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Adulticidal Activity of *Hyptis suaveolens*, *Chenopodium ambrosioides* and *Lippia adoensis* Leaf Extracts and Essential Oils against *Anopheles gambiae* (Diptera: Culicidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KMO, LY and ENN designed the study. Authors KMO, JDL and LY performed the statistical analysis. Authors LY and ENN wrote the protocol. Author KMO wrote the first draft of the manuscript. Authors KMO, LY, JDL, PS and ENN managed the analyses of the study. Authors KMO and LY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was undertaken to evaluate the adulticidal efficacy of the methanolic leaf extracts and essential oils of *Chenopodium ambrosioides, Hyptis suaveolens* and *Lippia adoensis* against adults of *Anopheles gambiae*. A chemical profile of each plant extracts (qualitative phytochemical

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screening) and essential oil (GC/MS analysis) was also determined. Doses of 125, 250, 500 and 1000 mg/bottle of plant extracts and 25, 50, 100 and 200 mg/bottle of essential oils were prepared and tested using CDC bottles. In each plant extract, alkaloids, flavonoids, saponins, tannins, phenolic groups, terpenoids, fats and oils were presents. Major chemical constituents of essential oils were thymol (27.09%), sabinene (18.93%) and 4-carene (52.88%) in *L. adoensis*, *H. suaveolens* and *C. ambrosioides*, respectively. *L. adoensis* extract (at 1000 mg/bottle) and essential oil of *H. suaveolens* (at 200 mg/bottle) were the most potent causing 100% mortality of *An. gambiae* adults, 24 h post-exposure. Methanolic extract of *L. adoensis* (LC_{50}= 20.20 mg/bottle) was the most effective compared to other extracts. Similarly, essential oil of *H. suaveolens* (LC_{50}= 5.27 mg/bottle) was revealed as the most toxic on *An. gambiae* adults compared to other oils. Therefore, the extracts of *L. adoensis* and essential oil of *H. suaveolens* showed a high activity and should be recommended to be promoting as natural bioinsecticides to control mosquito adults.

Keywords: Adulticidal; extract; Essential oil; Chenopodium ambrosioides; Hyptis suaveolens; Lippia adoensis.

1. INTRODUCTION

Mosquitoes are regarded as public enemies because of their biting annoyance, noise nuisance, sleeplessness, allergic reactions and disease transmission during their biting and feeding activities [1]. In most mosquito species, only the female transmits diseases such as malaria, yellow fever, dengue haemorrhagic fever, West Nile virus and encephalitis to human beings and animal, particularly in tropical and subtropical countries [2]. Among these mosquito borne diseases, malaria remains the most dangerous infection causing millions of deaths annually in sub-Saharan Africa. In sub-Saharan African countries, *Plasmodium* spp. parasites causing malaria is principally transmitted through the bites of *An. gambiae* females. According to WHO report of 2020, approximately 229 million malaria cases and 409,000 deaths from the disease were recorded worldwide in 2019 [3]. In Cameroon, 6291256 malaria cases with 11233 deaths were reported in 2019 [3].

Approaches undertaken to control malaria largely relied on interruption of the disease transmission cycle by either killing mosquito immature stages in their breeding sites or indoor/outdoor mosquito adults using synthetic chemicals. Malaria control strategies in sub-Saharan countries include the combination of insecticide treated nets and indoor residual spraying seemed to be for the moments effective as a control measure [4]. However, mosquito resistance to the currently-used insecticides and the emergence of multi drug-resistant strains of parasites has escalated the malaria problem in the affected countries. Most of synthetic insecticides have been noted to have high mammalian toxicity and possess deleterious impact on non-target insects as well as mosquito resistance development towards these insecticides limiting their success in vector control [4,5].

Lately, there has been intense search for effective phytochemicals to substitute or reduce the uses of synthetic insecticides. As reason, plant derived products are effective, target specific, ecologically safe, biodegradable, cost-effectiveness and are rich in diverse phytochemicals able to overcome the development of resistance by the target pests [6]. Several plants have been proven to possess toxic effect against the immature stages and mosquito adults and these phytochemical components responsible for that toxic effect include saponins, flavonoids, tannins, alkaloids, glycosides, steroids, terpenoids, hydrogenate and dehydrogenate monoterpenes [7]. However, miscellaneous research papers have reported the efficacy of plant extracts and essential oils against *An. gambiae* larvae. In Cameroon, recent studies include the leaf methanolic extracts and essential oils of two *Cymbopogon* species which were potent against *An. gambiae* [8]. A synergistic action of methanolic extract and essential oils of *Callistemon rigidus* combined with *Eucalyptus camaldulensis* against *An. gambiae* larvae was reported by Younoussa et al. [9].

Chenopodium ambrosioides L. (Chenopodiaceae) is a perennial herb native from America [10]. Its leaf powder and essential oils were toxic against *S. zeamais* [11]. A significant ovicidal and repellent potential of *C. ambrosioides* against *An. gambiae* was reported by [12]. Larvicidal activity of leaf methanolic extract and essential oils the plant was also reported by Oumarou et al. [13].

