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Larvicidal activities of hydro-ethanolic extracts of three Cameroonian medicinal plants against *Aedes albopictus*

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ABSTRACT

Objective: To investigate the larvicidal activity of *Syzygium guineense* (Myrtaceae) (*S. guineense*), *Monodora myristica* and *Zanthoxylum heitzii* (Rutaceae) (*Z. heitzii*) extracts against *Aedes albopictus* (*Ae. albopictus*).

Methods: The larvicidal activity of the hydro-ethanolic extracts from these plant species was assessed at three different concentrations (50, 100 and 200 mg/L) on first-instar of *Ae. albopictus* larvae in comparison with untreated controls. Mortality rate was recorded daily for a period of 12 days. The values of LC_{50} and lethal time killing 50% of the tested individuals (LT_{50}) were calculated using the log-probit analysis.

Results: The root extract of *S. guineense* exhibited the best activity with 100% mortality after 8 days of treatment at 200 mg/L, followed by the fruit extract of *Z. heitzii* with 83.33% mortality at the same concentration. Nonetheless, larvae were most susceptible to the fruit extract of *Z. heitzii* both in terms of LC_{50} (39.89 mg/L) and LT_{50} (145.68 h). A statistically significant difference between the control and the group treated at 200 mg/L was noticed in all the extracts.

Conclusions: The present study shows that the hydro-ethanolic extracts of *S. guineense*, *Monodora myristica* and *Z. heitzii* tested have significant larvicidal activity. These preliminary results are of great interest and some of these plant species can be proposed for the formulation of new bioinsecticides to control *Ae. albopictus* populations.

1. Introduction

Mosquitoes are known vectors of several disease-causing pathogens. *Aedes albopictus* (*Ae. albopictus*) (Diptera: Culicidae) is known as a mosquito species with an invasive behavior and a competent vector of various viruses highly dangerous to human health [1]. It is just second to *Aedes aegypti* as vector of dengue fever which is endemic in large areas of Africa, America and South East Asia [2,3]. Moreover, *Ae. albopictus* has gained the position of the most important public health vector species in Southern Europe. In fact, it was responsible for the recent occurrence of autochthonous epidemics of chikungunya and dengue viruses in this region [4]. Today, about two fifths of the world's population are at risk of dengue with cases reported in more than 100 countries [4]. Increased urbanization, migration, poor environmental sanitation, low investment in prevention and community participation and persistent use of control methods having limited efficacy are some of the major causes of emergence and re-emergence of vector-borne diseases in developing countries [5]. At present, since there are no effective vaccines against dengue and chikungunya [6], the most recommended strategy to interrupt pathogen transmission cycles relies on control strategies focusing on the mosquito vectors.

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The current mosquito control methods are mainly based on synthetic insecticides usually considered as the first line of action owing to their quick action. However, repeated application of chemical control often results in an unintended artificial selection of resistant mutants within the vector population [5,7]. In addition, many cases of lethal and sublethal pesticide poisoning of humans have occurred [8]. Besides leading to a constant increase in the cost of application due to development of genetic resistance, the continuous use of synthetic insecticides also causes ecological imbalance manifested by pollution of the environment and destruction of non-target organisms [9].

In addition to the different approaches proposed for adult mosquito control [10], developing an efficient strategy targeting larval stage is considered a key objective as part of the integrated mosquito management [11].

Plants may be an alternative source of agents for the eco-sustainable control of mosquitoes at the larval stages. The use of plant extracts has several appealing features since they are less hazardous, safer for non-target organisms, rich in bioactive chemicals, and biodegradable [12–15]. In the last two decades, a great attention has been given by scientists on the larvicidal properties of plant extracts and essential oils [16], but very few studies have been carried out on *Ae. albopictus* over a long period of treatment [17–20].

