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Evaluation of Larvicidal Activity of Selected Plant Extracts and Essential Oil against *Musca domestica* and *Anopheles arabiensis*

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Abstract: *Larvicidal activity of aqueous, hydroethanolic extracts and essential oil from selected plant species of Southern Mozambique: Annona muricata, Annona squamosa, Annona senegalensis, Lantana camara, Strychnos henningsii, Strychnos madagascariensis, Strychnos spinosa, Sclerocarya birrea, Securidaca longepedunculata, Eucalyptus citriodora and Cymbopogon citratus was tested against the third instar larvae of Anopheles arabiensis and Musca domestica.*

Aqueous and hydroethanolic extracts were obtained by Soxhlet extraction and essential oil was obtained by hydrodistillation. Phytochemical screening has been conducted using standard methods and essential oils have been analyzed qualitatively by GC-MS. Larval mortality was observed after 24 h of exposure.

Phytochemical screening revealed the presence of flavonoids, steroids, triterpenoids, saponins and tannins in all hydroethanolic extracts. Methylsalicylate (88%) was the major compound identified in Securidaca longepedunculata essential oil; humulene (22%) the major compound identified in essential oil of Lantana camara and methyl hexadecanoate (40%) the major compound identified in the essential oil of Strychnos spinosa. Hydroethanolic extracts showed higher activity than aqueous extracts and Anopheles arabiensis larvae showed higher susceptibility to extracts and essential oils than Musca domestica larvae.

These results suggest that the larvicidal activity of the extracts are related to the presence of the identified metabolites that act synergistically or individually to cause larval mortality.

Keywords: *Musca domestica, Anopheles arabiensis, larvicidal, phytochemical.*

1. INTRODUCTION

Insects have created many upheavals in society. Their control has been one of the greatest challenges nowadays, taking into account their rapid reproduction and growth. About one in six humans around the world are affected by diseases transmitted by insects, such as typhus, malaria, sleeping sickness, river blindness, as well as bites and allergies [1]. In Mozambique, malaria and diarrheal diseases are considered to be one of the main public health concerns. Such diseases are transmitted by the mosquito *Anopheles* and the housefly *Musca domestica* L. respectively, the latter being a mechanical vector. More than 100 pathogens are associated with the houseflies such as bacteria, protozoa, viruses and metazoan parasites [2].

The housefly is categorized as an important contributor to the dissemination of various infectious diseases, such as cholera, typhoid, shigellosis, bacillary dysentery, tuberculosis and infantile diarrhoea [2] while malaria is caused by parasites of the genus *Plasmodium* and transmitted by infected mosquitoes of the genus *Anopheles* [3]. Malaria has been one of the largest global public health problems with a higher incidence in Sub-Saharan Africa [4]. In the last decade, malaria prevalence has been increasing at an alarming rate, especially in developing countries. According to recent reports, 3.3 billion people are at risk of infection, of which 1.2 billion are at high risk [5]. The world malaria report 2019 highlights the burden of malaria among two most at risk groups: pregnant women and children in Africa. In 2018, 11 million pregnant women in sub-Saharan Africa were infected by malaria, and children under 5 years accounted for 67% of all malaria deaths. In the same year, an estimated 228 million cases occurred worldwide and 405,000 deaths were reported [6].

The control of mosquitoes and flies, as well as other insect vectors of diseases is an essential requirement in the control of epidemic diseases. The management and control of such insects has been based on the use of synthetic insecticides, such as organochlorines, organophosphates, carbamates and others [7].The most popular chemical methods involve application of insecticides with low toxicity that target mosquito or housefly larvae (larvicide) and adults (adulticide). Larval control by source reduction and routine application of chemical larvicides, such as organophosphate compounds and insect growth regulators, is considered as fundamental intervention [8]. However, the development of resistance to the most of insecticides used and problems caused by organosynthetic insecticides on the environment and non-target organisms have stimulated the search for new improved, economical and environmentally-friendly pest control strategies [8],[9]. A Study conducted in Mozambique, Maputo Province, Manhiça District, revealed that *Anopheles funestus* were increasing their resistance to insecticides and over 90% of mosquitoes survived to deltamethrin or lambdacyhalothrin exposure, and almost three-quarters of mosquitoes survived an exposure to permethrin[10].

Pesticides and repellents based on plants material have gained a lot of attention in recent years. Several plants have been reported to have insecticidal activity and can be prepared in powders, extracts and oils for insect control. The phytochemicals derived from plant resources can act as larvicides, adulticides, repellents, ovipositional attractants and therefore may be alternative sources of mosquito and housefly larval control agents [11]. The plants constitute a rich source of bioactive compounds that are biodegradable into non-toxic products [12]. Natural insecticides such as pyrethroids, nicotine, rotenone, among others, have been extensively used in pest control. Limonoids, such as azadirachtin and gedurin present in species Meliaceae and Rutaceae are recognized for their insecticidal effects, and are widely used in the formulation of insecticides throughout the world [13].

Several researchers have reported the effect of *Lantana camara* extracts and essential oils as larvicides, adulticides and repellents against a variety of mosquitoes and housefly *M. domestica* [14],[15],[16],[17],[18]. Essential oils from *Cymbopogon citratus* and *Eucalyptus citriodora* showed high larvicidal effect against mosquito *Aedes aegypti* and housefly *M. domestica* larvae [19],[20],[21],[22]. The genus *Strychnos* is a member of the Loganiaceae family comprising about 200 species. Plant species of the genus *Strychnos* have been used in folk medicine and in arrow and dart poisons in many parts of the world [23],[24]. Extracts from *Strychnos nox*-vomica revealed to be toxic to Sitophilus *oryzae and* showed larvicidal activity against larvae of *Ae. aegypti*, *Culex quinquefasciatus* and *Anopheles stephens* [25]. *Strychnos spinosa* unriped fruit pulp aqueous extracts revealed to be toxic to cattle ticks [26]. Oil extracted from the nut of *Sclerocarya birrea* presented around 70-78% of oleic acid [27], which revealed to be effective against mosquitoes larvae of *Ae. aegypti* and *Cx. Quinquefasciatus*[28]. *Annona squamosa* is reported to be toxic and having antifeedant activity against *Plutella xylostella* L. (Lepidoptera: Plutellidae) and *Trichoplusiani* (Hübner) (Lepidoptera: Noctuidae) [29] and showed larvicidal activity against *Ae. aegypti* and *Ae. atropalpus* [30]. *A. muricata* inhibits larval and pupal growth of *P. xylostella* [31]. Extracts of seeds and roots of *Securidaca longepedunculata* revealed growth inhibition of *S. zeamais* and *C. maculatus* [32], and showed larvicidal activity against *Ochlerotatus triseriatus* and insecticidal and repellent activity against *P. truncatus e T. castaneum* [33].

