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Larvicidal activities of ethanolic extracts of *Hyptis suaveolens* Linn (Lamiaceae) and *Azadirachta indica* (Meliaceae) leaves and their phytochemical properties in malaria vector control in Dogbo district in South-western Benin, West Africa

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Abstract

The use of chemical insecticides causes important damages to environment and human health and there is a need to search for alternative solutions. This study aims to investigate on phytochemical properties of *Hyptis suaveolens* Linn (Lamiaceae) and *Azadirachta indica* (Meliaceae) leaves and to evaluate their larvicidal activities in the malaria vector control in couffo department in south-western Republic of Benin. Larvae of *Anopheles gambiae* s.l mosquitoes were collected from breeding sites using the dipping method from April to July 2022 during the great rainy season in Dogbo district. A batch of twenty (20) larvae of fourth instar were exposed to ethanolic extracts of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves with different concentrations of 1 mg/liter, 2mg/liter, 3 mg/liter, 4 mg/liter and 5 mg/liter in some glass jars or plastic test cups of same dimensions covered with small cutting untreated net and in some control jars containing no trace of these ethanolic extracts. Larval mortality was recorded after 24 hours, 48 hours and 72hours exposure. The results showed that the optimal concentration of ethanolic extract of *Hyptis suaveolens* Linn which kills 100% of *Anopheles gambiae* s.l. larvae was 4 mg/liter whereas the optimal concentration of ethanolic extract of *Azadirachta indica* which kills 100% of *Anopheles gambiae* s.l. larvae was 2 mg/liter. *Hyptis suaveolens* Linn and *Azadirachta indica* leaves possess some phytochemical properties with larvicidal activities on larvae of *Anopheles gambiae* sensu lato.

Keywords: *Hyptis suavelens*; *Azadirachta indica*; Malaria control; Benin

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1. Introduction

The World Health Organization (WHO) estimated to 229 million the number of cases of malaria and to 409,000 the number of death having occurred worldwide in 2019. The same year, 94% of all malaria deaths occurred in sub-Saharan African countries [1], where malaria control consumes a major part of the national health budgets [2,3].

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, other non-target populations, their non-biodegradable nature, higher rate of biological magnification through ecosystem and increasing insecticide resistance on a global scale [4,5]. Thus, the environmental protection act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature [6]. It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friedly alternatives such as biological control of vectors has become the central focus of the control programmme instead of the chemical insecticides.

Conventionally, synthetic insecticides such as organochlorines, carbamates, organophosphates, pyrethroids, temephos, fenthion, malathion and dichlorodiphenyltrichloroethane (DDT) were expensive, leaving a residual effect, adapting resistance, non-biodegradable, toxicity to non-target organisms [7]. These problems urged the researchers for an expeditious search for new alternatives. Botanical-based insecticides were currently one of the most promising approaches, much research currently being devoted to plant extracts for the development of sustainable botanical insecticides [8]. Plant extracts contain a mixture of several chemical active ingredients and thus may be able to effectively kill the mosquito through a different mechanism [9]. For the past two decades, numerous researcheshave been conducted on the biological activity of plant extracts against larvae of mosquitoes and insects, in that few plant extracts were commercialized. This shows that plant extracts were environmentally safe, non-toxicity against humans and other organisms [10].

Very few researches were published on the use of essential oils in *Anopheles gambiae* s.l. larvae tolerance in Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to evaluate the larvicidal activities of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves and then investigate their phytochemical properties in malaria vector control in Dogbo district in South-western Benin.

2. Material and methods

2.1. Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district (Figure1). The southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km^2 and belongs to geographic region of ADJA.The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. We took these factors into account to evaluate the larvicidal activities of ethanolic extract of *Hyptis suaveolens* Linn and *Azadirachta indica* leaves and then investigate their phytochemical properties in malaria vector control in Dogbo district in South-western Benin. Couffo has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

Figure 1 Map of Republic of Benin showing Dogbo District

2.2. Mosquito sampling

Anopheles gambiae s.l .mosquitoes were collected from April to July 2022 during the great rainy season in Dogbo district. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles (Figure 2). The samples were then carried out to the Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) in Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) located in Dogbo district.

Figure 2 Breeding site of *Anopheles gambiae* s.l. larvae surveyed in Dogbo district

2.3. Collection of the plant leaves

The leaves of *Hyptis suaveolens* and *Azadirachta indica* were collected in their predilection areas in Dogbo district.