Syzygium guineense (Wild.) (*S. guineense*) is widely distributed in tropical regions and is used in African traditional medicine. This plant is used to treat epilepsy, stomachache, diarrhea, malaria, coughs, broken bones, wounds, asthma, sore throat, intercostal pain and as a tonic. The powdered bark is used as an antispasmodic, purgative [21]. The antibacterial properties of the aqueous extract of *S. guineense* have been demonstrated against different strains of bacteria responsible for diarrhea [22]. Ethanol extracts of the stem barks of *S. guineense* showed molluscicidal activities and cardiovascular properties, mainly due to the reduction of blood pressure [23]. Antibacterial activity of triterpenes isolated from *S. guineense* has been demonstrated [24]. Other biological properties such as anti-inflammatory, analgesic and immunological activities of different parts of *S. guineense* have been reported [25]. The chemical composition of essential oil from *S. guineense* was also investigated [26]. A recent study demonstrated that leaves, stem barks and roots of *S. guineense* had antioxidant properties and were rich in polyphenols [27].

Zanthoxylum heitzii (Aubrév. & Pellegr.) (*Z. heitzii*) is one of the cultivar of *Fagara zanthoxyloides* found in the West region of Cameroon. Its fruit is used as a spice for the preparation of "nkui" and "Nah poh", two traditional dishes of Cameroon [28]. It is also used as medicinal plant in Central Africa for the treatment of many diseases such as cancer, syphilis, malaria and other urogenital infections [29]. The bark of *Z. heitzii* is used as insecticides and revealed some activities against cardiac infections [30,31]. Fagaricine, an aqueous extract formulation from root of *Z. heitzii*, was demonstrated as an immune-restorative phytomedicine to treat immunodeficiency as well as an effective antioxidant and *in vitro* antisickling agent [32,33]. A recent study demonstrated that fruit and bark extracts of *Zanthoxylum* induced mitochondrial apoptosis and G0/G1 phase arrest in human leukemia HL-60 cells [34].

Several molecules were isolated from the stem bark of *Z. heitzii*. Among them, two amides (heitziamide A and heitziamide B) and two phenylethanoids (heitziethanoid A and heitziethanoid B) and *trans*-fagaramide, arnottianamide, iso-γ fagarine, iso-skimmianine, arctigenin methyl ether, savinin, (+)-eudesmin, (+)-sesamin, lupeol, lupeone, β-sitosterol, stigmasterol and stigmasterol-3-O-β-D-glucopyranoside [35].

Monodora myristica (Dunal) (*M. myristica*) is widely used especially to relieve toothache as well as in the treatment of dysentery. When roasted and ground, the seeds are rubbed on the skin for skin diseases [36].

Despite the important biological properties described, these plants have never been investigated as potential source of larvicidal molecules effective against mosquitoes. The objective of this study was to evaluate the insecticide effectiveness of the Cameroonian species *S. guineense*, *M. myristica* and *Z. heitzii* against *Ae. albopictus* larvae.

2. Materials and methods

2.1. Plant material and extract preparation

S. guineense, *Z. hertzii* and *M. myristica* were collected respectively in Bafia (centre region), Bachingou (west region) and Mount Kalla (centre region), identified at the National Herbarium of Cameroon by Mr. NANA and referenced under the following numbers: *S. guineense* (roots and leaves) (20899/SRF/Cam), *Z. heitzii* (fruits, leaves and barks) (1441/SRF) and *M. myristica* (fruit) (27690/SRF/Cam). The collected plant materials were air-dried and ground into fine powder. One hundred grams of each powder was macerated in 1 L of aqueous–ethanolic mixture 70:30 (v/v) for 72 h at room temperature and the obtained solution was filtered with Whatman No. 1 filter paper. The residue was further macerated twice under the same conditions. The filtrates obtained from each extraction were mixed and concentrated under vacuum. The obtained extracts were kept at 4 °C until use.

2.2. Ae. albopictus rearing

Ae. albopictus colony were derived from individuals collected in Central Italy and reared at the Laboratory of Sustainable Management of the Agro-ecosystems of Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA; Rome, Italy). Colonies were maintained in the laboratory under standard conditions at (27 ± 1) °C; (75 ± 10)% relative humidity, and with a 14:10 h photoperiod. Larvae were reared at a density of one larva per 5 mL water, in plastic trays containing dechlorinated water, provided with an aeration tube and supplemented with a powder obtained by crushing dry cat food (Friskies® adults). Adults were maintained in rearing cages (40 cm × 40 cm × 40 cm) and offered 10% sucrose. Females were provided with fresh defibrinated bovine blood using a thermostatic apparatus [37]. Eggs were oviposited on paper towels in cups partially filled with water. Strips of towel paper were submerged in a hatching broth for 5 h to obtain the first-instar larvae.

