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Extraction, Characterization and Larvicidal Activity of Essential Oil and Hydrosol from *Sida acuta* Burm. f. Leaves Grown in Nigeria

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ABSTRACT

Mosquitoes transmit the malaria parasite, causing diseases and thousands of deaths annually. The major problems associated with the use of chemicals for the control of mosquitoes include the development of resistance to these chemicals by the *plasmodium* parasite and their undesirable toxic effects on humans and the environment. In this research, the volatile oil was extracted (in n-hexane) from the air-dried leaves of *Sida acuta* by hydrodistillation method. The hydrosol was extracted with n-hexane in the ratio 10:2. The oils were analysed using Gas Chromatography–Mass Spectrometry (GC-MS).The larvae of the mosquito species were exposed to five different test concentrations of the volatile oil and hydrosol oil of *Sida acuta* and were assayed. The larval mortality was observed and IC_{50} value was calculated using the probit analysis test. The GC–MS chromatograph of the oil revealed a total of 17 volatile constituents. Heneicosane (13.73%), docosane (11.11%), 1-iodohexadecane (10.06%) and hexa-hydrofarnesyl acetone (9.55 %) were the predominant compounds. The hydrosol oil comprised of 11 volatile constituents with triacontane (16.76 %), phytane (16.33 %), tricosane (14.91%) and octane (13.04 %) as the most abundant major constituents. The oils displayed varying degrees of larvicidal activities against the larvae, with highest larval mortality percentage of 80 % and 70% at 500 mg/L respectively in comparison to chloroquine, positive control (90%) and hexane, negative control (0%). The results show that the essential oil and hydrosol of *Sida acuta* Burm. f. possesses strong larvicidal properties.

Keywords: Hydrodistillation, Larvicidal, *Sida acuta*, Volatile oil, Hydrosol.

Introduction

Mosquitoes transmit the malaria parasite, causing diseases and thousands of deaths annually. Currently, the control of malaria relies heavily on synthetic insecticides and antimalarial agents. However, the major problems associated with the use of chemicals for the control of mosquitoes include the development of resistance to these chemicals by the plasmodium parasite and their undesirable toxic effects on humans and the environment.

Sida acuta (family: Malvaceae) commonly known as broom/wire weed is a perennial shrub is widely distributed in the subtropical regions where it frequently dominates bushes, pastures, cultivated lands, wastelands, roadsides and lawns.[1] Traditionally, *Sida acuta* is often used to treat diseases such as; fever, headache, skin diseases, diarrhea and dysentery. The oil extracted from this plant is also used to alleviate pain.[2] The plant is widely used for its various pharmacological properties. Indoloquinoline alkaloids which are present in the plant have a good antimicrobial activity against Gram-positive bacteria.[3] Traditionally *Sida acuta* is used in the form of extracts/powder/paste by tribal populations of India for treating common ailments like cough and cold, fever, stomach, kidney and liver disorders, pains, inflammations and wounds.[4] Alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenoids, anthraquinones and steroids are found in the leaf, stem and root of the plant at varying levels and all the plant part exhibited inhibitory activities against *Pseudomonas aeruginosa*, *Micrococcus varians*, *Candida albicans* while the root extract gave highest inhibition of *Escherichia coli*, *Salmonella typhi* and *Aspergillus flavus* at 500 mg/ml.[5] Various parts of *Sida acuta* have been reported in many studies to be used by indigenous people from tropical countries to manage some health problems: rheumatic affections, azoospermia, oligospermia and spermatorrhea, leucorrhoea, wounds, sciatica, nervous and heart diseases, cold, cough, asthma, tuberculosis and respiratory diseases, disorders of the blood, bile and liver, elephantiasis, hemorrhoids, ulcers, gastric disorders and abdominal pain, headache, fever and malaria, skin diseases, worms, diarrhea and dysentery, venereal diseases, renal

