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 diarrhoeal 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].
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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 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 antifeedant activity against *Sitophilus oryzae* and *S. zeamais* [13], [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 nux-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* unripped 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 *Ct. Quinquefasciatus*[28]. *Anonna 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 longapedunculata* 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* and *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. longapedunculata, 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. longapedunculata* 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 Soxhlet 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 40°C and 55°C 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 4°C 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 250°C. The initial temperature was set to 50°C in the first 5 minutes and then increased to 220°C at a heating speed of 4°C /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. Domestic)
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 LC50 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.

| Table 1. Results of Qualitative Phytochemical Screening |
|-------------------------------------------------------|
| Plant Extracts           | Secondary Metabolites |
|                         | Alk. | Flav. | Ster. | Trit. | Card. glyc. | Coum. | Sap. | Tan. | Ant. |
| A. muricata             | HEE  | +     | +     | +     | +      | +      | +    | -    | -    |
| A. senegalensis         | HEE  | +     | +     | +     | -     | +      | +    | -    | -    |
| A. squamosa             | HEE  | +     | +     | +     | +      | +      | +    | -    | -    |
| L. camara               | HEE  | +     | +     | +     | -     | +      | +    | -    | -    |
| S. birrea               | HEE  | +     | +     | +     | -     | +      | +    | -    | -    |
| S. henningsii           | HEE  | +     | +     | +     | -     | +      | +    | -    | -    |
| S. madagascariensis     | HEE  | +     | +     | +     | -     | +      | +    | -    | -    |
| 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 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 streynos 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 stem bark 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 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)-fytol 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 limonene (22%), 1-(1-methylethyl)- 4-methylenedicyclo[3.1.0] hexane (21%), caryophyllene (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 caryophyllene, 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
The essential oil from *C. citratus* grown in Kenya was dominated by monoterpenic 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 monoterpenic 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.

### Table 2. 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.
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(LC₅₀ = 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. stephensi and Cx quinquefasciatus. The presence of lantadene triterpenoids and furanonaphthoquinones 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 (LC₅₀ = 634.25 ppm) and 73.30 ± 5.80% for aqueous extract (LC₅₀ = 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 %; LC₅₀ = 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. hemingisi (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 LC₅₀ 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 nits [66]. Essential oil of E. citriodora grown in Vietnam showed larvicidal activity with LC₅₀ 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 LC₅₀ value of 113.75 ppm and exhibited LC₅₀ 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. longipedunculata (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. longipedunculata 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 also been reported [18]. In this study, essential oil from *L. camara* leaves showed higher larvicidal activity (LC₅₀ = 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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