Hyptis suaveolens L. (Lamiaceae), native from America is one of 400 species of the *Hyptis* cosmopolitan genera which are indexed in the world**.** This plant is variously used in ethnomedicine for many disease treatments [14]. The essential oil and methanolic extract of *H. suaveolens* showed a potent larvicidal activity against *An. gambiae* [13].

Lippia adoensis Hochst belonging to Verbanaceae family is an herbaceous plant comprising 41 genera and includes about 220 species of grasses, shrubs and small trees [15]. Nukenine et al. [16] reported the efficacy of plant powder against *Sitophilus zeamais* Motsch. The essential oil and methanolic extract of *L. adoensis* were toxic against *An. gambiae* larvae [13].

The present study aimed to investigate the adulticidal activity of methanolic extract and essential oils of *C. ambrosioides, H. suaveolens* and *L. adoensis* against the adults of *An. gambiae* under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Plant Collection

Fresh green leaves of *C. ambrosioides* and *H. suaveolens* were collected from Dang (latitude 7°24.94'N, longitude 13°32.87'E and altitude 1093 masl), while the leaves of *L. adoensis* were harvested from Mbé (latitude 7°51.28'N, longitude 13°35.51'E, and altitude 601 masl), Vina division in the Adamaoua region of Cameroon in June 2016. Plant species were identified by professor Mapongmetsem Pierre Marie, a botanist of the Faculty of Science, University of Ngaoundere, Cameroon and then were confirmed at the National Herbarium of Cameroon at Yaounde through the comparison to the voucher specimen No. 33300HNC in comparison with the plant material sample of CSA No. 259 for *C. ambrosioides* and No. 6929/SRF/Cam compared with Letouzey No. 6101 voucher sample for *H. suaveolens* and No. 3921SRFK/Cam compared with Letouzey No. 382 voucher sample for *L. adoensis*. The leaves of the three plant species were shade-dried for 10 days at ambient laboratory conditions (24 ± 2°C; 76 ± 4% HR), grounded in the wood mortar, and passed through 0.4 mm mesh size sieve. Each plant powder obtained was stored in the glass bottles at the ambient temperature until their use for extraction.

2.2 Plant Methanol Extraction

The cold maceration was performed for plants extraction. Extract of each plant species was obtained by macerating 500 g of each plant powder in 2500 mL of methanol. Each plant maceration was stirred twice a day for 72 h and then filtered with Whatman paper No. 1. The methanol in each filtrate was evaporated in open air, and the dried methanol plant extract was stored in the dark glass in the refrigerator set at 4°C until its use for bioassay and phytochemical screening. The extraction yield was calculated following the formula:

> Extraction yield (%) = Weight of plant extract obtained (g) / Weight of plant powder used X 100

2.3 Qualitative Phytochemical Screening of the Methanol Extracts

The methanol extracts of *C. ambrosioides, H. suaveolens* and *L. adoensis* were submitted to the qualitative phytochemical screening tests to identify some anti-insect phytochemical compounds such as alkaloids, flavonoids, saponins, tannins, phenolic group, terpenoids, steroids, fats and oils. Methods performed by Harborne [17], Evans and Trease [18] and Prashant et al. [19] were followed to determine these phytochemicals.

2.4 Essential Oils Extraction

Essential oils were extracted immediately from the harvested fresh plant leaves by hydro-distillation process using Clevenger apparatus for 3 hours. Each plant essential oil was separated from water using separating funnel and traces of water were discarded using anhydrous sodium sulphate and then stored in airtight dark glass bottle until needed for GC/MS analysis and bioassay. The extraction yield of each plant essential oil was determined according to the following formula:

> Extraction yield (%) = Weight of essential oil obtained / Weight of plant powder used X100

2.5 GC/MS Analysis of Essential Oils

Essential oils were analysed for chemical components identification using an Agilent Technologies 6850 gas chromatograph coupled to a mass detector 5973 and a 7683B Series Injector Auto-sampler. Each plant essential oil (1

µL) sample was diluted in 1 mL of hexane and 1 µL of each preparation sample was injected in split less mode. The column was 5% phenylmethylpolysyloxane (30 m x 0.25 mm; film thickness 0.25 µm). Injector temperature was kept at 200°C. Components were separated in the oven following a temperature gradient starting from 50°C and kept for 7 min; then raised to 300°C (10°C/min) and kept at this temperature for 4 min. Helium was used as carrier gas with a flow of 1.1 mL/ min. The mass detector settings were as follow: ionization voltage of 70 eV; scan rate of 2.91 scan/s; mass range of 50-500 and transfer line at 230°C. The output data were elaborated using MSD ChemStation and the NIST deconvolution software AMDIS. Essential oil components were identified whether by the comparison of their relative retention times and mass fragmentation with those of authentic standards or through computer matching against NIST98, as well as retention indices as calculated according to Kovats, for alkanes C9-C24 compared with those reported by Adams [20].