Figure 3 Tree of *Hyptis suaveolens*

Figure 4 Tree of *Azadirachta indica*

2.4. Plant leaves extraction

To prepare botanical pesticide of *Hyptis suaveolens* or *Azadirachta indica*, we collected fresh green leaves of both plants and we washed them with tap water. The leaves were dried outside of the laboratory at ambient temperature in a class room for a period of 3 days. Then, the dried leaves were crushed or grounded into powder with an electronic mix and a weight of 100g of the leave powder of each plant were extracted with 250 milliliters of ethanol for a period of 48 hours at temperature of 25°C. Each extract was then filtered with the aid of Whatman No. 1 filter paper. Then, the mixture were dried and stored in some labeled bottles for bioassays.

2.5. Bioassays

A batch of 20 larvae of fourth instar reared in the insectary of the Department of Sciences and Agricultural Techniques was added to each of five glass jars or test cups of same dimensions containing the dilutions of 1.0mg/liter, 2.0mg/liter, 3.0mg/liter, 4.0 mg/liter and 5.0mg/liter respectively of ethanolic extract of *Hyptis suaveolens* or *Azadirachta indica* leaves previously obtained and stored. These tests cup were covered with small cutting untreated net. At each range of dilutions there is a corresponding control. The control jars contained no trace of ethanolic extracts of *Hyptis suaveolens* or *Azadirachta indica* leaves.

Four replicates were set up and an equal number of controls were set up simultaneously with distilled water. The test containers were held at 25-28°C.

Larval mortality was recorded after 24hours, 48hours and 72hours exposure. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

2.6. Performing of phytochemical screening

The dried ethanolic extract of *Hyptis suaveolens* and *Azadirachta indica* leaves was separately investigated for secondary metabolites according to the laid down rules [11, 12].

2.7. Statistical analysis

Analysis using t-test was performed with 95% confidence interval in SPSS version 16.0 (SPSS Inc., Chicago, IL). The p-value acquired by t-test for all cases of this study is less than 5%.

3. Results and discussion

3.1. Evaluation of larvicidal effect of ethanolic extract of *Hyptis suaveolens* leaves on larvae of *Anopheles gambiae* s.l

The analysis of figure 5 showed that after the exposure of larvae of *Anopheles gambiae* s.l. to ethanolic extract of *Hyptis suaveolens* leaves, alive and moribund larvae were registered again with the concentrations of 1mg/liter, 2mg/liter and 3mg/liter. However, the exposure of the same larvae to the concentration of 4 mg/liter of ethanolic extract of *Hyptis suaveolens* leaves, showed that all these larvae were died after 24 hours exposure and no moribund was registered at the 48 hours and 72 hours mortality recording.

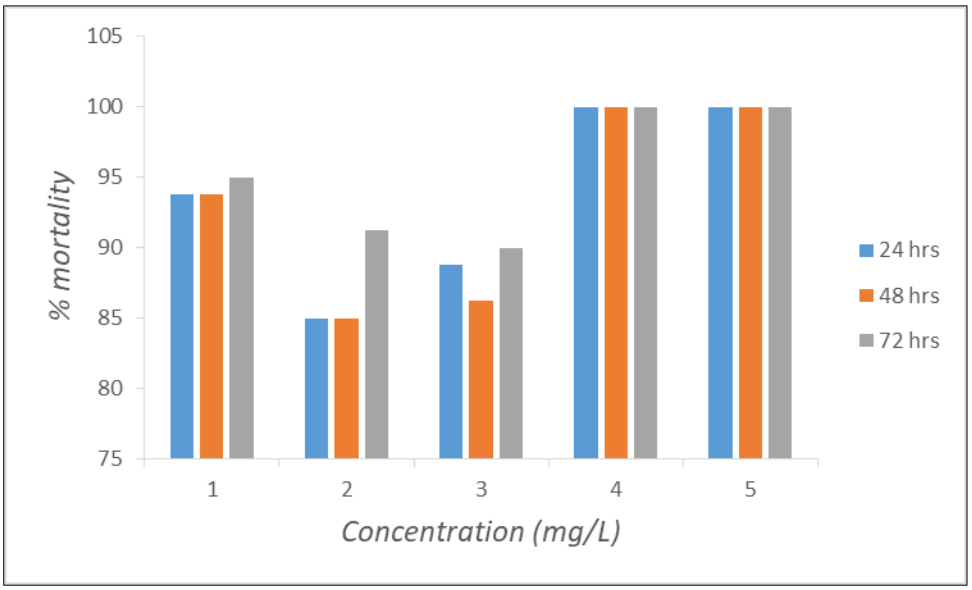

Figure 5 Larvicidal activity of *Hyptis suaveolens* leaves on larvae of *Anopheles gambiae* s.l

3.2. Evaluation of larvicidal effect of ethanolic extract of *Azadirachta indica* leaves on larvae of *Anopheles gambiae* s.l.