2.3. Larvicidal bioassay

Each crude extract was tested at 200, 100 and 50 mg/L by mixing 20 first instar *Ae. albopictus* in 25 mL of each mixture of

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extract solution. As control, 20 larvae were exposed to a 1% acetone solution because this solution had been used to dissolve the extract. Larvae were fed at regular intervals of 24 h. Each container was daily monitored for larval mortality over a period of 12 days. The experiment was replicated three times and the mean of one triplicate was recorded in this study.

2.4. Statistical analysis

Kruskal–Wallis non parametric test ($P < 0.05$) followed by a *post hoc* Dunnett C was used for testing the larvicidal activity of each extract at various concentrations and times of exposure. Log-probit regression analysis was used to determine LC_{50} and lethal time (LT_{50}) killing 50% of the tested individuals. The interaction between concentration and time was studied using the Univariate General Linear Model test and Fisher test. Analyses were performed using the Statistical Software SPSS version 16 for Windows.

3. Results

Larval mortality varied with the extract concentration, the plant part and the time of exposure. In comparison with the control, all of the tested extracts were active against *Ae. albopictus* larvae but showed different levels of toxicity. Figure 1 present the percentages of mortality induced by each tested extract depending on the exposure time and concentration. Among the extracts (Figure 1A,E and F), significant differences ($P < 0.05$) were observed from 192 h (8th day) when using the following extracts: roots and leaves of *S. guineense*, barks of *Z. heitzii* and leaves of *M. myristica* (Kruskal–Wallis analysis with Dunnett C as *post hoc*). The percentages of mortality were significantly different ($P < 0.05$) when the time of exposure of the larvae to the extracts reached 192 h, 240 h and 288 h. A statistically significant difference was always found between control and the extracts at 200 mg/L concentration. Moreover, only the root extract of *S. guineense* showed a statistically significant difference of induced mortality when comparing 50 and 200 mg/L concentrations (Figure 1A). Overall, the root extract of *S. guineense* was the most potent as it induced 100% mortality at 200 mg/L within the 8th day of exposure (Figure 1A), followed by the bark extract of *Z. heitzii* (Figure 1E) inducing 83.33% mortality at the same concentration. In general, all of the tested extracts exhibited a strong significant correlation ($P < 0.001$) with a coefficient higher than 0.90. The plots resulting from the Univariate General Linear Model test checking the effect of the interaction of time and concentration on the percentage of induced mortality are also shown in Figure 1. Since this kind of diagrams generally inform about the progression due to the interaction, they may show two forms of lines, parallel or non parallel, which indicate a significant or not significant interaction respectively. Then, we can assume that between 144 h and 192 h, the interaction could be significant with all the tested extracts. This was confirmed by Fisher test that showed a significant difference between 144 h and 192 h with high coefficients with respect to root and leaf extracts of *S. guineense* (Fisher: 9.54, 0.01; 10.80, 0.01), fruit, leaf and bark extract of *Z. heitzii*