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inflammation, toothache and snake bites.[6] The antiplasmodial and safety profile of alkaloid, flavonoid and phenol extracts of *Sida acuta* (300 and 600 mg/kgbw) on hepatic and renal integrity of rats produced parasitaemia suppression of 50.83%, 33.50% and 64.64%, respectively. The integrity of hepatocytes and renal cells increased with increase extract concentrations and no degenerative changes were observed in all treatment groups.[7] In another research the antiplasmodial effect of extracts of crude ethanol and alkaloid of *Sida acuta* leaf investigated in *Plasmodium berghei* infected mice showed a reduction on the fourth day of treatment of the Plasmodium parasite level for the crude ethanol extract and extracted crude alkaloids to 18.0 and 5.0 parasite per field respectively compared to chloroquine (8.2 parasite level per field). The control untreated group showed increased parasitemia level of 110 parasites per field within the same duration.[8] Several reports abound in literature on the biological potencies and uses of crude extracts and isolated constituents of sida acuta, but there are no reports on the uses of the volatile oils of the same plant. It is on this premise that this research is focused on the search for natural volatile oil-based control agent for malaria, which pose no (or reduced) risks to humans and other organisms.

Materials and Methods

Plant material
Sida acuta were collected in July, 2019 from Ijobo area of Ikorodu, Lagos State, Nigeria. The botanical identification and authentication was done at the Herbarium of the Department of Botany, University of Lagos, Nigeria and voucher specimen was deposited for future reference with voucher number LUTH7958. The fresh leaves were air-dried to a constant weight for a period of one week and pulverized using a mechanical grinder, Micron Glacis GC fine grinding mill prior to extraction.

Extraction of volatile oil
Essential oil from air-dried leaves of *S. acuta* was extracted by hydrodistillation using the modified Clevenger-type apparatus. The extraction was carried out by loading 400 g of *S. acuta* leaves in 1 litre of water in the round bottomed compartment of the hydrodistillation setup and heating for 4 hours at a temperature of about 70^{o}C. The oil was dried over anhydrous sodium sulfate and stored in a sealed vial prior to analysis[9].

Extraction of hydrosol
The hydrosol which was the floral water left in the round bottomed flask was extracted with n-hexane. This was done to isolate any volatile oil still present in it. To every 10 mL of the hydrosol measured into a separatory funnel, 2 mL of n-hexane was added, shaken and the n-hexane fraction dried over anhydrous sodium sulphate (Sigma-Aldrich, USA) and stored in a refrigerator prior to analysis.

GC-MS analyses of volatile oil and hydrosol oil
The chemical composition of the essential oil and hydrosol oil extracted from *S. acuta* leaves were determined using gas chromatography-mass spectrometry (GC-MS).[10,11] GC/MS analyses was performed on a Perkin Elmer Turbo mass Clarus 600 Instrument at 70 eV ionization energy with a mass range of 40–500 amu, employing an Elite-5 column (5% phenyl and 95% dimethylpolysiloxane) of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness (PerkinElmer, USA). Helium was used as a carrier gas at a flow rate of 1 mL/min. The initial temperature was 60°C (1 min), this was increased to 240°C at the rate of 6°C/min, and remained at 240°C for 6 min, and then continued to increase to 250°C at the rate of 10 °C/min, with a final stage of 10 min at 250°C. The oven temperature was programmed from 50°C to 250°C at a 5°C/min dynamic rate, and remained for 15 min at 250 °C. Each sample (0.1 uL diluted in n-hexane in the ratio 4:1) was injected with a splitless mode.

Identification of constituents present in volatile oil and hydrosol
Identification of components in the volatile oil and hydrosol oil was based on the comparison of their mass spectra and retention time with those in literature and by computer matching with NIST 2020 and WILEY libraries as well as by comparison of the fragmentation patterns of the mass spectral data with those reported in the literature.[12,13] Relative percentage amounts of the volatile oil components were evaluated from the total peak area (TIC) by apparatus software. Average values of three replicates along with their standard deviation values are presented and discussed.