2.6 Mosquito Strain

To obtain *An. gambiae* adults, eggs of that mosquito species were collected from the Organization of Coordination for the Fight against Endemic Diseases in Central Africa (OCEAC) of Yaounde in Cameroon. In the Laboratory of Applied Zoology of the University of Ngaoundere, mosquito eggs were transferred into the buckets containing tap water to hatch into larvae. Larvae were fed with TetraMin® (Tetra GmbH, Germany) and reared following the standard procedure of mosquito rearing adopted by WHO [21]. After emergence, mosquito adults were fed with 10% sucrose solution and maintained at 28 ± 2°C, 75 ± 5% relative humidity under 12 h light and 12 h dark photoperiod. Mosquito adults aged 5-6 days were used for the adulticidal bioassay.

2.7 Adulticidal Test Using CDC Bottles

Transparent glass bottles (250 mL) were used for the bioassay and were coated from the inside with different doses of the plant extracts or essential oils according to the CDC protocol [22]. For each plant extract or essential oil, stock solutions were prepared by dissolving them in the acetone solvent. To obtain the concentrations of 25, 50, 100 and 200 mg/bottle of essential oils; 125, 250, 500 and 1000 mg/bottle of extracts, the plant products were dissolved in adequate quantity of acetone to make 10 mL of total solution. In each 1 mL these solutions would contain 25, 50, 100 and 200 mg of the essential oils and 125, 250, 500 and 1000 mg of extracts. Four stock solutions for the different concentrations were made. Bottle coated with only 1 mL of acetone constituted a negative control and Bi-one™ (DDVP) applied at the recommended concentration of 1000 mg/bottle was used as positive control. Bottles in a lying position were left for 24 h in open air of the laboratory for drying. According to Aizoun et al. [23] method, a mouth aspirator was used to transfer 15 mosquito adults into each test bottle including the control bottles. At start time (Time 0), the bottles were examined to count the number of dead and alive mosquitoes. The number of mosquitoes dead or alive was subsequently recorded every 15 minutes up to 24 h or in less time if all the insects died. However, data were grouped such that mortality counts were reported for 1, 6, 12, 18 and 24 h post-treatment. The mortality in the control bottle was taken into consideration at 24 hours (end of the bioassay) when reporting the results of the bioassay. The bioassay results were discarded, if mortality in the control bottle at the end of the test was >10%. Mosquitoes were considered dead if they can no longer stand. Mortality percentage was calculated and Abbott's formula [24] was applied for mortality correction whenever required according to the following formulae:

Mortality (%) = number of dead larvae in test\control / total number of larvae used X100

Corrected mortality (%) = Number of dead larvae in control – Number of dead larvae in test X 100

2.8 Statistical Analyses

The percentage of mortality data were subjected to the ANOVA procedure using SPSS 16.0 software. Tukey test (P=0.05) was applied for mean separation. Lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality of *An. gambiae* larvae 24 h after treatment application were determined using Probit analysis [25] with SPSS 16.0 software.

3. RESULTS

3.1 Extraction Yield (%)

The extraction yield of *C. ambrosioides*, *H. suaveolens* and *L. adoensis* methanolic extracts

and essential oils are presented in Table 1. From 500g of each plant powder used, *L. adoensis* methanolic extract yielded 9.76% slightly higher than 7.24 and 5.77% yields obtained for *C. ambrosioides* and *H. suaveolens*, respectively. *C. ambrosioides* yield (1.8%) was also moderately superior to *L. adoensis* (1.2%) and *H. suaveolens* (0.44%) extraction yields.

3.2 Chemical Compositions of Leaf Methanolic Extracts

The methanolic extracts of *C. ambrosioides* and *L. adoensis* revealed the presence of alkaloids, flavonoids, saponins, tannins, phenolic groups, terpenoids, fats and oils (Table 2). The same phytochemical components were also presents in the methanolic extract of *H. suaveolens* except saponins.

3.3 Chemical Composition of Essential Oils

Generally, *H. suaveolens* contained more chemical groups than *C. ambrosioides* and *L. adoensis.* The total percentage of the identified compounds was high in *H. suaveolens* (98.82%) followed by *L. adoensis* (96.41%) and *C. ambrosioides* (85.26%) (Table 3). The major compounds in *C. ambrosioides* included 4-carene (52.88%), p-cymene (29.03%) and γ-terpinene (1.23%). In *H. suaveolens* essential oil, sabinene (18.93%), γ-terpinene (11.52%), tumerol (9.80%), terpinene-4-ol (9.22%) and 3-carene (7.29%) were the major phytochemical components. In *L. adoensis* essential oil, thymol (27.09%), O-cymene (10.93%), Cis-carvyl acetate (8.44%), myrterol (7.54%) and verbenone (6.32%) were revealed as the major phytochemical constituents.