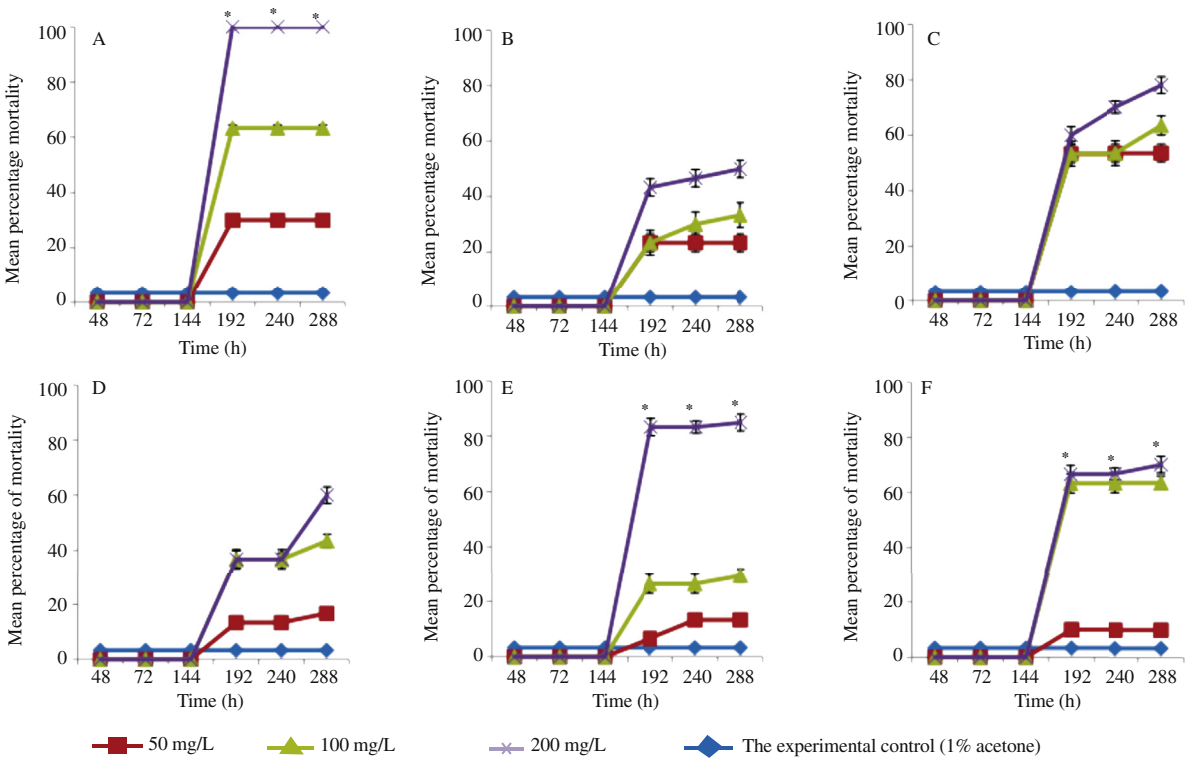

Figure 1. Mortality induced by different plant extracts on *Ae. albopictus* larvae.
A: *S. guineense* root extract; B: *S. guineense* leaf extract; C: *Z. heitzii* fruit extract; D: *Z. heitzii* leaf extract; E: *Z. heitzii* bark extract; F: *M. myristica* fruit extract. Kruskal–Wallis non parametric test ($P < 0.05$) was used for testing the larvicidal activity of each extract at various concentrations and times of exposure followed by a *post hoc* Dunnett C test. *: Significant difference ($P < 0.05$) of means at 200 mg/L compared to the control from 192 h up to 288 h. Univariate General Linear Model test and Fisher test were used for the interaction between concentration and time.

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(Fisher: 257.39, 0.01; 5.66, 0.01; 7.96, 0.01), leaf extract of *M. myristica* (Fisher: 11.34, 0.01).

LT_{50} values were calculated at different concentrations. Among all the tested extracts, the fruit extract of *Z. heitzii* had the lowest LT_{50} value of 145.68 h at the highest concentration (200 mg/L) followed by the root extract of *S. guineense* with a LT_{50} of 192.00 h at the same concentration (Table 1). Generally, the results showed that the higher the concentration of the extract the lesser the exhibited LT_{50}. Similar results were observed with the LC_{50}. Longer time of exposure led to lesser LC_{50} values. Regarding the LC_{50} value, the fruit extract of *Z. heitzii* showed the lowest LC_{50} value among all the tested extracts (Table 2). This extract exerted the best larvicidal activity among all the tested extracts with LC_{50} values of 47.94, 44.50 and 39.89 mg/L, at 192, 240 and 288 h respectively. Second to this extract was the root extract of *S. guineense*.

Table 1

LT_{50} values calculated for the tested plant extracts at three different concentrations based on the log-probit regression analysis.

Extracts	Concentration (mg/L)	LT_{50} (h)
S. guineense (root)	50	–
	100	200.00
	200	192.00
S. guineense (leaf)	50	–
	100	242.74
	200	235.75
Z. heitzii (bark)	50	930.00
	100	927.00
	200	222.00
Z. heitzii (leaf)	50	410.00
	100	409.00
	200	265.00
Z. heiztii (fruit)	50	–
	100	177.57
	200	145.68
M. myristica (fruit)	50	–
	100	–
	200	–

–: Evaluation not possible.

Table 2

LC_{50} values calculated for the tested plant extracts at three different time based on the log-probit regression analysis.