The rising interest for developing plant based insecticides as an alternative to chemical insecticides stimulated the undertaking of the current study which is a comparative study of the larvicidal potential of aqueous and ethanolic extracts of *L. camara*, *S. birrea*, *A. squamosa*, *A. muricata*, *A. senegalensis*, *S. spinosa*, *S. madagascariensis* and *S. henningsii* against 3rd instar larvae of *M. domestica* and *An. Arabiensis*. Larvicidal activity of essential oils from *S. longepedunculata*, *C. citratus*, *E. citriodora* and *L. camara* was also assessed against larvae of *M. domestica* and *An. arabiensis*.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material and Identification

Fresh leaves of *A. senegalensis* and *S. henningsii* were collected in Matutuine District, roots of *S. longepedunculata* and leaves of *L. camara, S. birrea, S. madagascariensis and S. spinosa* were collected in Marracuene District, *C. citratus* and *E. citriodora* were collected in Namaacha District, *A. squamosa* and *A. muricata* were collected in Maputo City, Mozambique. The plants were identified at the Herbarium Unit of the Department of Biological Sciences, Eduardo Mondlane University. The

leaves and roots were dried at room temperature for a period of 30 days in the Department of Chemistry, Eduardo Mondlane University. The dried leaves and roots were ground into fine powder using an electrical grinder and stored in closed plastic containers at room temperature prior to extraction.

2.2. Extraction

The extracts were obtained by Sohxlet extraction using ethanol 70% and distilled water to obtain the hydroethanolic and aqueous extracts respectively. The hydroethanolic and aqueous extracts were concentrated in the rotary evaporator under 40ºC and 55ºC respectively. Essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus and dried over anhydrous sodium sulphate. The extracts and essential oils obtained were stored at 4ºC until further use.

2.3. Preliminary Phytochemical Analysis

The phytochemical tests were carried out by using standard methods as described by [34] with modifications. The metabolites screened were alkaloids, flavonoids, cardiac glycosides, tannins, saponins, coumarins, steroids, triterpenoids and anthraquinones.

2.4. Analysis of Essential Oils by GC – MS

Qualitative analysis for identification of the chemical components present in the essential oils was performed on a Gas Chromatography – Mass Spectrometry (GC-MS) system comprising a Gas Chromatographer (Agilent GC 106 System 7820A) equipped with Mass Spectrometer (Agilent detector 107 5977B). The column was a HP-5MS (30 m x 0.25 mm ID x 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1mL/min. The injection temperature was programmed to 250ºC. The initial temperature was set to 50ºC in the first 5 minutes and then increased to 220ºC at a heating speed of 4ºC /min. The injection was of 1μL with a split ratio of 10:1. Electron ionization energy of 70 eV was used for GC-MS detection. The identification of the chemical compounds was performed by comparison with the library's database (NIST 14 Library). The relative percentages of the constituents were expressed as percentage by peak area.

2.5. Insect Rearing

Adults flies of *M. domestica* were collected in Maputo, Southern Mozambique, by using a sweeping net. They were transported into a small cage and reared under laboratorial conditions. Adult flies were maintained in cages (20 cm x 30 cm x 20 cm) and provided with granulated sugar, Petri dishes containing cotton pads soaked in milk powder dissolved in water (10% w/v) and jars (500 mL) containing larval medium for egg laying. Larval medium consisted of dry milk powder, bran, minced fish meat and water according to the method described by [35] with modifications. After 24 h, the larvae hatched in the larval medium, and were allowed to feed in the medium, until reaching the 3rd stage. A part of the larvae was left until pupation and hatching of adult flies. The subsequent generation of flies was used to obtain other larvae.

An. arabiensis larvae were obtained from Manhiça Health Research Centre (CISM).

2.6. Larvicidal activity (*An. arabiensis*)

The biological assay for larvicidal activity against *An. arabiensis* was performed using the WHO procedure [36], with minor modifications. From the crude extract, several concentrations from 10 ppm to 10.000 ppm were prepared. Ten larvae of the third stage were introduced into a 250 mL Beaker containing 200 mL of test solution. Ethanol was used as negative control. Mortality was recorded after 24 h. The bioassay was done in triplicate.

2.7. Larvicidal Activity (M. Domestica)

The larvicidal activity was evaluated by the immersion method [37], where 10 third instar larvae were immersed in 10 mL of each test solution for 30 s and then transferred to a filter paper in a Petri dish (9.0 cm). Larval mortality was recorded after 24 h. The same experiment was performed for positive control (cypermethrin) and negative control (ethyl alcohol/ distilled water). Each test was replicated three times.

2.8. Statistical Analysis

The results obtained were expressed as Mean ± SD. Data obtained in the larvicidal activity tests were subjected to analysis of variance (ANOVA). In the case of significant treatment effects, the averages were compared by Tukey test at 5% significance level with the aid of MINITAB19 statistical program and Microsoft Excel 2013 was used to determine LC_{50} of the extracts and essential oils.

3. RESULTS AND DISCUSSION

3.1. Qualitative Phytochemical Screening

The results of the phytochemical screening of the extracts of the seven plants studied are summarized in Table 1.