Larvicidal activity
Test Organism
The larvae of *Anopheles stephensi* was obtained from different breeding sites including: gutters, open drains, domestic run-offs, abandoned ponds, discarded tanks, tyres and plastics. The larvae was collected with the aid of plastic dippers and stored in the Vector Control Laboratory, Department of Zoology, University of Lagos, Nigeria.

Larvicidal bioassay
Twenty (20) larvae each of the mosquito specie were exposed to five different test concentrations (50, 100, 200, 300 and 500 mg/L in n-hexane) of the essential oil and hydrosol of *Sida acuta* each and were assayed. All trials were conducted at ambient temperatures that ranged between 23^{o}C-27^{o}C. The larval mortality was observed after (10, 15, 20, 30, 60) minutes of exposure respectively. Hexane and chloroquine were used as negative and positive controls respectively

Statistical analysis
The average values of three replicate percentage compositions of the oils were calculated and presented. The IC_{50} value was calculated using the u SAS Proc Probit analysis test[14,15].

Results and Discussion

The volatile oil of *Sida acuta* leaves and its hydrosol oil were yellow and pale -coloured with camphoraceous aroma and percentage yield of 0.80% and 0.20 % respectively, an indication that the leaves is richer in oil than hydrosol. GC-MS analysis of the essential oil and hydrosol oil of *Sida acuta* revealed a total of 17 and 11 volatile constituents accounting for 99.99 % and 100 % of the total oil of the essential oil and hydrosol respectively as shown in (Table 1). This indicates that the essential oil of *S. acuta* had more chemical constituents than its hydrosol. The constituents of the essential oil and its hydrosol were made up of mostly hydrocarbons and their derivatives. The predominant compounds present in the volatile oil were heneicosane (13.73%), docosane (11.11%), 1-iodohexadecane (10.06%) and hexahydrolfarnesyl acetone (9.55 %), while the hydrosol oil had triacontane (16.76%), phytane (16.33 %), tricosane (14.91%) and octacosane (13.04%). This could be attributed to the fact that most of the volatile constituents of the oil may have been extracted during the hydrodistillation process. The present study revealed that the essential oil of *Sida acuta* had leaves more chemical compounds than its hydrosol. Two chemical components; tricosane and 1-iodohexadecane were present in both the essential oil and hydrosol, though in slightly varying quantities while the two oils showed a marked difference in their chemical composition as some constituents were either present in one method and absent in the other. Several authors have reported different volatile constituents of essential oils obtained from different plant species which are in agreement with the volatile constituents obtained in this study. The essential oil of *Thaumatococcus danielli* was reported to contain predominantly long chain saturated hydrocarbons and its derivatives;[16] this is similar to the essential oil and hydrosol constituents revealed in this study. Long-chain alkanes are toxic to the insects[17]. Eicosene, heneicosane and tetracosane which were

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present in the oil from this study were also the major constituents in the essential oil of *Azadirachta indica* leaves[18]. Thymol, phytol, hexahydrofarnesyl acetone and docosane which were detected in the essential oil of *S. acuta* from this study were among the 119 compounds listed as sources of anti-insect properties from the 10 *Eucalyptus* species analyzed in Tunisia[19]. The insecticidal properties of hydrocarbons of the aliphatic series from methane to hexacosane have been studied. It was established that the insecticidal activity of the hydrocarbons increases with an increase in their molecular weight[20]. The volatile oil and the hydrosol oil of *Sida acuta* leaves showed varying degree of larvicidal activities at different concentrations and time (Tables 2-5). The volatile oil showed a higher larval mortality rate at each concentration and time compared to its hydrosol oil. At 60 mins, 8, 11, 12, 15 and 16 mosquito larvae were killed by 50, 100, 200, 300 and 500 mg/L of the volatile oil respectively, while 5, 7, 8, 11 and 14 mosquito larvae were killed by 50, 100, 200, 300 and 500 mg/L of the hydrosol oil at the same respectively (Tables 2-3)

