Int. j. adv. multidisc. res. stud. 2024; 4(2):503-509

Received: 02-02-2024
Accepted: 12-03-2024

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Cleistopholis Patens's Extract: An Alternative to Synthetic Insecticides against *Anopheles Gambia*

[1] Adesina Temilade F, [2] Adesina Femi P

[1, 2] Department of Biology, Federal University of Technology Akure, Ondo State, Nigeria

Corresponding Author: **Adesina Femi P**

Abstract

In this study, the toxic effects of the methanolic and aqueous extract obtained from the tree bark of *Cleistopholis patens* (Benth.) were evaluated against *Anopheles gambiae* Giles larvae and adult. Five treatment concentrations of *C. patens* extracts (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml) were evaluated against *A. gambiae*'s larvae for contact effect and for the fumigant effect on adults. Knockdown and mortality data were obtained at intervals of 15, 30, 45, 60, 75, and 90 minutes. The result showed that 0.4 ml and 0.5 ml concentrations of the methanolic extract achieved absolute (100 %) larvae mortality at 60- and 75-minutes post-exposure. Likewise, after 90 minutes, 100% mortality was observed at 0.3 ml – 0.5 ml concentration. For aqueous extract treatments, larvae mortality recorded at 90 minutes ranged from 10 – 16.67% without significant difference ($p>0.05$). The high mortality of *An. gambiae*'s adult at 90 minutes using methanol extract was 36.67 %, while aqueous extract was 30.00 %. LC_{50} and LC_{90} recorded for methanol extract on larvae were 0.10 ml and 0.31 ml, respectively, while aqueous extract was 0.817 ml and 1.186 ml respectively. For *An. gambiae*'s adult, LC_{50} and LC_{90} recorded for methanol extract were 0.689 ml and 1.251 ml, respectively while aqueous extract was 1.428 ml and 2.575 ml respectively. This study suggests that aqueous and methanolic extracts of *C. patens* possess insecticidal activity against *An. gambiae*. Thus, we recommend further investigation of its component to correlate insecticidal activity with the chemical constituent.

Keywords: Malaria, Mosquito, Solvent Extract and Botanical Insecticide

Introduction

The principal vector of malaria is the mosquito, and it is transmitted to humans by the female of the genus *Anopheles* [1], especially *Anopheles gambiae* [2]. Together with AIDS, malaria is one of the causes of mortality in the population of Africa, South Asia, and Latin America [3]. It contributes a large part of the continued impoverishment of the population in Africa. With more infection burden on under 5 years old children and pregnant women [4]. The World Health Organisation (WHO) has stated malaria to be a life-threatening disease, accounting for 409 000 deaths among 229 million malaria cases reported for 2019. Statistically, 67% (274 000) of the deaths were children that are under 5 years of age, 94% of the malaria and death cases were from African regions, and Nigeria happens to share the highest malaria global death (23%) from Africa [5].

While the research on effective vaccines against malaria is still ongoing, conventional insecticides and repellents are seem effective and widely used to prevent the transmission of this arthropod – borne diseases by preventing the vector from having contact with humans. Repellents like IR3535, picaridin and DEET (N, N-diethyl-3-methylbenzamide), and insecticides; diazon, permetrin and Dichlorodiphenyltrichloroethane – DDT (banned) are the most common mosquito repellent/insecticide formulations available on the market, which has been the gold standard in repelling/eliminating mosquitoes and other biting insects [6-8]. Unfortunately, these synthetic chemicals have some shortcomings. Afify and Potter [8] stated that DEET and other mosquito synthetic repellents mode of action is unclear yet, and human toxicity reactions of DEET after it applications vary from mild to severe [9], such as skin irritation (erythema and pruritis), disturb sensory, motor, memory and learning abilities [10, 11]. Others have resulted in the development of genetic resistant strains, poisoned the environment and non- target organisms [12]. To avoid these adverse effects, research on plant extract that can replace synthetic chemicals is very much crucial.