The analysis of figure 6 showed that after the exposure of larvae of *Anopheles gambiae* s.l. to ethanolic extract of *Azadirachta indica* leaves, alive and moribund larvae were registered again with the concentration de 1mg/liter. However, the exposure of the same larvae to the concentration of 2 mg/liter of ethanolic extract of *Azadirachta indica* leaves showed that all these larvae were died after 24 hours exposure and no moribund was registered at the 48 hours mortality recording.

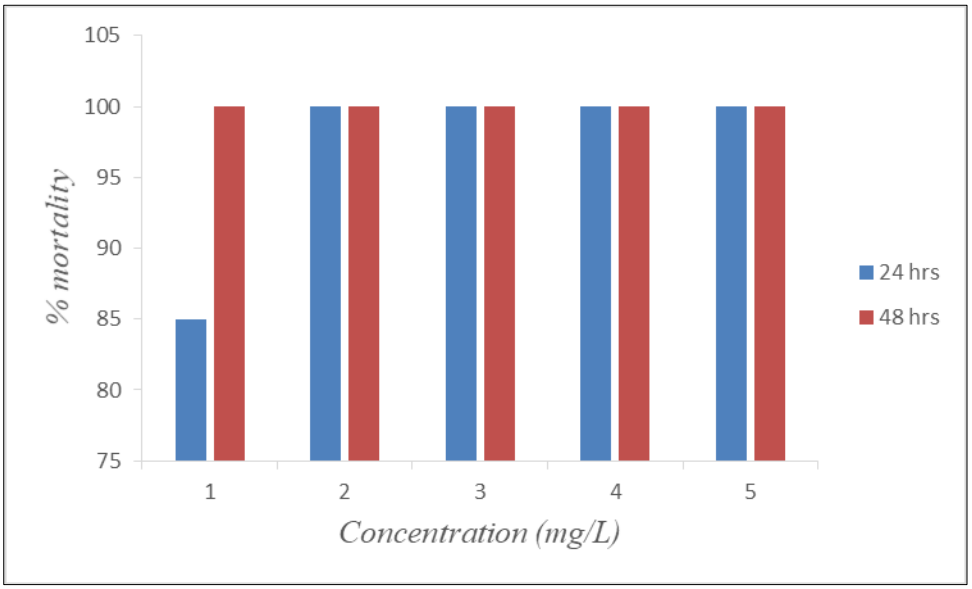

Figure 6 Larvicidal activity of *Azadirachta indica* leaves on larvae of *Anopheles gambiae* s.l

3.3. Phytochemical screening

The results of the qualitative characterization of different phytochemical compounds containing in the powder of *Hyptis suaveolens* and *Azadirachta indica* leaves are showed in table 1.

Table 1 Qualitative evaluation of phytochemical compounds containing in *Hyptis suaveolens* and *Azadirachta indica* leaves

Phytochemical compounds		***Azadirachta indica***	***Hyptis suaveolens***
Alkaloids		+	+
Polyphenolic compounds	Catechic tannins	+	+
	Gallic tannins	-	+
	Flavonoids	+	+
	Anthocyans	+	+
	Leuco-anthocyans	-	+
Quinonic derived		-	-
Saponins		-	-
Terpenes		+	-
Steroids		-	-
Cyanogenic derived		-	-
Mucilages		+	+
Coumarins		+	-
Reducer compounds		-	+
Free anthracenic		-	-
Combined anthracenic	O-heterosids	-	-
	C-heterosids	-	-
	Cardiotonic derived	-	-

+ = presence - = absence

The analysis of table 1 showed that the alkaloids and mucilages were present in both *Hyptis suaveolens* and *Azadirachta indica* leaves. Regarding the polyphenolic compounds such as catechic tannins, flavonoids and anthocyans, they were present in both *Hyptis suaveolens* and *Azadirachta indica* leaves whereas gallic tannins and leuco-anthocyans were present in *Hyptis suaveolens* leaves but absent in *Azadirachta indica* leaves. Quinonic derived, saponins, steroids, cyanogenic derived and free anthracenic were all absent in *Hyptis suaveolens* and *Azadirachta indica* leaves. Regarding the terpenes and coumarins, they were present in *Azadirachta indica* leaves but absent in *Hyptis suaveolens* leaves whereas reducer compounds were present in *Hyptis suaveolens* leaves but absent in *Azadirachta indica* leaves. Regarding combined anthracenic such as O-heterosids, C-heterosids and cardiotonic derived, they were absent in both *Hyptis suaveolens* and *Azadirachta indica* leaves.

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friedly in nature.