The volatile oil and hydrosol oil of *S.acuta* showed considerable *in vitro* cytotoxicity against *Anopheles stephensi* with LC_{50} values 106.40 mg/L - 80.00 % killing and 101.22 mg/L -70 % killing at 500.00 mg/L respectively (Tables 4 - 5). Comparatively, the essential oil showed higher larvicidal activity than the hydrosol of *Sida acuta*. At 60 mins,12, 12, 13, 17 and 18 mosquito larvae were killed by 50, 100, 200, 300 and 500 mg/L of the standard drug (chloroquine) respectively with LC_{50} value of 87.11 mg/L - 90.00 % killing, 10 % higher than that of the essential oil and 20 % higher than that of the hydrosol of *S. acuta*. The negative control (n-hexane) showed no larvicidal property. The larvicidal activity of the volatile oil and hydrosol oil of *Sida acuta* leaves, in a similar manner as the standard drug, were dose dependent. There are several reports on the larvicidal activity of different constituents of *sida acuta*. For instance, heneicosane is known as an insect pheromone and it is effective against the mosquito vector, *Aedes aegypti*.

Table 1: Chemical composition (%) of volatile oil and hydrosol oil from *Sida acuta* leaves

Compounds[a]	RI[b]	Essential oil (%)	Hydrosol (%)	m/z values
Thymol	1262	4.40	-	150,135,128,121,115,107,91
Cis-2-methyl-7-octadecene	1854	1.42	-	266,235,208,125,97,69,43
Neophytadiene	1774	6.54	-	278,263,193,137,68,82,57
1-Bromodocosane	2504	-	6.53	388,309,211,135,97,71,57
Hexahydrolfarnesyl acetone	1754	9.55	-	268,250,124,109,71,58,43
Phytane	1753	-	16.33	282,239,197,85,71,57,43
Phytol	2045	3.19	-	296,278,235,123,81,71,57
Citronellyl propanoate	1402	4.67	-	155,138,123,109,95,81,69
2-(octadecyloxy)Ethanol	2328	7.69	-	311,296,283,111,97,71,57
1-Iodohexadecane	2026	10.06	5.46	352,225,196,169,85,71,57
Heneicosane	2109	13.73	-	296,266,239,155,99,57,43
Tetracosane	2407	-	2.69	338,309,253,85,71,57,43
Carbonic acid, eicosyl vinyl ester	2541	2.52	-	325,280,180,125,85,71,57
Docosane	2208	11.11	-	310,280,239113,71,57,43
2-Methyl hexacosane	2641	3.02	-	365,337,183,113,85,71,57
Hexacosane	2606	-	7.58	366,323,239,183,113,85,57
Tricosane	2307	7.57	14.91	324,294,141,85,71,578,43
Octacosane	2804	-	13.04	394,323,225,85,71,57,43
Nonacosane	2904	-	3.53	408,366,337,183,141,85,71
Hentriacontane	3103	2.15	-	436,394,351,280,97,71,57
Tritriacontane	3301	5.81	-	464,434,407,239,127,71,57
Tritetracontane	4295	4.02	-	604,518,404,322,127,71,57
2,6,11-trimethyl Dodecane	1320	-	6.20	212,196,154,85,71,57,43
3-Eicosene, (E)	2107	2.54	-	280,252,180,97,69,57,43
Triacontane	3003	-	16.76	422,323,253,225,197,71,57
Nonahexacontanoic acid	7236	-	6.97	334,291,253,210,71,54,44
Total Percentage (%)		99.99	100	
No of Compounds		17	11	

a- Compounds are listed in order of elution from capillary column coated with Elite-5 column.
b- Retention Indices on fused capillary column coated with Elite-5 column.

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Table 2: Larvicidal activity of the volatile oil from *S. acuta* leaves on *A. stephensis* larvae in comparison with Positive control.