3.4 Adulticidal Efficacy in the Laboratory Conditions

3.4.1 Toxicity methanolic extracts

The methanol extracts of *C. ambrosioides*, *H. suaveolens* and *L. adoensis* caused a significant mortality *An. gambiae* adults, 24 h post-exposure (Table 4). The toxic activity of these three plant species significantly (P<0.05) augmented with the increasing concentrations. Globally, no adult mortality was recorded in the negative control while 100% mortality of *An. gambiae* adults was registered in the positive control (DDVP 1000 mg/bottle). Treated with the methanolic extract of *C. ambrosioides*, the mortality of mosquito adults varied significantly ($F_{(5;18)}$ = 1670; P<0.001) from 76.66% at 125 mg/bottle to 100% at 500 mg/bottle. The adult mortality of *An. gambiae* significantly ($F_{(5;18)}$ = 497.28; P<0.001) ranged from 50% (at 125 mg/bottle) to 100% at 500 mg/bottle when tested with *H. suaveolens* extract. With *L. adoensis* extract, the adult mortality significantly ($F_{(5;18)}$ = 3480; P<0.001) ranged from 83% (at 125 mg/bottle) to 100% (at 250 mg/bottle). At the lowest dose of 125 mg/bottle, the mortality percentages of 76.66, 50 and 83% were recorded with *C. ambrosioides, H. suaveolens* and *L. adoensis* leaf methanolic extracts, respectively.

Among the three plant extracts tested against *An. gambiae* adults, *L. adoensis* methanol extract (LC_{50} = 20.20 mg/bottle was revealed as the most toxic against *An. gambiae* adults compared to methanolic extract of *C. ambrosioides* (LC_{50} = 37.19 mg/bottle) and *H. suaveolens* (LC_{50} = 65.92 mg/bottle) (Table 4).

3.4.2 Toxicity of essential oils

Each essential oil of the three plant species caused a significant (P<0.001) adulticidal activity against *An. gambiae* adults. The efficacy of the essential oil of *C. ambrosioides, H. suaveolens* and *L. adoensis* significantly increased with the augmenting concentration and exposure time (Fig. 1). After 24 h post-treatment with the essential oil of *H. suaveolens*, 100% mortality of *An. gambiae* adults was registered at all tested doses while a significantly ($F_{(5;18)}$ = 850.6; P<0.001) mortality rate of mosquito adults ranging from 90.33% at 25 mg/bottle to 100% at 50 mg/bottle and significantly ($F_{(5;18)}$ = 104.70; P<0.001) varying from 60% (at 25 mg/bottle) to 100% (at 200 mg/bottle) were registered with *C. ambrosioides* and *L. adoensis* essential oils, respectively after 24 h post-exposure. After 1 h post exposition, 8.33, 12.66 and 0% mortality were recorded at the lowest dose (25 mg/bottle) with *C. ambrosioides, H. suaveolens* and *L. adoensis* essential oils, respectively, while 100% mortality of *An. gambiae* adults was recorded with the highest tested concentration (200 mg/bottle) of the three plant species as well as the positive control applied at 1000 mg/bottle.

The values of LC_{50} of the three plant essential oils tested of *An. gambiae* adults decreased with increasing exposure time (Table 5). Among the plant essential oils tested, the *H. suaveolens* with the lowest value of LC_{50}= 5.27 mg/bottle was the most potent on *An. gambiae* adults compared to

C. ambrosioides (LC_{50} = 10.52 mg/bottle) and *L. adoensis* (LC_{50} = 17.06 mg/bottle) essential oils after 18 h post-exposure.

