Fractions S1-S1a and S2-S2a showed no larvicidal activity while fraction S3 resulted in 13% mortality; this mortality was low compared to the parent fraction (fraction S), therefore suggesting that the compounds act in a synergistic manner thus yielding the best result when combined than when isolated (Table 5).

The GC-MS analysis of fraction S identified five major compounds; oleic acid, columbin, benzene acetaldehyde, naphthalene and fucosterol (Table 6, Figures 1 and 2). In a previous research, it was reported that columbin; a compound isolated from *Aristolochia albida* rhizome inhibits phospholipase A_2-catalysed hydrolysis of Red Blood Cell (RBC) which was linked to its antivenom properties[12] and also its

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trypanocidal effect *in-vitro* and *in vivo* in *Trypanosoma brucei* infected mice.[11] Columbin was also among the compounds identified in the GC-MS results of the most potent fraction (fraction S). However, the larvicidal effect was likely due to synergy of fractions S1-S1a, S2-S2a and S3.

Control of mosquito vector can be achieved through focusing on reduction efforts on the larval stage prior to dispersal or acquisition of the disease and interrupting the life cycle before it can cause harm.[45] Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are man-made and can be easily identified.[46]

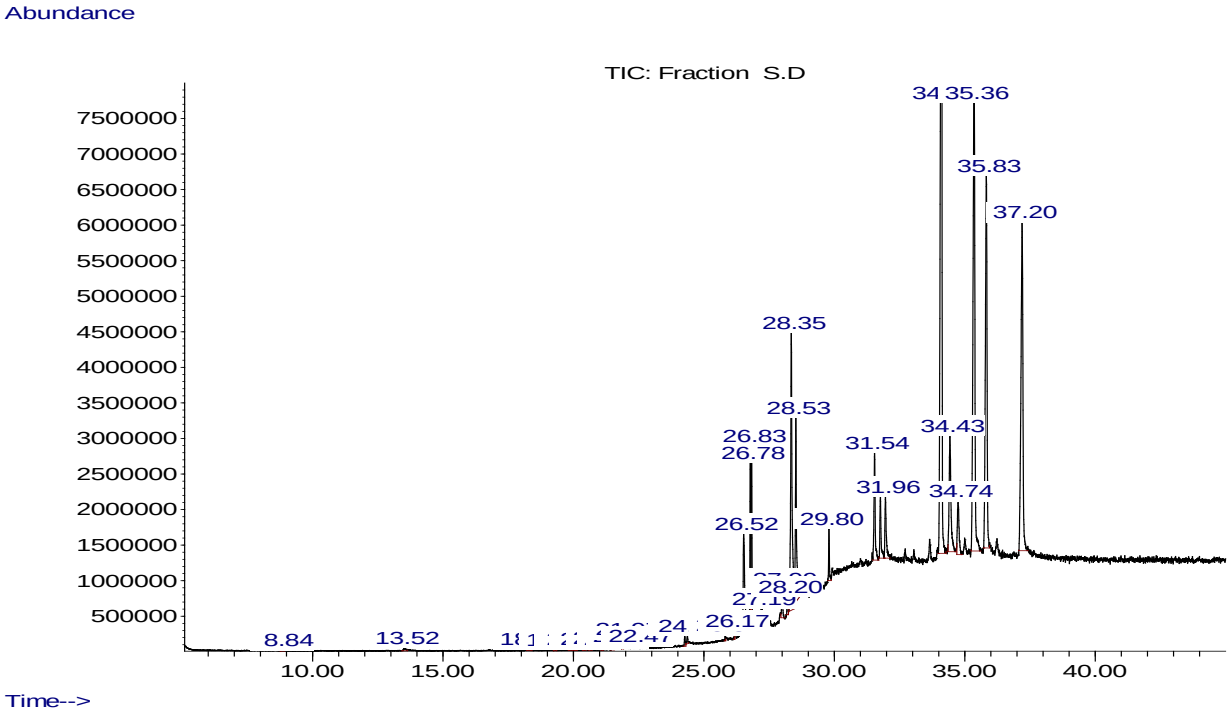

Figure 1: GC-Mass spectra of fraction S of chloroform extract of *Aristolochia albida* rhizome.

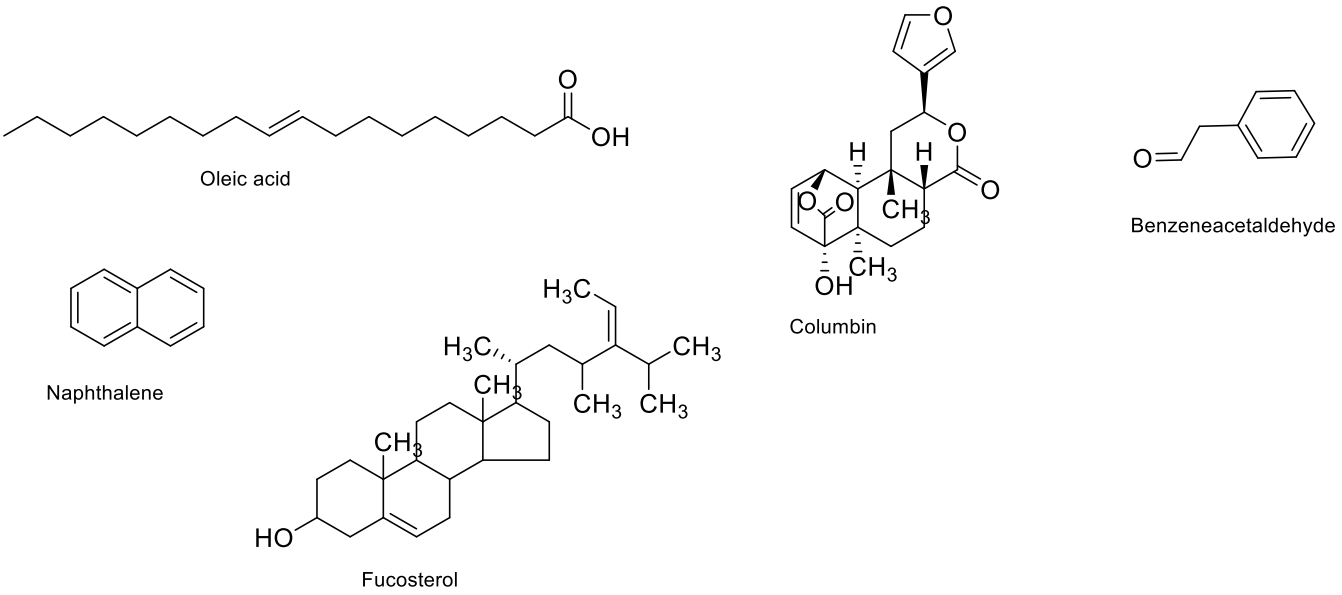

Figure 2: Chemical structures of compounds identified from the GC-MS analysis of Fraction S.

Conclusion

The data obtained in this study has provided information on the larvicidal activity of crude extracts and partially purified fractions of *Aristolochia albida* rhizome, thus indicating a potential for use in the control of mosquito larvae. The chloroform extract and fractions exhibited the highest larvicidal activity against *Culex quinquefasciatus* larvae; this may be due to the presence of phytochemicals such as terpenes (columbin and fucosterol) identified in the plant rhizome.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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