Plant extracts have been proved to have antibacterial, antifungal and insecticidal properties, as well as being effective, safe to users, and also inexpensive to use [13-15]. Recently, extracts of several plants including neem (*Azadirachta indica*), croton (*Codiaeum variegatum*), citronella grass (*Cymbopog annardus*), basil (*Ocimum* sp), *Clerodendrum capitatum*, *Bridelia*

machrantha, clove (*Syzygium aromaticum*), prickly straggler (*Solanum trilobatum*), musk basil (*Moschosma polystachyum*) and thyme (*Thymus vulgaris*) have been studied as possible mosquito insecticides and repellents [16-19].

Genus *Cleistopholis* comprises of three species: *C. staudii*, *C. glauca* and *C. patens*, and it belongs to Annonaceae family. They can be found in the tropical forest of Western and Central Africa. For instance, *C. patens* is widely present in Angola, Burkina Faso, Cameroon, Cote d'lvoire, Democratic Republic of Congo, Ghana, Liberia, Senegal, Uganda, Togo and Nigeria. Traditionally, the plant is used as medicine to treat tuberculosis, headaches, stomach aches, diarrhea, swellings, edemas, panaris bronchitis, hepatitis, menstrual irregularities and in friction against rickets in children [15, 20]. The result of the Udem *et al*. [21] study, validated the use of *C. patens* aqueous leaf extract as a hypolipidemic and hypocholesterolemic agents in native medicine. Furthermore, studies carried out on solvent extracts of *C. patens* led to isolation and structure elucidation of several secondary metabolites: Terpenes, alkaloids and oligorhamnosides [22]. The chemical compositions of *C. patens* essential oils isolated by other studies from various parts of the plant in Nigerian, Cote d'lvoire and Cameroon are presented in Table 1 [23-25]. Thus, this study aimed to investigate the potential of *Cleistopholis patens* on the control of *Anopheles gambia;* a malaria vector.

Table 1: Chemical compositions of essential oils extracted from *C. patens* parts

Parts	Chemical components	Composition (%)
Trunk bark oil	*p*-cymene	13.4%
	Myrcene	12.0%
	δ-cadinene (Cameroon)	28.7
	α-copaene (Cameroon)	16.9
	Germacrene B	7.4 – 20.6
	Germacrene D	0 – 25.4
	(*E*)-β-caryophyllene	0.4 – 69.1
	β-pinene	0 – 57
	β-elemol	0.1 – 29.9
	α-pinene	0.1 – 30.6
	α-phellandrene	0 – 33.2
	Juvenile hormone III	0 – 22.9
	Sabinene	0 – 20.3
Fruit oil	Linalool	23.1%
	Trans- linalool oxides (tetrahydrofuran)	17.7
	Cis-- linalool oxides (tetrahydrofuran)	17.0
Leaf oil	(*E*)-β-ocimene	0.1 – 33.2
	Linalool	0.1 – 38.5
	(*E*)-β-caryophyllene	0.3 – 39.3
	β-pinene	Traces – 59.1
	Germacrene B	0 – 21.2
	Germacrene D	0.0 – 33.1
	Sabinene	Traces – 54.2
Root oil	Patchoulenone	0 – 70.5
	β-pinene	0 – 51.9
	α-pinene	0.2 – 25.7
	Bornyl acetate	0.5 – 31.2
	Juvenile hormone III	0.3 – 22.2
	β-elemol	0 – 18.8

Materials and methods

Collection of plant materials

The tree bark of *Cleistopholis patens* was obtained from a farm at Modebiayo camp, Ondo East Local Government Area, Ondo, Ondo State.

Preparation of plant powder

The tree bark was cut into smaller sizes and was air dried for a week before it was pounded using pestle and mortar into a form that can be milled using the milling machine. After being milled thoroughly, it was sieved in 600 nm laboratory sieve to remove any form of particles that might be present. It was kept in an airtight plastic container and stored at an ambient temperature of 28 ±2°C and 75 ±5% relative humidity.

Extraction of plant oil

The methanolic extract of the powder of *Cleistopholis patens* of the tree bark was done using a Soxhlet apparatus in which the active ingredients in the plant bark were removed and the filtrate was concentrated using Rotary evaporator. Thereafter, it was air dried to remove the trace of the organic solvent used.