Extracts	Time of exposure (h)	LC_{50} (mg/L)
S. guineense (roots)	192	66.80
	240	66.80
	288	66.80
S. guineense (leaves)	192	–
	240	274.74
	288	214.08
Z. heitzii (roots)	192	229.00
	240	266.00
	288	114.00
Z. heitzii (leaves)	192	288.00
	240	288.00
	288	139.84
Z. heitzii (fruits)	192	47.94
	240	44.50
	288	39.89
M. myristica (fruit)	192	113.93
	240	113.93
	288	110.92

–: Evaluation not possible.

4. Discussion

The interest in developing pesticides of natural origin has increased during recent years because of the drawback of synthetic chemical pesticides, such as the impact on environment and the toxicity to non target organisms including humans and due to the development of resistance in targeted insect populations. Specifically, the possibility to exploit available low cost and renewable raw material, like wastes, for a possible individual use in urban area against mosquito vectors may lead to develop promising control strategies especially in developing countries [38]. Several plant extracts have been reported to be biologically active against insect pests [39,40]. Herein, the larvicidal activity of some Cameroonian plant extracts (roots and leaves of *S. guineense*, barks of *Z. heitzii* and leaves of *M. myristica*) against *Ae. albopictus* has been investigated.

The larvicidal efficacy of the tested extracts was dose dependent. The biological activity of the plant extracts is generally known to be due to the presence of various bioactive phytomolecules present in the plant, including alkaloids, terpenoids, and phenolics [41]. However, the difference in toxicity expressed by the different plant species and parts may be due to the quantitative and qualitative variation in the chemical composition of the plant extracts. *S. guineense* induced full mortality (100%) of *Ae. albopictus* larvae within 8 days of exposure at 200 mg/L. A study conducted on the sheep ked parasite using the same extract reported about 47% mortality within 24 h of exposure [39].

The mortality depends on time of exposure, plant species and chemical composition. The variation in chemical composition could explain the differences in LC_{50} and LT_{50} values since the higher the concentration, the lesser the LT_{50}, according to the extract and the part of the plant used. Likewise, in general, the higher the time of exposure the lesser the LC_{50}. The fruit extract of *Z. heitzii*, exerted the best larvicidal activity among all the tested extracts with LC_{50} values of 47.94, 44.50 and 39.89 mg/L at 192 h (6th day), 240 h (10th day) and 288 h (12th day) respectively, followed by the root extract of *S. guineense*. The variation of LC_{50} and LT_{50} values could be due to differences in the levels of toxicity of the specific insecticidal components in each plant extract. However, only the isolation and identification of the pure molecules exerting the larvicidal activity may allow evaluating more thoroughly these differences.

Various plant extracts have been tested as potential mosquito larvicides [42,43]. The larvicidal and repellent effects of *Albizzia amara* and *Ocimum basilicum* against *Aedes aegypti* at different concentrations were assessed. The petroleum ether extract of *Ageratum conyzoides* leaves exhibited larvicidal activity with a LC_{50} value of 425.60 and 267.90 mg/L after 24 and 48 h of exposure, respectively [44]. Compared to the above results, the present study revealed low values of LC_{50} between 192 h and 288 h of exposure. Similarly, another study reported the larvicidal effects of a neem cake methanol extract against *Ae. albopictus* larvae exposed to 50 and 100 mg/L for 20 days [20]. Different fractions of increasing polarity were also successfully tested by the same authors following the method described by Nicoletti *et al.* [45] and showing a significant larvicidal activity against the *Ae. albopictus*. Moreover, according to works of Bilal *et al.* [46] on the larvicidal activity of selected plant extracts against *Ae. albopictus*, all the extracts showed moderate larvicidal activity. The lowest LC_{50} was found in *Coriandrum sativum*, *Nigella sativa* and

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Syzygium aromaticum at a dose of 363.7, 377.5 and 403.4 mg/L, respectively, after 24 h exposure, while the amount of extracts used reduced to 263.9, 300.8 and 342.2 mg/L, respectively, after 48 h. In terms of lethal time response again *Coriandrum sativum*, *Nigella sativa* and *Syzygium aromaticum* showed less time to produce 50% mortality (14.28, 17.77 and 17.99 h). Yadav *et al*. [47] identified two plant species *Vernonia cinerea* and *Prosopis juliflora* as potential larvicide against *Ae. albopictus*.