Table1. *Results of Qualitative Phytochemical Screening*

Plant Extracts		Secondary Metabolites								
		Alk.	Flav.	Ster.	Trit.	Card. glyc.	Coum.	Sap.	Tan.	Ant.
A. muricata	HEE	+	+	+	+	+	+	+	+	+
	AE	-	+	-	-	-	+	+	+	-
A. senegalensis	HEE	+	+	+	+	-	+	+	+	+
	AE	-	-	-	-	-	+	+	+	-
A. squamosa	HEE	+	+	+	+	+	+	+	+	+
	AE	-	-	-	-	-	-	-	+	-
L. camara	HEE	+	+	+	+	-	+	+	+	+
	AE	+	+	+	+	-	-	+	+	-
S. birrea	HEE	-	+	+	+	+	-	+	+	+
	AE	-	+	-	-	+	-	+	+	+
S. henningsii	HEE	+	+	+	+	-	+	+	+	-
	AE	+	+	+	+	-	+	+	+	-
S. madagascariensis	HEE	+	+	+	+	-	-	+	+	-
	AE	+	+	-	-	-	-	+	+	-
S. spinosa	HEE	+	+	+	+	+	-	+	+	+
	AE	-	+	+	+	-	-	-	+	-

Legend: *HEE: Hydroethanolic extract, AE: Aqueous extract, Alk.: Alkaloids, Flav: Flavonoids, Ster: Steroids, trit: triterpenoids, Ant: Anthraquinones, Coum.: coumarins, card.glyc.: cardiac glycosides, sap.: saponins, Tan.: tannins, Ant.: anthraquinones, +: detected, - : not detected*

The phytochemical screening revealed the presence of flavonoids, tannins, saponins, steroids and triterpenoids in all hydroethanolic extracts. Alkaloids were also detected in tested hydroethanolic leaves extracts except from *S. birrea.* Previous reports on phytochemical screening of *S. birrea* extracts sustained the results obtained in this study. The absence of alkaloids was observed *by* [38] in the bark of *S. birrea* and by [39] in methanol and ethanol extracts of leaf, stem bark and root of *S. birrea*. Alkaloids were not detected in all aqueous extracts in the current study. The presence of alkaloids, flavonoids, steroids, triterpenoids, coumarins, saponins, tannins and anthraquinones in the hydroethanolic leaf extract of *L. camara* corroborates with the results previously reported in the literature. [40] reported that the leaf extract of *L. camara* contained alkaloids, glycosides, steroids, saponins, flavonoids, coumarins, tannins, carbohydrates, hydroxyanthraquinones, anthraquinone glycosides, proteins, phytosteroids, fixed oils, fats and triterpenoids. The preliminary phytochemical screening of methanol, ethanol and ethyl acetate leaf extracts of *L. Camara* showed the presence of steroids, flavonoids, tannins, glycerol, and saponins [40].

In addition to *S. birrea* and *L. camara*, 6 other plants subjected to phytochemical screening in this study, were 3 species of the genus *Annona* (*muricata*, *squamosa* and *senegalensis*) and 3 species of the genus *Strychnos* (*spinosa*, *henningsii* and *madagascariensis*). In the current study, most of the secondary metabolites identified in hydroethanolic leaf extracts of the genus *Annona*: alkaloids, flavonoids, steroids, triterpenoids, coumarins, tannins and anthraquinones are similar to those reported by other researchers [41],[42], [43],[44]. Several studies carried out on different parts of *A. squamosa* led to the identification of a variety of secondary metabolites. From the phytochemical screening of the leaves, were identified alkaloids, phenols, saponins, glycosides and vitamins in aqueous,

methanol, chloroform and petroleum ether extracts [41]. Phytochemical studies extensively carried out on different parts of A. muricata, revealed that, until 2018, 212 secondary metabolites had been isolated and identified, such as acetogenins, alkaloids, phenolic compounds and megastigmanes [42].

Phytochemical screening carried out on extracts of *A. senegalensis* revealed the presence of various secondary metabolites including tannins, flavonoids, saponins, alkaloids, glycosides, steroids, volatile oils and anthocyanins [43],[44].

The qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, steroids, triterpenoids, saponins and tannins in all hydroethanolic extracts of the three strychnos species studied in the present work. These results are in consonance with those reported by other researchers [45],[46],[47]. The genus Strychnos is well-known for its contents in alkaloids to which is mainly attributed their pharmacological and toxicological properties [45].

The presence of alkaloids, steroids and terpenoids, tannins, reducing sugars and saponins has been reported in the leaf and stembark extracts of *S. spinosa* [46]. Phytochemical screening of the aqueous bark extract of *S. henningsii* revealed the presence of phenols, flavonoids, alkaloids, saponins , glycosides and tannins [47].

3.2. GC-MS Analysis of Essential Oils

Five essential oils (*E. citriodora, C. citratus*, *L. Camara*, *S. longepedunculata* (root bark) and S. spinosa*)* were subjected to qualitative GC-MS analysis for identification of their chemical components. The yields of the essential oils obtained by hydrodistillation were 5.20% for *E. citriodora*, 1.40% for *C. citratus*, 0.81% for *S. longepedunculata*, 0.29% for *L. camara* and 0.04% for *S. spinosa*.

In the essential oil from the roots of *S. longepedunculata* was indentified methyl salicylate as the major component (88%). This major compound was reported as a characteristic constituent of root bark from *S. longepedunculata* growing in Benin, Senegal, Ghana and BurkinaFaso, and of leaf from trees growing in Nigeria [48],[49].

[50] reported for the first time the composition of the leaf essential oil of *S. Spinosa* and identified 22 compounds in it. The main constituents were palmitic acid (34.3%), linalool (16.0%), (E)-phytol (6.7%) and (E)-geraniol (4.0%). Among the identified compounds, they reported that only 4 were already known to occur in other plants of this species: palmitic and linoleic acids in the seed oil, eugenol and nonacosane in the fruit pericarp and two others (squalene and benzaldehyde) were already identified in other Strychnos species and the remaining 16 constituents were identified for the first time from the *Strychnos* genus. In the present study, qualitative analysis by GC-MS of *S. Spinosa* leaf essential oil led to the identification of a large number of fatty acid derivatives, the major compounds being: methyl hexadecanoate (40%), methyl (Z)-6-octadecenoate (29%), methyl (E)-9-octadecenoate (7%) and 6,10-dimethyl-5,9-undecadien-2-one (5%). Linalool, (E)-fitol and geraniol were also identified in this study but in very low quantities.