The aqueous extract was obtained by dissolving 500 g of *C. patens* bark powder in 250 ml of dechlorinated water. After several hours of thorough mixing, this concoction was then filtered using a muslin cloth to separate the fibrous material and other large particles.

Collection and breeding of mosquito

A shallow container with a larger surface area was established outside, under a partial shade. The container was filled with rain water and water from the Fisheries and Wildlife's aquarium FUTA. Small quantity of industrial yeast was sprinkled on the surface and allowed to decompose slowly; this was added to nourish the developing larvae. They were transferred to a screened cage of 20 x 20 x 20 cm, where the adults emerged. At emergence, *A. gambiae* was identified using Gilles and De-Mellon's (26) key.

After emergence, female mosquitoes obtained blood meal from caged immobilized Albino rats. This is to make their egg fertile while male mosquitoes were fed on a 10% sucrose solution. The egg or mass were kept to continue to the next generation.

Test of essential oil on *Anopheles gambiae* adults

Mosquitoes used in this study were laboratory-raised *female Anopheles gambiae*. The insects were reared as described above. The larvae were reared at 26–28 °C and fed on yeast. The adults were maintained in 10% glucose solution and the females fed on rat blood thrice a week. Rearing temperatures and relative humidity in the adult insect were 26–28 °C and 70–80% respectively.

Bioassay for the larvicidal activity

Bioassay for the larvicidal activity was carried out using Alouni *et al*. [27] procedures with slight modifications. Ten (10) larvae each, were introduced into small plastic dishes containing 50 ml of dechlorinated water. The treatment set was respective concentrations of the plant extracts (0.1 ml,

0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml). A control was used for each solvent extract, containing only larvae. Mortality counts of larvae were monitored at regular intervals i.e. 15, 30, 45, 60, 75 and 90 minutes after treatment. The treatments were replicated three times. The percentage mortality was calculated and corrections for mortality were done when necessary, using Abbot's (28) formula. Larvae are considered dead if they settle and remain motionless in the bottom of the plastic dishes after being probed with a needle.

Adulticidal bioassay

Adulticidal bioassay was performed by using a clean glass beaker. Different concentrations (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml) of the extract were impregnated on separate filter papers (diameter 6.5 cm), allowed to dry and then placed in a beaker. Twenty female mosquitoes were released into the beaker, and there were three replicates per concentration. Beakers were tightly covered with netting materials and filter paper without treatment served as control. Mosquito mortality was determined at 15, 30, 45-, 60-, 75- and 90-minute exposure. Mosquito mortality was recorded as dead if it was lying on its back or side and was unable to maintain flight after a gentle tap on the bottom.

Statistical Analysis of Data

The data were analyzed using one way Analysis of Variance (ANOVA) for determination of larvae and adult mortality. The differences between the treatments were determined by Tukey's HSD (Honestly Significant Difference) at $p > 0.05$. LC_{50} and LC_{90} were determined using the Log Probit analysis test.

Result

The larvicidal effect of *Cleistopholis patens* on *Anopheles gambiae*

The larvicidal effect of *Cleistopholis patens* on *Anopheles gambiae* with methanol and aqueous solvent extraction was shown in Table 2 and 3 respectively. In Table 2, 0.5 ml concentration was significantly different ($p < 0.05$) from control (0.00 %), 0.1 ml (10.00 %) and 0.2 ml (10.00 %) but not from others at 15 minutes of exposure. At 30 minutes, mortality ranged from 26.67 % to 53.33 % and control (0.00 %) was significantly different ($p < 0.05$) from other treatments, except 0.1 ml concentration (26.67 %). There was no significant difference between 0.1 ml (40.00 %) and 0.2 ml (43.33 %), likewise among 0.3 ml (70.00 %), 0.4 ml (80.00 %) and 0.5 ml (83.33 %) at 45-minute exposure. At 0.4 ml and 0.5 ml concentration, there was absolute mortality (100 %) by 60- and 75-minutes exposure. Likewise, in 90 minutes, 100 % mortality was observed from 0.3 ml to 0.5 ml concentration.