4. DISCUSSION

Indoor residual spraying with synthetic insecticides and the use of impregnated mosquito bed net are the common control methods used sub-Saharan countries to control mosquito adults. In the present investigation, methanol extracts of *C. ambrosoides, H. suaveolens* and *L. adoensis* demonstrated a significant adulticidal activity against *An. gambiae*. Globally, the insecticidal activity of the three plant methanol extracts against mosquito adults was dose-dependent and significantly increased when concentrations are gradually augmented. In the same way, *Eclipta alba* and *Andrographis paniculata* extracted with various solvents showed a high adulticidal activity of the methanol extract of these plants against the malarial vector, *Anopheles stephensi* [26]. The methanol leaf extract of *Pithecellobium dulce* exhibited also a high insecticidal activity against *Cx. quinquefasciatus* and *Ae. aegypti* with LC_{50} values were 149.81 and 172.37 mg/L recorded, respectively [27]. In Ethiopia, study conducted by Bekele et al. [28] showed high mortality *An. arabiensis* adults exposed to the methanol leaf extract of *Oreosyce africana* and methanol fruit extract of *Piper capense* with LC_{50} values of 18.74 and 24.30 ppm, respectively.From South Africa, Mavundza et al. [29] reported high mortality up to 98 and 86% of *An. Arabiensis* mosquito adults, when exposed respectively to dichloromethane and ethanol extracts of *Aloe ferox*. Nathan et al. [30] reported also the adulticidal activity of of *Dysoxylum malabaricum* methanol leaf extract against *An. stephensi*. Crude hexane, benzene, ethylacetate, acetone and methanol leaf extracts of *Acalypha alnifolia* exhibited each a significant adulticidal activity and the highest mortality was registered against *An. stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus* [31]. Indeed, plant extracts usually contain phytochemicals like terpenoids, flavonoids, alkaloids, steroids, tannins and phenols which possess insecticidal properties against insect vectors. The variation in activity between the plant methanolic extracts tested could be attributed to their qualitative and quantitative variation in phytochemical contents such as phenolic compounds, saponins, flavonoids, alkaloids, tannins, steroids, that possess insecticidal properties. They may singly or jointly contribute to induce toxic effect against mosquito adults [32]. These phytochemicals might be acted like pyrethroid insecticide by deactivating the acetylcholinesterase enzyme leading to the appearance of neurotoxic symptoms such as hyper excitation, convulsion followed by the paralysis and then the insect pest death [33,34].

Table 1. Extraction yield (%) of methanolic leaf extracts and essential oils of *Chenopodium ambrosioides*, *Hyptis suaveolens* and *Lippia adoensis*

Extraction type	Plant species	Plant powder/Fresh leaves used (g)	Yield (%)
Methanolic Extract (cold maceration)	*C. ambrosioides*	500	7.24
	H. suaveolens	500	5.77
	L. adoensis	500	9.76
Essential Oil (hydrodistillation)	*C. ambrosioides*	200	1.8
	H. suaveolens	200	0.44
	L. adoensis	200	1.2

Table 2. Trend of some phytochemical components of leaf methanolic extracts of *Chenopodium ambrosioides, Hyptis suaveolens* and *Lipiia adoensis*

Phytochemical Components	*H. suaveolens*	*C. ambrosioides*	*L. adoensis*
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	-	+	+
Tannins	+	+	+
Phenolic group	+	+	+
Steroids	+	+	+
Fats and oils	+	+	+
Terpenoids	+	+	+

- = absent; + = present

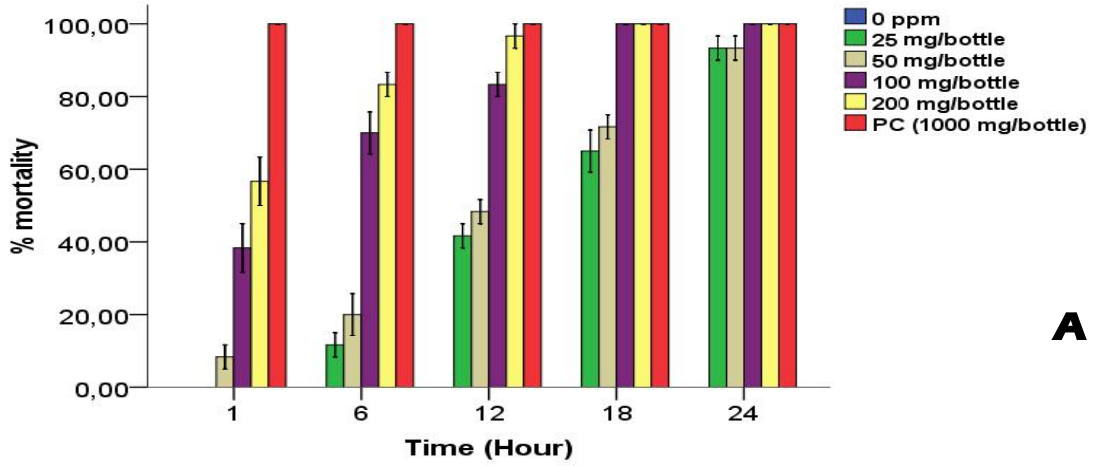
Table 3. Chemical compounds of leaf essential oil of *Hyptis suaveolens*, *Chenopodium ambrosioides* and *Lippia adoensis*