L. camara essential oil seems to possess a huge diversity of compounds, which makes its essential oil a complex mixture of volatile compounds. The major constituents found in this study were humulene (22%), 1-(1-methylethyl)- 4-methylenebicyclo[3.1.0] hexane (21%), caryophylene (12%), eucalyptol (9%), 3,7,11-trimethyl-(E)-1,6,10-dodecatrien-3-ol (9%), (1R)-2,6,6-trimetilbicyclo[3.1.1] hept-2-ene (5%), Terpinen-4-ol (4%), (1S)-6,6-dimetil-2-methylenebicyclo[3.1.1] heptane (3%). Different classes of compounds have been described in the literature and it is reported that the composition may be influenced by the geographical location, age, season, climate[51] and daytime collection. Compounds such as caryophylene, γ-muurolene, γ-elemene, γ-terpinene, copaene, eucalyptol, 3-carene, β-pinene were indicated as the main constituents of *L. camara* essential oil of Bangladesh [52]. There is a great variation in chemical composition of *L. camara* essential oil reported up to now from the different parts of the world [15],[53].

The chemical composition of essential oils of *E. citriodora* and *C. citratus* is well documented in the literature. Citronellal, isopulegol are the main components of *E. citriodora* grown in Uruguay [54]. The chemical composition of the essential oil from Algerian *E. citriodora* leaves analyzed by GC-MS revealed the presence of 22 compounds, the dominant ones were citronellal (69.77 %), citronellol

(10.63 %) and isopulegol (4.66 %) [55]. Essential oil from leaves of *E. citriodora* grown in Congo Brazzaville showed two main constituents (citronellal 57.1-75.4 % and citronellol 8-11 %) out of 64 identified constituents (> 0.1 %) [56]. In the present study, the main components identified by GC-MS analysis in essential oil from *E. citriodora* leaves were citronellal (94%), 13-tetradecen-11-yn-1-ol (3%) and 2,2'-(1,4-butanediyl)bisoxirane (2%).

The essential oil from *C. citratus* grown in Kenya was dominated by monoterpene hydrocarbons which accounted for 94.25% of the total oil and characterised by a high percentage of geranial (39.53%), neral (33.31%) and myrcene (11.4%) [57]. In the essential oils from *C. citratus* collected from Brazil and Cuba, the main components were the isomers geranial (53.2% - 51.4%) and neral (36.37% - 35.21%), and the monoterpene myrcene (6.52%) observed only in the Cuban sample. In the essential oil from Sudan, the three main components were citral (34.8%), neral (30.72%) and β-myrcene (11.28%) [58]. In the present study, the major compounds identified by GC-MS in the *C. citratus* essential oil were geranial (E-citral) (55%), neral (Z-citral) 26% and β-myrcene (7%).

3.3. Larvicidal Activity

LC_{50} values of ethanolic and aqueous extracts from *L. camara*, *A. muricata*, *A. squamosa*, *A. senegalensis*, *S. spinosa*, *S. henningsii*, *S. madagascariensis*, *S. birrea* and essential oils from *L. camara*, *S. longepedunculata*, *E. citriodora* and *C. citratus* against 3rd instar larvae of *M. domestica* and *An. arabiensis* are shown in Table 2.

Table2. *LC_{50} values of plant extracts and essential oils against An. Arabiensis and M. domestica larvae*

Plant material	Extract/essential oil	LC_{50} (ppm)	
		An. arabiensis	*M. domestica*
A. muricata	Hydroethanolic	664.53	4023.76
	Aqueous	848.23	6516.34
A. senegalensis	Hydroethanolic	1081.85	5294.30
	Aqueous	1352.08	6313.92
A. squamosa	Hydroethanolic	764.10	4874.45
	Aqueous	961.08	5871.34
S. henningsii	Hydroethanolic	4883.97	5864.14
	Aqueous	*Nd*	7642.33
S. madagascariensis	Hydroethanolic	*Nd*	7591.69
	Aqueous	*Nd*	8490.72
S. spinosa	Hydroethanolic	3256.13	7156.38
	Aqueous	*Nd*	*Nd*
S. birrea	Hydroethanolic	634.25	4923.20
	Aqueous	798.56	7295.18
L. camara	Hydroethanolic	75.22	3422.02
	Aqueous	134.31	4450.73
	Essential oil	24.61	2382.01
C. citratus	Essential oil	102.44	2482.33
E. citriodora	Essential oil	113.75	2296.67
S. longepedunculata	Essential oil	142.37	2792.50

Nd: not determined

In larval assay, it was found that hydroethanolic extracts were more effective than aqueous extracts and the percentage of larval mortality was dose-dependent, i.e., the larval mortality increased with the increase of the extract concentration. The larvicidal activity varied according to plant species and *An. arabiensis* larvae showed higher susceptibility to plant extracts than *M. domestica* larvae. No larval mortality was observed for the negative control, while the positive control (Cypermethrin) exhibited 100% larval mortality. These results are in agreement with those reported on the effect of plant extracts on larval mortality of mosquitoes and houseflies [3],[14],[59],[60].

Among the extracts tested, *L. camara* leaf extracts exhibited the highest larval mortality, reaching 100% at 200 ppm for hydroethanolic extract (LC_{50} =75.22 ppm) and 87.70 ± 5.80% for aqueous extracts (LC_{50} = 134.31 ppm) against *An. arabiensis* larvae, and 80.00 ± 0.00% larval mortality at 6000 ppm for hydroethanolic extract (LC_{50} = 3422.02 ppm) and 66.70 ± 5.80% for aqueous extract

(LC_{50} = 4450.73 ppm) against *M. domestica* larvae. The larvicidal activity of ethanolic extract of *L. camara* against 3rd instar larvae of *M. domestica* has been already reported by [60] who indicated that ethanolic extract of *L. camara* leaves caused complete larval mortality (100%) at the concentration of 2600 ppm and the larval mortality decreased to 16 % at 600 ppm. [61] reported that *L. camara* methanol extract was more effective than aqueous, acetone, chloroform and ethanol extracts against 4th instar *Ae. aegypti, An. stephensis* and *Cx quinquefasciatus*. The presence of lantadene triterpenoids and furanonaphtoquinones in *Lantana sp* has been linked to its mosquito larvicidal properties [61].