The mode of action of most of the plant extracts on mosquito larvae is still unknown. As an example, a previous research documented that phytochemicals could interfere with the proper functioning of the mitochondria particularly at the proton transferring site [48]. Some bioactive molecules of plant extracts have been found to primarily affect the mid-gut epithelial surface and secondarily the gastric caeca and the Malpighian tubules of the mosquito larvae [49].

Among the tested plant extracts, the 30% ethanol extracts of *Z. heitzii* (fruits in particular) and *S. guineense* (roots and leaves) demonstrated remarkable larvicidal activity against *Ae. albopictus*. These plants extracts are therefore promising as alternatives to synthetic insecticides in mosquito control programs, thus providing the basis to use the plant extracts against *Ae. albopictus*.

Future steps in the validation of these extracts as possible raw materials for the development of a new domestic insecticide will be the use of two to fourth instar larvae, the study of the physiological effects on the larvae, the determination of the chemical composition of the most effective extracts, the isolation of the most active fractions to possibly identify the molecules inducing the observed larvicidal effect and histopathological studies proving how the active substance act on the targeted hosts.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Gratz NG. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 2004; **18**: 215-27.
[2] Knudsen AB. Global distribution and continuing spread of *Aedes albopictus*. *Parassitologia* 1995; **37**: 91-7.
[3] Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Dis* 2007; **7**: 76-85.
[4] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature* 2013; **496**: 504-7.
[5] Maciel-de-Freitas R, Avendanho FC, Santos R, Sylvestre G, Araújo SC, Lima JB, et al. Undesirable consequences of insecticide resistance following *Aedes aegypti* control activities due to a dengue outbreak. *PLoS One* 2014; **9**(3): e92424.
[6] Weaver SC, Osorio JE, Livengood JA, Chen R, Stinchcomb DT. Chikungunya virus and prospects for a vaccine. *Expert Rev Vaccines* 2012; **11**: 1087-101.
[7] Marcombe S, Farajollahi A, Healy SP, Clark GG, Fonseca DM. Insecticide resistance status of United States populations of *Aedes albopictus* and mechanisms involved. *PLoS One* 2014; **9**(7): e101992.
[8] Forget G. Pesticides, necessary but dangerous poison. Ottawa: IDRC; 1989. [Online] Available from: https://idl-bnc.idrc.ca/dspace/bitstream/10625/24094/1/108896.pdf [Accessed on 25th June, 2015]
[9] Carriger JF, Rand GM, Gardinali PR, Perry WB, Tompkins MS, Fernandez AM. Pesticides of potential ecological concern in sediment from South Florida canals: an ecological risk prioritization for aquatic arthropods. *Soil Sediment Contam* 2006; **15**: 21-45.
[10] Bourtzis K, Dobson SL, Xi Z, Rasgon JL, Calvitti M, Moreira LA, et al. Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. *Acta Trop* 2014; **132**: S150-63.
[11] Rose RI. Pesticides and public health: integrated methods of mosquito management. *Emerg Infect Dis* 2001; **7**: 17-23.
[12] Jaswanth A, Ramanathan P, Ruckmani K. Evaluation of mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus* Say. *Indian J Exp Biol* 2002; **40**: 363-5.
[13] Park K. *Park's textbook of preventive and social medicine*. 20th ed. Jabalpur: Banarsidas Bhanot Publishers; 2009.
[14] Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(2): 124-9.
[15] Allison LN, Dike SK, Opara FN, Ezike MN, Amadi AN. Evaluation of larvicidal efficacy and phytochemical potential of some selected indigenous plant against *Anopheles gambiense* and *Culex quinquefasciatus*. *Adv Biosci Biotechnol* 2013; **4**: 1128-33.
[16] Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Curr Res* 2011; **3**: 296-304.
[17] Liu XC, Liu QY, Zhou L, Liu QR, Liu ZL. Chemical composition of *Zanthoxylum avicennae* essential oil and its larvicidal activity on *Aedes albopictus* Skuse. *Trop J Pharm Res* 2014; **13**: 399-404.
[18] Yadav R, Tyagi V, Tikar SN, Sharma AK, Mendki MJ, Jain AK, et al. Differential larval toxicity and oviposition altering activity of some indigenous plant extracts against dengue and chikungunya vector *Aedes albopictus*. *J Arthropod Borne Dis* 2014; **8**: 174-85.
[19] Akono Ntonga P, Baldovini N, Mouray E, Mambu L, Belong P, Grellier P. Activity of *Ocimum basilicum, Ocimum canum*, and *Cymbopogon citratus* essential oils against *Plasmodium falciparum* and mature-stage larvae of *Anopheles funestus*. *Parasite* 2014; **21**: 33.
[20] Benelli G, Canale A, Conti B. Eco-friendly control strategies against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae): repellency and toxic activity of plant essential oils and extracts. *Pharmacologyonline* 2014; **1**: 44-51.
[21] Malele RS, Moshi MJ, Mwangi JW, Achola KJ, Munenge RW. Pharmacological properties of extracts from the stem bark of *Syzygium guineense* on the ileum and heart of laboratory rodents. *Afr J Health Sci* 1997; **4**: 43-5.
[22] Ashebir M, Ashenafi M. Assessment of the antibacterial activity of some traditional medicinal plants on some food-borne pathogens. *Ethiop J Health Dev* 1999; **13**: 211-6.
[23] Oketch-Rabah HA, Dossaji SF. Molluscicides of plant origin: molluscicidal activity of some Kenyan medicinal plants. *South Afr J Sci* 1998; **94**: 299-301.
[24] Djoukeng JD, Abou-Mansour E, Tabacchi R, Tapondjou AL, Boud H, Lontsi D. Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae). *J Ethnopharmacol* 2005; **101**: 283-6.
[25] Ior LD, Otimenyin SO, Umar M. Anti-inflammatory and analgesic activities of the ethanolic extract of the leaf of *Syzygium guineense* in rats and mice. *IOSR J Pharm* 2012; **2**: 33-6.
[26] Noudogbessi JP, Yédomonhan P, Sohounhloué DC, Chalchat JC. Chemical composition of essential oil of *Syzygium guineense*