No.	Compounds	% composition		
		L. adoensis	***H. suaveolens***	***C. ambrosoides***
1	p-thymol	0.66	/	/
2	∆-cadinene	/	0.12	/
3	∆-elemene	/	5.15	/
4	1,3,8-para-menthatriene	/	Tr	/
5	1,8-cineole	/	0.17	/
6	12-Oxabicyclo-1,5,5,8 tetramethyl-[9.1.0]- dodeca-3,7-diene	0.7	/	/
7	1-azabicyclo-2,2,2-octan-3-one	/	0.26	/
8	1-bromo-octane	/	2.92	/
9	2,2,3,5,6-pentamethyl-3-heptane	/	0.7	/
10	2-acetyl-cyclohexanone	/	Tr	/
11	2-carene	/	0.18	/
12	3-carene	/	7.29	2.12
13	4,8-dimethyl-3,7-nonadien-2-ol	/	0.13	/
14	4-carene	/	/	52.88
15	4-hydroxy-3-methylacetophenone	0.23	/	/
16	8,13-bietadien-18-ol	/	0.2	/
17	Abieta-8,12-diene	/	0.11	/
18	Abietatrene	/	0.49	/
19	Acetate trans-totarol	/	0.12	/
20	Artemisia ketone	2.7	/	/
21	Bicyclogermacrene	/	0.11	/
22	Carvacrol	/	0.18	/
23	Carvone	2.19	/	/
24	Caryophyllene	3.25	/	/
25	Caryophyllene epoxide	4.44	/	/
26	Caryophyllene oxide	0.18	/	/
27	Cis-Carvyl Acetate	8.44	/	/
28	Cis-para- menth-2-en-1-ol	/	0.16	/
29	Cis-sabinene hydrate	0.41	0.3	/
30	Cyclooctanone	/	0.4	/
31	E-caryophyllene	/	5.83	/

No.	Compounds	% composition		
		L. adoensis	***H. suaveolens***	***C. ambrosoides***
32	E-isovalencenol	/	Tr	/
33	Germacrene A	/	0.23	/
34	Germacrene B	/	0.41	/
35	Germacrene D	0.14	/	/
36	Linalool propanoate	/	0.44	/
37	Longifolol	/	0.1	/
38	Methyl linoleate	/	0.14	/
39	Myrtenol	7.54	/	/
40	Myrtenyl acetate	0.77	/	/
41	Nerolidol	0.16	/	/
42	n-pentadecanol	/	0.25	/
43	O-Cymene	10.93	/	/
44	P-cymen-2-ol	3.47	/	/
45	P-cymene	/	/	29.03
46	Phenyl ethyl octanoate	/	0.15	/
47	Phyllocladene	/	0.35	/
48	Sabinene	/	18.93	/
49	Sandaracopimarinol	/	1.15	/
50	Sesquithujene	/	0.84	/
51	Terpinen-4-ol	0.58	9.22	/
52	Terpinolene	/	2.16	/
53	Thymol	27.09	0.16	/
54	Tricyclene	/	4.92	/
55	Tumerol	/	9.8	/
56	Umbellulone	0.17	/	/
57	Verbenone	6.32	/	/
58	Z-α-trans-bergamatol	/	0.15	/
59	α-chamigrene	0.19	/	/
60	α -copaene	/	0.14	/
61	α-guaiene	/	0.39	/
62	α-humulene	0.85	/	/
63	α-Phellandrene	0.5	0.11	/
64	α-Pinene	2.23	3.41	/

No.	Compounds	% composition		
		L. adoensis	***H. suaveolens***	***C. ambrosoides***
65	α -terpeneol	/	0.35	/
66	α-thujene	/	2.06	/
67	β-bisabolene	0.34	/	/
68	β-E-ocimene	/	0.15	/
69	β-farnesene	3.66	/	/
70	β-linalol	5.9	/	/
71	β-macrocarpene	/	0.15	/
72	β-myrcene	0.93	/	/
73	β-Phellandrene	/	0.57	/
74	β-Pinene	/	5.75	/
75	β-sesquiphellandrene	/	Tr	/
76	γ-patchoulene	/	Tr	/
77	γ-terpinene	1.44	11.52	1.23
Total identified		96.41	98.82	85.26