Concentration (mg/L) of the volatile oil of *S. acuta*						Concentration (mg/L) of positive control				
Time (min)	**50**	**100**	**200**	**300**	**500**	**50**	**100**	**200**	**300**	**500**
10	1	1	2	3	6	2	3	5	5	6
15	1	3	4	6	8	3	6	7	8	9
20	3	6	7	10	11	6	9	10	11	13
30	5	6	10	13	14	9	10	11	14	14
60	8	11	12	15	16	12	12	13	17	18
		Number of deaths					Number of deaths			

Table 3: Larvicidal activity of the hydrosol oil from *S. acuta* leaves on *A. stephensis* larvae in comparison with positive control

Concentration (mg/L) of the hydrosol oil of *S. acuta*						Concentration (mg/L) of positive control				
Time (min)	**50**	**100**	**200**	**300**	**500**	**50**	**100**	**200**	**300**	**500**
10	0	0	2	2	5	2	3	5	5	6
15	0	1	2	5	6	3	6	7	8	9
20	2	3	5	8	9	6	9	10	11	13
30	3	6	7	9	11	9	10	11	14	14
60	5	5	8	11	14	12	12	13	17	18
		Number of deaths					Number of deaths			

Table 4: Percentage Mortality and IC_{50} of the volatile oil from *S. acuta* leaves on *A. stephensis* larvae in comparison with controls

% Mortality for volatile oil						% Mortality for positive control				
	Time (min)					Time(min)				
Concentration (mg/L)	**10**	**15**	**20**	**30**	**60**	**10**	**15**	**20**	**30**	**60**
50	5	5	15	25	40	10	15	25	25	30
100	5	15	30	30	55	15	30	35	40	45
200	10	20	35	50	60	30	45	50	55	65
300	15	30	50	65	75	45	50	55	70	70
500	30	40	55	70	80	60	60	65	85	90
IC_{50}					106.41					87.11

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Table 5: Percentage Mortality and IC_{50} of Hydrosol oil from *S. acuta* leaves on *A. stephensis* larvae in comparison with controls

Concentration (mg/L)	% Mortality for hydrosol oil					% Mortality for positive control				
	Time (min)					Time(min)				
	10	15	20	30	60	10	15	20	30	60
50	0	0	10	15	25	10	15	25	25	30
100	0	5	15	30	35	15	30	35	40	45
200	10	10	25	35	40	30	45	50	55	65
300	10	25	40	45	55	45	50	55	70	70
500	25	30	45	55	70	60	60	65	85	90
IC_{50}					101.22					87.11

Tritetracontane appeared in a significant amount (7.69 %) as one of the major constituents of *Coleus aromaticus* essential oil and it showed significant larvicidal activity against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.[21] The larvicidal activity of the essential oil of *Coccinia grandis* against mosquito vectors was attributed to the major components: tetracosane and eicosene or synergistic action of the major and minor components present in the oil.[22] Thymol is a natural substance increasingly used as an alternative to pesticides. It provided complete repellency toward *Anopheles stephens*i. Also, egg-laying ability by female adults of *Anopheles stephensi* was much significantly reduced when exposed to vapours of thymol,[23] hence this supports the use of thymol containing insecticides as a safer means of insect vector control. Neophytadiene was identified as one of the major constituents in the essential oil of *Acalypha segetalis.*[24]

Conclusion

There have been traditional claims that the essential oil of the leaves of *Sida acuta* could be used as a mosquito repellent. Therefore, there was a need to scientifically justify this claim. The essential oil of *Sida acuta* may indeed be a very useful breakthrough in the demand for alternative safer mosquito control agents; this can be attributed to the various volatile constituents detected in the plant and larvicidal potential shown by the oil. The results justify the use of this in the traditional control of insect vectors. This implies that the essential oil and hydrosol of *Sida acuta* could be exploited in the development of plant sources as mosquito repellants which are considered to be non-toxic, biodegradable and environmental friendly. The larvicidal properties could be attributed to the presence of compounds such as; thymol, eicosene, heneicosene and tetracosane Thus, the volatile oil of *Sida acuta* is presented as an excellent potential source of novel insecticides.

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Conflict of Interest

We declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

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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