In the current study, *S. birrea* leaf extract also exhibited a good larvicidal activity against *An. arabiensis* reaching 83.30 ± 5.80% of larval mortality at 1000 ppm for hydroethanolic extract (LC_{50} = 634.25 ppm) and 73.30 ± 5.80% for aqueous extract (LC_{50} = 798.56 ppm). However there is no significant difference between both extracts (aqueous and hydroethanolic extracts of *S. birrea*) at 1000 ppm as well as no significant difference between aqueous and hydroethanolic extracts of *S. birrea* and hydroethanolic extract of A. *muricata* (80.00 ± 0.00 %; LC_{50} = 664.53 ppm) against *An. arabiensis* larvae.

At the same concentration of 1000 ppm, among *Annona* species, *A. muricata* hydroethanolic extract showed higher larval mortality against *An. arabiensis* (80.00 ± 0.00%) than *A. squamosa* (63.30 ± 5.80%) and *A. senegalensis* (40.00 ± 0.00%). However, at 1500 ppm there is no significant difference between the three hydroethanolic extracts against *An. arabiensis* larvae with larval mortality 100.00 ± 0.00 %; 93.30 ± 5.80%; 86.70 ± 5.80% respectively. The activity of *Annona* species has been related to acetogenins [62] which are reported to be responsible for larvicidal/insecticidal activity of these species besides other compounds which can interact synergistically to promote larval mortality.

The *Strychnos* species tested showed a weak larvicidal activity against *An. arabiensis* larvae in comparison with other species tested. But against *M. domestica* larvae, there is no significant difference in percentage of mortality between hydroethanolic extracts of *S. henningsii* (70.00 ± 10.00%), *A. squamosa* (76.70 ± 5.80%), *A. senegalensis* (73.30 ± 5.80%) and *S. birrea* (73.30 ± 5.80%) at 8000 ppm. [3] reported that at 500 µg/mL, the seed ethanolic extract of *S. birrea* did not show any larvicidal activity. These results are in contrast with our findings which may be explained by the variation in the plant part used and the geographical location [3].

The insecticidal properties of the four essential oils tested in this study have been previously reported mainly against different species of mosquitoes. Essential oil of *E. citriodora* exhibited larvicidal activity against *Cx. quinquefasciatus* larvae with LC_{50} of 245.5 mg/mL [63],[64] and against *Lutzomyria longipalpis* larvae with 100% larval mortality at 6.5 mg/mL [65]. *E. citriodora* essential oil has also shown acaricidal activity against larvae of *Amblyomma cajennense* and *Anocentor nitens* [66]. Essential oil of *E. citriodora* grown in Vietnam showed larvicidal activity with LC_{50} value of 104.4 ppm against *Ae. aegypti* larvae [19] and essential oil of *E. citriodora* from Benin exhibited larvicidal activity of 0.9% against *An. gambiae* [63]. In the current study, *E. citriodora* essential oil showed a good larvicidal activity against *An. arabiensis* with LC_{50} value of 113.75 ppm and exhibited LC_{50} value of 2296.67 ppm *against M. domestica* larvae. The presence of citronellal, citronellol and isopulegol in the essential oil of *E. citriodora* has been related to its insecticidal activity against *An. gambiae* [63]. Similarly the high content of citronellal in the essential oil of *E. citriodora* analysed in this study could explain its larvicidal activity against *An. arabiensis* and *M. domestica*.

Previous studies worldwide reported the efficacy of *Cymbopogon* species essential oils against mosquito species. Larvicidal activity of *C. citratus* essential oil has been demonstrated on *Ae. aegypti* [67],[19], *An. gambiae* [63],[68] and *An. arabiensis* [69]. The insecticidal activity of *C. citratus* has been also reported on *M. domestica* [70],[37]. Insecticidal and larvicidal activities of *C. citratus* have been attributed to citral (mixture of two isomers: geranial (E-isomer) and neral (Z-isomer). The presence of high content of geranial and neral in *C. citratus* essential oil in this study could explain the larvicidal activity against *An. arabiensis* and *M. domestica*.

The volatile component of *S. longepedunculata* (methylsalicylate) has been reported to exhibit repellent and toxic effects against *S. zeamais* and was identified as the compound responsible of the fumigant property of the root of the plant [71]. The presence of methyl salicylate as the main compound identified in essential oil of the root bark of *S. longepedunculata* in the present study, could explain the larvicidal activity against *An. arabiensis* and *M. domestica*. The essential oil from

roots of *S. longepedunculata* appeared to be the less active against the larvae of *An. arabiensis* and *M. domestica* in comparison with *L. Camara*, *E. citriodora* and *C. citratus* leaf essential oils as shown in Table 2.

L. camara essential oil has been reported to possess mosquito larvicidal and adulticidal activity against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluviatilis*, and *An. stephensi* [71]. Larvicidal activity of *L. camara* against *M. domestica* has been also reported [18]. In this study, essential oil from *L. camara* leaves showed higher larvicidal activity (LC_{50} = 24.61 ppm) against *An. arabiensis* than other essential oils tested.

4. CONCLUSION

The Chemical constituents identified in the extracts and essential oils of plants tested in the present study can be linked to their larvicidal activity against *An. arabiensis* and *M. domestica*. The hydroethanolic extracts showed higher larval mortality against *An. arabiensis* and *M. domestica* than aqueous extracts, and *An. arabiensis* 3rd instar larvae showed higher susceptibility to extracts and essential oils than *M. domestica* larvae. Results from the current study are supported by some reported in the literature and suggest that *L. camara* extracts and essential oil have the potential to be used as larvicide for controlling mosquito and housefly population. Thus, the results revealed that aqueous and hydroethanolic extracts from *L. camara*, *S. birrea*, and *Annona* species can be used as an alternative of organosynthetic pesticides for controlling *An. arabiensis* at the larval stage. The present results can be useful for the rural population to control mosquito and housefly population using local resources.