Tr= traces



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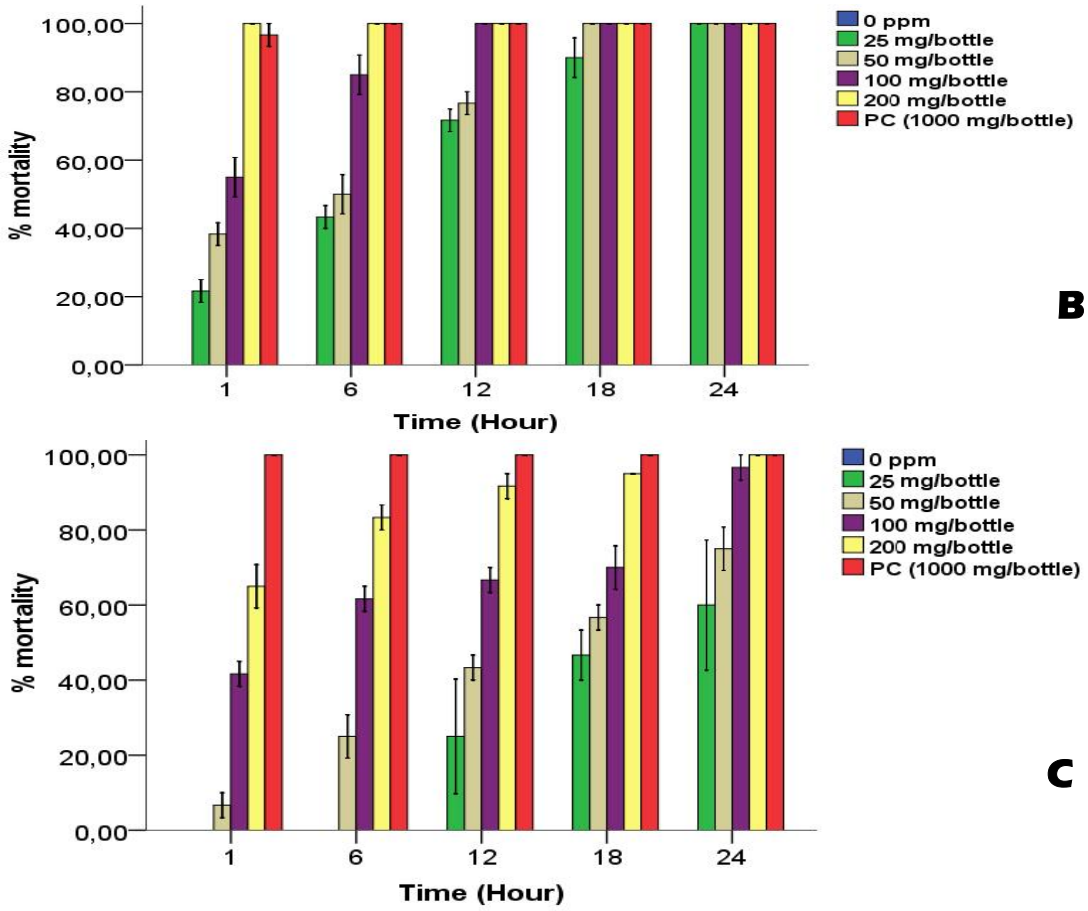

Fig. 1. Mortality of *Anopheles gambiae,* adults treated with different concentrations of *Chenopodium ambrosioides* (A), *Hyptis suaveolens* (B) and *Lippia adoensis* (C) leaf methanolic extracts after 1, 6, 12, 18 and 24 h post treatment in the laboratory conditions (25±2° C, 72±5% r.h.); PC= Positive control (DDVP)

Table 4. Mortality percentage of mosquito adults and LC_{50} as well as LC_{90} (mg/bottle) values of *C. ambrosoides, H. suaveolens* and *L. adoensis* leaf methanol extracts against *An. gambiae* adults in the laboratory conditions (25±2°C, 72±5% r.h.)

Plant species	Conc	% mortality	Slope±SE	R^2	LC_{50} (95% LFL)	LC_{90} (95% UFL)	χ^2
C. ambrosioides	0	0.00±0.00d	3.09±0.35	0.52	37.19 (28.83-44.07)	96.65 (87.92-108.03)	7.44^{ns}
	125	76.66±1.66c					
	250	93.33±1.66b					
	500	100.00±0.00a					
	1000	100.00±0.00a					
	DDVP (1000 mg/bottle)	100.00±0.00a					
	$F_{(5, 18)}$	1670***					
H. suaveolens	0	0.00±0.00d	3.58±0.25	0.62	65.92 (56.02-74.49)	150.23 (130.98-182.41)	21.59*
	125	50.00±2.88c					
	250	78.33±3.33b					
	500	100.00±0.00a					
	1000	100.00±0.00a					
	DDVP (1000 mg/bottle)	100.00±0.00a					
	$F_{(5, 18)}$	497.28***					
L. adoensis	0	0.00±0.00c	2.59±0.48	0.33	20.20 (9.10-29.59)	63.05 (51.03-73.02)	9.44^{ns}
	125	83.33±1.66b					
	250	100.00±0.00a					
	500	100.00±0.00a					
	1000	100.00±0.00a					
	DDVP (1000 mg/bottle)	100.00±0.00a					
	$F_{(5, 18)}$	3480***					

*Mean of mortality percent ± standard error within a column followed by the same letter did not differ significantly according to Tukey test at P= 0.05. ^{ns}P>0.05; *P<0.05; ***P<0.001; CI= Confidence interval; SE= Standard error; R^2=Coefficient of determination; χ^2 = Chi-square; Number of replicates: 4*

Table 5. LC_{50} and LC_{90} values (mg/bottle) of *Chenopodium ambrosioides, Hyptis suaveolens* and *Lippia adoensis* essential oils against adults of *Anopheles gambiae* under laboratory conditions (25±2°C, 72±5% r.h.)