It is simple to prepare ethanolic extracts of plant leaves which have given good results in the current study regarding their use in the control of larvae of *Anopheles gambiae* sensu lato. In addition, the use of ethanolic extracts of *Hyptis suaveolens* and *Azadirachta indica* leaves is not expensive.

In the current study, the exposure of larvae of *Anopheles gambiae s.l.* to ethanolic extract of *Hyptis suaveolens* leaves, showed again alive and moribund larvae with the concentrations of 1mg/liter, 2mg/liter and 3mg/liter. However, the exposure of the same larvae to the concentration of 4 mg/liter of ethanolic extract of *Hyptis suaveolens* leaves, showed that all these larvae were died after 24 hours exposure and no moribund was registered at the 48 hours and 72 hours mortality recording. So, the concentration of 4 mg/liter is that optimal to kill the larvae of *Anopheles gambiae* s.l. with ethanolic extract of *Hyptis suaveolens* leaves. The results obtained in the current study corroborated with those obtained

by Dawet *et al.* [13] who had compared the larvicidal effects of *Hyptis suaveolens* and *Chenopodium ambrosoides* on *Anopheles* mosquito larvae. These authors had shown that the extract of *Hyptis suaveolens* had the most elevated larvicidal activity against *Anopheles* mosquito larvae.

In the current study, the exposure of larvae of *Anopheles gambiae* s.l. to ethanolic extract of *Azadirachta indica* leaves, showed again alive and moribund larvae with the concentration de 1mg/liter. However, the exposure of the same larvae to the concentration of 2 mg/liter of ethanolic extract of *Azadirachta indica* leaves, showed that all these larvae were died after 24 hours exposure and no moribund was registered at the 48 hours mortality recording. So, the concentration of 2mg/liter is that optimal to kill the larvae of *Anopheles gambiae* s.l. with ethanolic extract of *Azadirachta indica* leaves. The results obtained in the current study confirmed those obtained by Okumu *et al.*[14] who had shown that *Azadirachta indica* oil has good larvicidal properties against *Anopheles gambiae* s.s and at very low concentrations, is a threat for adult emergence. Our results also confirmed those obtained by Wahedi *et al.* [15], who had evaluated the larvicidal activity of aqueous and ethanol extracts of *Azadirachta indica* seed on 2nd and 3rd instar larvae of the malaria vector, Anopheles mosquito. Their results revealed that neem seed extracts are effective larvicide against *Anopheles* mosquito larvae, because of its high toxicity to *Anopheles* mosquito larvae. The high rates of larval mortality observed within 72 hours in all the concentrations indicated its high toxicity to mosquito larvae. Although there was a remarkable difference in the impact of the concentrations and timing of their activity. Ethanol extract exhibited superiority in controlling *Anopheles* larvae as 100% mortality was recorded within 24 hours of exposure.

Recent studies carried out by Aïzoun *et al.* [16, 17]also had given goods results regarding lethal effects of aqueous extract of lemon (*Citrus limon)* and of coconut oil on *Anopheles gambiae* sensu lato larvae tolerance in malaria vector control in Dogbo district.

Many phytochemical compounds such as alkaloids, tannins, flavonoids and so on, present in the leaves of *Hyptis suaveolens* contributed to the dead of larvae of *Anopheles gambiae* s.l. The results obtained in the current study corroborated with those obtained by Ohimain *et al.* [18] who reported the presence of these phytochemical compounds in the leaves of *Hyptis spp* which conducted to high toxicity on larvae of *Anopheles gambiae.*

In the same way, the current study showed that many phytochemical compounds such as alkaloids, tannins, flavonoids and so on, present in the leaves of *Azadirachta indica* contributed to the dead of larvae of *Anopheles gambiae* s.l. Our results also corroborated with those obtained by Adewole *et al*. [19] who reported the presence of phytochemical compounds such as alkaloids, flavonoids, saponins and terpenes at different concentrations in the leaves of *Azadirachta indica* which conducted to high toxicity on larvae of mosquito.

Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. According to Bowers *et al*. [20], the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system.

4. Conclusion

Both ethanolic extracts of *Hyptis suaveolens* and *Azadirachta indica* leaves are found to be effective against the larvae of *Anopheles gambiae* sensu lato in laboratory conditions in the current study. These extracts are very active on mosquito larvae, therefore more effort must be done in order to explore the potentiality of these plant parts available for botanical insecticide preparing. Researches must also be carried out in field conditions by mosquito larvae breeding sites treatment with these ethanolic extracts in a context where it is useful to search for alternative solutions to damages cause by chemical insecticides to environment and human health.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest among the authors.

Statement of ethical approval

The study follows proper ethical procedures.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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