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REFERENCES

[1] George C. McGavin. Expedition Field Techniques INSECTS and other terrestrial arthropods. London: Royal Geographical Society with IBG; 2007.
[2] Chintalchere J. M., Lakare S., and Pandit R. S. Bioefficacy of Essential Oils of Thymus Vulgaris and Eugenia Caryophyllus Against Housefly, Musca. Int Q J Life Sci. 2013;8(3):1029–34.
[3] Mavundza E. J., Maharaj R., Chukwujekwu J. C., Finnie J. F., and Staden J. Van. Larvicidal activity against Anopheles arabiensis of 10 South African plants that are traditionally used as mosquito repellents. South African J Bot. 2013;88:86–9.
[4] WHO Global Malaria Programme. Fact Sheet: World Malaria Report 2015. World Malar Rep. 2015;
[5] WHO Global Malaria Programme. World malaria report 2014. In 2015. p. 14–9.
[6] WHO Global. World malaria report 2019 [Internet]. WHO Regional Office for Africa. 2019. Available from: https://www.who.int/news-room/fact-sheets/detail/malaria
[7] Scott J. G., Alefantis T. G., Kaufman P. E., and Rutz D. A. Insecticide resistance in house flies from caged-layer poultry facilities. Pest Manag Sci. 2000;56(2):147–53.
[8] Chansang A., Champakaew D., Junkum A., Jitpakdi A., Amornlerdpison D., Aldred A. K., Riyong D., Wannasan A., Intirach J., Muangmoon R., and Pitasawat B. Synergy in the adulticidal efficacy of essential oils for the improvement of permethrin toxicity against Aedes aegypti L. (Diptera: Culicidae). Parasites and Vectors. 2018;11(1):1–16.
[9] Abbas M. N., Sajeel M., and Kausar S. House fly (Musca domestica), a challenging pest; biology, management and control strategies. Elixir Entomol. 2013;64:19333–8.
[10] Glunt K. D., Abílio A. P., Bassat Q., Bulo H., Gilbert A. E., Huijben S., Manaca M. N., Macete E., Alonso P., and Paaijmans K. P. Long-lasting insecticidal nets no longer effectively kill the highly resistant Anopheles funestus of southern Mozambique. Malar J. 2015;14(1):1–7.
[11] Kamaraj C., and Rahuman A. A. Larvicidal and adulticidal potential of medicinal plant extracts from south India against vectors. Asian Pac J Trop Med. 2010;3(12):948–53.
[12] Rahuman A. A., Bagavan A., Kamaraj C., Saravanan E., Zahir A. A., and Elango G. Efficacy of larvicidal botanical extracts against Culex quinquefasciatus Say (Diptera: Culicidae). Parasitol Res. 2009;104(6):1365–72.
[13] Harve G., and Kamath V. Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against Aedes aegypti. Indian J Exp Biol. 2004;42(12):1216–9.