Mosquito species	Time (Hour)	Slope±SE	R^2	LC_{50} (95% LFL)	LC_{90} (95% UFL)	χ^2
C. ambrosioides	1	2.74±0.17	0.90	77.34(66.8-93.09)	226.32(168.99-352.73)	25.95**
	6	2.68±0.13	0.83	39.67(34.02-46.52)	119.11(92.94-170.94)	36.52***
	12	2.20±0.13	0.87	19.08(15.03-22.95)	72.89(56.52-107.68)	33.86***
	18	2.61±0.21	0.70	10.52(5.95-13.96)	32.48(25.52-50.96)	55.32***
	24	1.96±0.54	0.54	3.11(0.6-5.92)	14.03(8.80-18.32)	20.21*
H. suaveolens	1	2.58±0.13	0.98	30.86(24.02-39.26)	96.82(68.57-181.31)	79.64***
	6	2.44±0.15	0.93	18.25(13.76-22.46)	60.92(46.50-95.35)	50.92***
	12	2.41±0.21	0.71	8.58(4.13-11.96)	29.07(22.74-44.02)	46.71***
	18	3.51±0.07	0.28	5.27(2.50-7.18)	12.22(10.21-13.75)	8.74ns
	24	-	-	-	-	-
L. adoensis	1	3.18±0.18	0.93	68.47(61.08-78.14)	173.18(139.9-234.14)	22.31*
	6	3.28±0.16	0.88	43.52(39.62-50.20)	109.21(91.08-139.77)	30.08**
	12	2.18±0.12	0.90	28.02(23.40-33.05)	108.38(82.58-162.86)	30.72**
	18	1.62±0.12	0.96	17.06(11.88-21.77)	104.93(73.58-194.42)	31.74**
	24	2.54±0.19	0.65	11.00(7.22-14.05)	35.03(28.09-49.98)	40.45***

*Mean±SE, χ^2=Chi-square test; ^{ns}: non-significant (P>0.05); *: significant (P<0.01); **: moderately significant (P<0.01); ***: highly significant (P<0,001); LC= Lethal concentration; LFL: Lower Fiducial Limit; UFL: Upper Fiducial Limit; Number of replicates: 4; -: Not determined because of 100% mortality at all tested concentrations*

Essential oils of *C. ambrosoides, H. suaveolens* and *L. adoensis* exhibited also a significant adulticidal activity against the adults of three major vectors mosquito species assessed in the laboratory condition. Generally, the adulticidal efficacy of the plant essential oils against *An. gambiae* adults was concentration-dependent and significantly increased with the increasing concentrations. Similarly, essential oils of *Annona senegalensis* and *Boswellia dalzielii* were reported to be highly toxic against *An. gambiae* adults with LC_{50} values of 2.15 and 7.33 mg/bottle registered, respectively [35]. Adulticidal activity of *Lantana camara* essential oil evaluated against *Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluvialitis,* and *An. stephensi* on 0.208 mg/cm^2 impregnated filter papers caused mortality rate of 93.3%, 95.2%, 100%, 100%, and 100%, respectively [36]. *Citrus sinensis* oil was also proved to be significantly toxic against *Aedes albopictus* adults with LC_{50} values of 53.61, 11.07 and 3.41% after 6, 12 and 24 hours post-exposure, respectively [37]. Indeed, essential oils are complexes of many biologically active constituents such as terpenes, acyclic monoterpene alcohols, monocyclic alcohols, aliphatic aldehydes, aromatic phenols, monocyclic ketones, bicyclic monoterpenic ketones, acids, and esters having biological effects noticed in behavior modification (attraction/repellency) and contact toxicity for insects [38]. Previous works reported pure insecticide compounds, essential oils or pyrethroid insecticides in contact of insects may lead to neurotoxic symptoms such as hyperactivity, seizures and tremors accompanying by knock down effect andthen death of insects [34]. The different compound contained in essential oils may interfere with acetylcholinesterase enzyme acting as potent of the central nervous system where all cholinergic synapses are virtually located, and is responsible of the inhibition action of that enzyme [39].

5. CONCLUSION

Extracts and essential oils of *C. ambrosioides, H. suaveolens* and *L. adoensis* exhibited a significant adulticidal activity against *An. gambiae* adults. Phytochemicals revealed in the three plant extracts were alkaloids, flavonoids, tannins, phenolic groups, terpenoids, fats and oils. Major chemical constituents of *L. adoensis* essential oil were thymol, O-cymene and Cis-carvyl acetate, while sabinene, τ-terpinene and tumerol were the major constituents of *H. suaveolens* essential oil. Major constituents of *C. ambrosioides* included 4-carene and p-cymene. Globally, the leaf essential oils of three plants species were more potent than their methanolic extracts against *An. gambiae* adults. Among the plant extracts, *L. adoensis* extract was the most potent followed by *C. ambrosoides* and *H. suaveolens* extracts on mosquito adults. At all tested doses, essential oil of *H. suaveolens* was revealed as the most toxic compared to *C. ambrosoides* and *L. adoensis* essential oils against *An. gambiae* adults. Thus, methanol extract of *L. adoensis* and essential oil of *H. suaveolens* should be considered as a lead candidate for a formulation of new botanical adulticide to control both indoor and outdoor *Anopheles* species, vectors of malaria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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