(Willd.) DC. var. guineense (Myrtaceae) from Benin. *Rec Nat Prod* 2008; **2**: 33-8.

[27] Pieme CA, Ngoupayo J, Nkoulou CH, Moukette BM, Nono BLN, Ama Moor VJ, et al. *Syzyguim guineense* extracts show antioxidant activities and beneficial activities on oxidative stress induced by ferric chloride in the liver homogenate. *Antioxidants* 2014; **3**: 618-35.

[28] Tchiégang C, Mbougueng PD. [Chemical composition of spices used in the preparation of *Nah poh* and *Nkui* of West Cameroon]. *Tropicultura* 2005; **23**: 193-200. French.

[29] Mbaze LM, Poumale HM, Wansi JD, Lado JA, Khan SN, Iqbal MC, et al. Alpha-glucosidase inhibitory pentacyclic triterpenes from the stem bark of *Fagara tessmannii* (Rutaceae). *Phytochemistry* 2007; **68**: 591-5.

[30] Ntchapda F, Maguirgue K, Adjia H, Etet PF, Dimo T. Hypolipidemic, antioxidant and anti-atherosclerogenic effects of aqueous extract of *Zanthoxylum heitzii* stem bark in diet-induced hypercholesterolemic rats. *Asian Pac J Trop Med* 2015; **8**: 359-65.

[31] Moussavi N, Malterude KE, Mikolo B, Dawes D, Chandre F, Corbel V, et al. Identification of chemical constituents of *Zanthoxylum heitzii* stem bark and their insecticidal activity against the malaria mosquito *Anopheles gambiae*. *Parasit Vectors* 2015; **8**: 503.

[32] Mokondjimobe E, Joe MB, Barkha S, Dzeufiet PD, Chenal H, Otsudi'andjeka JB, et al. Fagaricine, a new immunorestorative phytomedicine from *Zanthoxylum heitzii*: preclinical and multicenter cohort clinical studies based on HIV-infected patients in six countries. *Phytopharmacology* 2012; **2**: 26-45.