[14] Rajan D. S., and Varghese T. A. An evaluation on the larvicidal efficacies of aqueous leaf extracts of Lantana camara and Catharanthus roseus against mosquito larvae. Int J Mosq Res. 2017;4(3):93–7.
[15] Ayalew A. A. Insecticidal activity of Lantana camara extract oil on controlling maize grain weevils. Toxicol Res Appl. 2020;4:1–10.
[16] Sharma A., Kumar S., and Tripathi P. Evaluation of the Larvicidal Efficacy of Five Indigenous Weeds against an Indian Strain of Dengue Vector, Aedes aegypti L. (Diptera: Culicidae). J Parasitol Res. 2016;2016:1–8.
[17] Hemalatha P., Elumalai D., and Janaki A. Larvicidal activity of Lantana camara aculeata against three important mosquito species. J Entomol Zool Stud. 2015;3(1):174–81.
[18] Fouda M., Hassan M., Shehata A., Hasaballah A., and Gad M. Larvicidal and Antifeedant Activities of Different Extracts from Leaves and Stems of Lantana camara (Verbenaceae) Against the Housefly, Musca domestica L. Egypt Acad J Biol Sci F Toxicol Pest Control. 2017;9(1):85–98.
[19] Manh H. D., Hue D. T., Hieu N. T. T., Tuyen D. T. T., and Tuyet O. T. The mosquito larvicidal activity of essential oils from cymbopogon and eucalyptus species in vietnam. Insects. 2020;11(2):1–7.
[20] Vera S. S., Zambrano D. F., Méndez-Sanchez S. C., Rodríguez-Sanabria F., Stashenko E. E., and Duque Luna J. E. Essential oils with insecticidal activity against larvae of Aedes aegypti (Diptera: Culicidae). Parasitol Res. 2014;113(7):2647–54.
[21] Chantawee A., and Soonwera M. Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly Musca domestica. Asian Pac J Trop Med. 2018;11(11):621–9.
[22] Kumar S., Mishra M., Wahab N., and Warikoo R. Larvicidal, repellent, and irritant potential of the seed-derived essential oil of Apium graveolens against dengue vector, Aedes aegypti L. (Diptera: Culicidae). Front Public Heal. 2014;2(147):1–6.
[23] Thongphasuk P., Suttisri R., Bavovada R., and Verpoorte R. Alkaloids and a pimarane diterpenoid from Strychnos vanprukii. Phytochemistry. 2003;64(4):897–901.
[24] Isa A. I., Awouafack M. D., Dzoyem J. P., Aliyu M., Magaji R. A. S., Ayo J. O., and Eloff J. N. Some Strychnos spinosa (Loganiaceae) leaf extracts and fractions have good antimicrobial activities and low cytotoxicities. BMC Complement Altern Med. 2014;14(1):1–8.
[25] Arivoli S., and Tennyson S. Larvicidal efficacy of Strychnos nuxvomica Linn.(Loganiaceae) leaf extracts against the filarial vector Culex quinquefanciatus Say (Diptera: Culicidae). World J Zool. 2012;7(1):6–11.
[26] Madzimure J., Nyahangare E. T., Hamudikuwanda H., Hove T., Belmain S. R., Stevenson P. C., and Mvumi B. M. Efficacy of Strychnos spinosa (Lam.) and Solanum incanum L. aqueous fruit extracts against cattle ticks. Trop Anim Health Prod. 2013;45(6):1341–7.
[27] Glew R. S., VanderJagt D. J., Huang Y. S., Chuang L. T., Bosse R., and Glew R. H. Nutritional analysis of the edible pit of Sclerocarya birrea in the Republic of Niger (daniya, Hausa). J Food Compos Anal. 2004;17(1):99–111.
[28] Gurunathan A., Senguttuvan J., and Paulsamy S. Evaluation of Mosquito Repellent Activity of Isolated Oleic Acid, Eicosyl Ester from Thalictrum javanicum. Indian J Pharm Sci. 2016;78(1):103–10.
[29] Leatemia J. A., and Isman M. B. Toxicity and antifeedant activity of crude seed extracts of Annona squamosa (Annonaceae) against lepidopteran pests and natural enemies. Int J Trop Insect Sci. 2004;24(2):150–8.
[30] Masetti A. The potential use of essential oils against mosquito larvae: A short review. Bull Insectology. 2016;69(2):307–10.
[31] Trindade R. C. P., da Silva P. P., de Souza Luna J., de Lima M. R. F., and Santana A. E. G. Larvicidal activity and seasonal variation of Annona muricata (Annonaceae) extract on Plutella xylostella (Lepidoptera: Plutellidae). Rev Colomb Entomol. 2011;37(2):223–7.
[32] Afful E., and Owusu E. O. Bioactivity of Securidaca longepedunculata Fres . against Callosobruchus maculatus Fab . (Coleoptera : Bruchidae) and Sitophilus zeamais Motsch (Coleoptera : Curculionidae). Int J Agric Sci Res. 2012;1(3):46–54.
[33] Georges K., Jayaprakasam B., Dalavoy S. S., and Nair M. G. Pest-managing activities of plant extracts and anthraquinones from Cassia nigricans from Burkina Faso. Bioresour Technol. 2008;99(6):2037–45.
[34] Sofowora A. Screening plants for bioactive agents. 2nd ed. House S, editor. Medicinal Plants and Traditional Medicinal in Africa. Ibadan: Spectrum Books; 1993. 134–156 p.
[35] Pavela R. Insecticidal properties of several essential oils on the house fly (Musca domestica L.). Phyther Res. 2008;22(2):274–8.
[36] WHO. Report of the WHO Informal Consultation on the "Evaluation and Testing of Insecticides." World Health Organization; 1996. p. 96.
[37] Sinthusiri J., and Soonwera M. Efficacy of herbal essential oils as insecticides against the housefly, Musca domestica L. Southeast Asian J Trop Med Public Heal. 2013;44(2):188–96.
[38] Virginie A., Pierre K. D., Francois M. G., and Franck A. M. Phytochemical Screening of Sclerocarya birrea (Anacardiaceae) and Khaya senegalensis (Meliaceae), Antidiabetic Plants. Int J Pharm Chem. 2016;2(1):1–5.

[39] Manzo L. M., Bako H. D., and Idrissa M. Phytochemical Screening and Antibacterial Activity of Stem Bark, Leaf and Root Extract of Sclerocarya birrea (A. Rich.) Hochst. Int J Enteric Pathog. 2017;5(4):127–31.
[40] AL-Safi A. E. Chemical constituents and pharmacological activities of lantana camara – A review. Asian J Pharm Clin Res. 2019;12(12):10–20.
[41] Saha R. Pharmacognosy and pharmacology of Annona squamosa: A review. Int J Pharm Life Sci. 2011;2(10):1183–9.
[42] Wahab S. M. A., Jantan I., Haque M. A., and Arshad L. Exploring the leaves of Annona muricata L. as a source of potential anti-inflammatory and anticancer agents. Front Pharmacol. 2018;9(661):1–20.
[43] Mustapha A. A. Annona senegalensis Persoon : A Multipurpose shrub, its Phytotherapic, Phytopharmacological and Phytomedicinal Uses. Int J Sci Technol. 2013;2(12):862–5.
[44] Okhale S., Akpan E., Fatokun O., Esievo K., Okhale S. E., Temitope Fatokun O., Folashade Kunle O., and Benefit Esievo K. Annona senegalensis Persoon (Annonaceae): A review of its ethnomedicinal uses, biological activities and phytocompounds Evaluation of Nigerian Medicinal Plants View project Aristolochic acids in herbal medicine View project Annona senegalensis Persoon (An. J Pharmacogn Phytochem. 2016;5(2):211–9.
[45] Frederich M., Tits M., and Angenot L. Indole Alkaloids from Strychnos Species and Their Antiplasmodial and Cytotoxic Activites. Chem Nat Compd. 2003;39(6):425–9.
[46] Ugoh S. H., and Bejide O. Phytochemical Screening and Antimicrobial Properties of the Leaf and Stem Bark Extracts of Strychnos Spinosa. Nat Sci. 2013;11(9):123–8.
[47] Oyedemi S., Bradley G., and Afolayan A. Antidiabetic activities of aqueous stem bark extract of strychnoshenningsii Gilg in streptozotocin-nicotinamide type 2 diabetic rats. Iran J Pharm Res. 2012;11(1):221–8.
[48] Alitonou G. A., Koudoro A. Y., Dangou J. S., Yehouenou B., Avlessi F., Adeoti S., Menut C., and Sohounhloue D. C. K. Volatile constituents and biological activities of essential oil from Securidaca longepedunculata Fers. Growing in Benin. Sci Study Res Chem Chem Eng Biotechnol Food Ind. 2012;13(1):33–42.
[49] Adebayo M. A., Karioti A., Skaltsa H., and Ogunwande I. A. Essential oils of nigeria II: Analysis of the leaf oil of securidaca longependuculata fers. J Essent Oil Res. 2007;19(5):452–4.
[50] Hoet S., Stévigny C., Hérent M. F., and Quetin-Leclercq J. Antitrypanosomal compounds from the leaf essential oil of Strychnos spinosa. Planta Med. 2006;72(5):480–2.
[51] Medeiros L. B. P., Rocha M. dos S., de Lima S. G., de Sousa Júnior G. R., Citó A. M. da. G. L., da Silva D., Lopes J. A. D., Moura D. J., Saffi J., Mobin M., and da Costa J. G. M. Chemical constituents and evaluation of cytotoxic and antifungal activity of Lantana camara essential oils. Brazilian J Pharmacogn. 2012;22(6):1259–67.
[52] Chowdhury J. U., Nandi N. C., and Bhuiyan M. N. I. Chemical composition of leaf essential oil of Lantana camara L . from. Bangladesh J Bot. 2007;36(2):193–4.
[53] Rana V. S., and Prasad D. Chemical Composition of the Leaf Oil of Lantana camara. J Essent Oil Res. 2005;17:1–4.
[54] Dellacassa E., Menéndez P., Moyna P., and Soler E. Chemical composition of Eucalyptus essential oils grown in Uruguay. Flavour Fragr J. 1990;5(2):91–5.
[55] Tolba H., Moghrani H., Benelmouffok A., Kellou D., and Maachi R. Essential oil of Algerian Eucalyptus citriodora: Chemical composition, antifungal activity. J Mycol Med. 2015;25(4):128–33.
[56] Loumouamou A. N., Silou T., Mapola G., Chalchat J. C., and Figuérédo G. Yield and composition of essential oils from eucalyptus citriodora x eucalyptus torelliana, a hybrid species growing in congo-brazzaville. J Essent Oil Res. 2009;21(4):295–9.
[57] Matasyoh J. C., Wagara I. N., Nakavuma J. L., and Kiburai A. M. Chemical composition of Cymbopogon citratus essential oil and its effect on mycotoxigenic Aspergillus species. African J Food Sci. 2011;5(3):138–42.
[58] Mahmoud M. A. GC-MS Analysis and Antimicrobial Screening of Essential Oil from Lemongrass (Cymbopogon citratus). Int J Pharm Chem. 2017;3(6):72–6.
[59] Machanguia P. C., and Munyemana F. Evaluation of Adulticidal , Larvicidal and Repellent Activity of Selected Medicinal Plant Extracts and Essential Oil against Musca domestica L. Sch Acad J Biosci. 2019;7(12):503–16.
[60] Fouda M., Hassan M., Shehata A., Hasaballah A., and Gad M. Larvicidal and Antifeedant Activities of Different Extracts from Leaves and Stems of Lantana camara (Verbenaceae) Against the Housefly, Musca domestica L. Egypt Acad J Biol Sci. 2017;9(1):85–98.
[61] Hemalatha P., Elumalai D., Janaki A., Velu K., Velayutham K., and Kaleena P. K. Larvicidal activity of Lantana camara aculeata against three important mosquito species. J Entomol Zool Stud. 2015;3(1):174–81.
[62] Isman M. B., and Seffrin R. Natural Insecticides from the Annonaceae: A Unique Example for Developing

Biopesticides. In: Advances in Plant Biopesticides. Springer India; 2007. p. 21–33.
[63] Bossou A. D., Mangelinckx S., Yedomonhan H., Boko P. M., Akogbeto M. C., Kimpe N. De, Avlessi F., and Sohounhloue D. C. K. Chemical composition and insecticidal activity of plant essential oils from Benin against Anopheles gambiae (Giles). Parasites and Vectors. 2013;6(1):337–53.
[64] Idris H. S., Lawal S. B., and Balarabe B. M. Larvicidal potentials of leaf and bark extracts of Eucalyptus camaldulensis (Schlect) and Eucalyptus citriodora (Hook) on Culex quinquefasciatus (Say) larvae. Entomol Res. 2008;38(4):243–9.
[65] Maciel M. V., Morais S. M., Bevilaqua C. M. L., Silva R. A., Barros R. S., Sousa R. N., Sousa L. C., Brito E. S., and Souza-Neto M. A. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. Vet Parasitol. 2010;167(1):1–7.
[66] Clemente M. A., De Oliveira Monteiro C. M., Scoralik M. G., Gomes F. T., De Azevedo Prata M. C., and Daemon E. Acaricidal activity of the essential oils from Eucalyptus citriodora and Cymbopogon nardus on larvae of Amblyomma cajennense (Acari: Ixodidae) and Anocentor nitens (Acari: Ixodidae). Parasitol Res. 2010;107(4):987–92.
[67] Phasomkusolsil S., and Soonwera M. Efficacy of herbal essential oils as insecticide against Aedes aegypti (Linn.), Culex quinquefasciatus (Say) and Anopheles Dirus (Peyton and Harrison). Southeast Asian J Trop Med Public Health. 2011;42(5):1083–92.
[68] Batti A. C. S., Younoussa L., Nukenine E. N., Oumarou M. K., and Fomena A. Larvicidal Activity of Two Cymbopogon Species Leaf Extracts and Essential Oils against Anopheles gambiae Gilles (Diptera: Culicidae). J Appl Life Sci Int. 2019;21(1):1–12.
[69] Karunamoorthi K., and Ilango K. Larvicidal activity of Cymbopogon citratus (DC) Stapf. and Croton macrostachyus Del. against Anopheles arabiensis Patton, a potent malaria vector. Eur Rev Med Pharmacol Sci. 2010;14:57–62.
[70] Kumar P., Mishra S., Malik A., and Satya S. Housefly (Musca domestica L.) control potential of Cymbopogon citratus Stapf. (Poales: Poaceae) essential oil and monoterpenes (citral and 1,8-cineole). Parasitol Res. 2013;112(1):69–76.
[71] Jayasekara T. K., Stevenson P. C., Hall D. R., and Belmain S. R. Effect of volatile constituents from Securidaca longepedunculata on insect pests of stored grain. J Chem Ecol. 2005;31(2):303–13.

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