[33] Pauline N, Cabral BN, Anatole PC, Jocelyne AM, Bruno M, Jeanne NY. The *in vitro* antisickling and antioxidant effects of aqueous extracts *Zanthoxyllum heitzii* on sickle cell disorder. *BMC Complement Altern Med* 2013; **13**: 162.

[34] Pieme CA, Santosh GK, Tekwu EM, Askun T, Aydeniz H, Ngogang JY, et al. Fruits and barks extracts of *Zanthozyllum heitzii* a spice from Cameroon induce mitochondrial dependent apoptosis and Go/G1 phase arrest in human leukemia HL-60 cells. *Biol Res* 2014; **47**: 54.

[35] Mbaze LM, Lado JA, Wansi JD, Shiao TC, Chiozem DD, Mesaik MA, et al. Oxidative burst inhibitory and cytotoxic amides and lignans from the stem bark of *Fagara heitzii* (Rutaceae). *Phytochemistry* 2009; **70**: 1442-7.

[36] Irvine FR. *Woody plants of Ghana with special reference to their uses*. London: Oxford University Press; 1961, p. 13-23.

[37] Calvitti M, Moretti R, Lampazzi E, Bellini R, Dobson SL. Characterization of a new *Aedes albopictus* (Diptera: Culicidae)-*Wolbachia pipientis* (Rickettsiales: Rickettsiaceae) symbiotic association generated by artificial transfer of the *w*Pip strain from *Culex pipiens* (Diptera: Culicidae). *J Med Entomol* 2010; **47**: 179-87.

[38] Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc* 1991; **7**: 210-37.

[39] Gemeda N, Mokonnen W, Lemma H, Tadele A, Urga K, Addis G, et al. Insecticidal activity of some traditionally used Ethiopian medicinal plants against sheep ked *Melophagus ovinus*. *J Parasitol Res* 2014; **2014**. 978537.

[40] Fayemiwo KA, Adeleke MA, Okoro OP, Awojide SH, Awoniyi IO. Larvicidal efficacies and chemical composition of essential oils of *Pinus sylvestris* and *Syzygium aromaticum* against mosquitoes. *Asian Pac J Trop Biomed* 2014; **4**: 30-4.

[41] Vindhya K, Sampath Kumara KK, Neelambika HS, Leelavathi S. Preliminary phytochemical screening of *Gardenia latifolia* Ait. and *Gardenia gummifera* Linn. *Res J Pharm Biol Chem Sci* 2014; **5**: 527-32.

[42] Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res* 2012; **135**: 581-98.

[43] Murugan K, Murugan P, Noortheen A. Larvicidal and repellent potential of *Albizzia amara* Boivin and *Ocimum basilicum* Linn against dengue vector, *Aedes aegypti* (Insecta:Diptera:Culicidae). *Bioresour Technol* 2007; **98**: 198-201.

[44] Sharma PD, Sharma OP. Natural products chemistry and biological properties of the *Ageratum* plant. *Toxicol Environ Chem* 1995; **50**: 213-32.

[45] Nicoletti M, Serafini M, Aliboni A, D'Andrea A, Mariani S. Toxic effects of neem cake extracts on *Aedes albopictus* (Skuse) larvae. *Parasitol Res* 2010; **107**: 89-94.

[46] Bilal H, Akram W, Din S, Khan IA, Hassan SA, Arshad M. Larvicidal activity of selected plant extracts against *Aedes albopictus* Skuse (Diptera: Culicidae). *Afr Entomol* 2012; **20**(1): 8-12.

[47] Yadav R, Tikar SN, Sharma AK, Tyagi V, Sukumaran D, Jain AK, et al. Screening of some weeds for larvicidal activity against *Aedes albopictus*, a vector of dengue and chikungunya. *J Vector Borne Dis* 2015; **52**: 88-94.

[48] Chakkaravarthy VM, Ambrose T, Vincent S, Arunachalam R, Paulraj MG, Ignacimuthu S, et al. Bioefficacy of *Azadirachta indica* (A. Juss) and *Datura metel* (Linn.) leaves extracts in controlling *Culex quinquefasciatus* (Diptera: Culicidae). *J Entomol* 2011; **8**: 191-7.

[49] Rey D, Pautou MP, Meyran JC. Histopathological effects of tannic acid on the midgut epithelium of some aquatic dipteral larvae. *J Invertebr Pathol* 1999; **73**: 173-81.

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