In Table 3 (aqueous extract), there was no mortality in *A. gambiae* recorded on the larvicidal effect of *Cleistopholis patens* in the first 15 minutes of the observation. It was in 0.4 ml (3.33%) and 0.5 ml (3.33%) concentration that mortality was recorded in 30 minutes. A mortality of 6.67 % was recorded for 0.3 ml, 0.4 ml and 0.5 ml concentration at 45 minutes exposure. At 60 minutes, there was no significant difference ($p > 0.05$) among 0.3 ml (6.67 %), 0.4 ml (10.00 %) and 0.5 ml (10.00 %). Concentration 0.5 ml (13.33 %) was not significantly different from 0.3 ml (10.00 %) and 0.4 ml (10.00 %). At 90 minutes, mortality recorded in the treatments ranged from 10.00 % to 16.67 % without significant difference ($p > 0.05$).

Adulticidal effect of *Cleistopholis patens* on *Anopheles gambiae*

Mortality effect of methanol and aqueous extract of *C. patens* on *A. gambiae* was recorded in Table 4 and 5 respectively. In Table 3, there was no significant difference ($p > 0.05$) among 0.2 ml (10.00 %), 0.3 ml (10.00 %), 0.4 ml (20.00 %) and 0.5 ml (20.00 %) in the first 15 minutes. Concentration 0.5 ml (26.67 %) was significantly different ($p < 0.05$) from other treatments at 30 minutes. There was no significant difference ($p > 0.05$) between 0.4 ml (26.67 %) and 0.5 ml (30.00 %) at 45 minutes. At 60 minutes, the mortality recorded range from 6.67 % to 30.00 % and there was no significant difference ($p > 0.05$) among 0.3 ml (23.33 %), 0.4 ml (26.67 %) and 0.5 ml (30.00 %). Likewise in 75 minutes, there was no significant difference ($p > 0.05$) among 0.3 ml (30.00 %), 0.4 ml (30.00 %) and 0.5 ml (33.33 %). There was no significant difference ($p > 0.05$) in *A. gambiae* mortality recorded among 0.2 ml (26.67 %), 0.3 ml (33.33 %), 0.4 ml (33.33 %) and 0.5 ml (36.67 %) at 90 minutes of exposure to methanol extract of *C. patens*.

Mortality of *A. gambiae* due to aqueous extract of *C. patens*, was 0.00 % for all the concentrations at first 15 minutes (Table 5). Mortality of 3.33 % was only recorded at 0.5 ml concentration at 30 minutes, and at 45 minutes for 0.4 ml and 0.5 ml concentration. There was no significant difference ($p > 0.05$) among the treatments at 60- and 75-minutes exposure but the highest mortality recorded was in 0.5 ml (13.33 % and 20.00 % respectively). At 90 minutes, the mortality of *A. gambiae* range was 13.33 % - 30.00 % and there was no significant difference among 0.2 ml (23.33 %), 0.3 ml (23.33 %), 0.4 ml (26.67 %) and 0.5 ml (30.00 %).

LC_{50} and LC_{90} of *Cleistopholis patens* on *Anopheles gambiae*

LC_{50} and LC_{90} of *C. patens* on *A. gambiae*'s larvae at 90 minutes exposure were shown on Fig 1. *C. patens* with water extraction have the highest lethal concentration while methanol extract has the lowest. LC_{50} and LC_{90} recorded for methanol extract were 0.101 ml and 0.310 ml respectively while water extract were 0.817 ml and 1.186 ml respectively.

For *A. gambiae*'s adult (Fig 2), LC_{50} and LC_{90} recorded for methanol extract were 0.689 ml and 1.251 ml respectively while aqueous extract were 1.428 ml and 2.575 ml respectively.

Table 2: Larvicidal effect of methanol extract of *Cleistopholis patens* on *Anopheles gambiae*

Concentration (ml)	Exposure Time (Mins)					
	15	30	45	60	75	90
0.0	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
0.1	10.00 ±0.00^{ab}	26.67 ±3.33^{ab}	40.00 ±5.77^{b}	56.67 ±3.33^{b}	80.00 ±5.77^{b}	86.67 ±3.33^{b}
0.2	10.00 ±5.77^{ab}	36.67 ±3.33^{b}	43.33 ±3.33^{b}	66.67 ±6.67^{bc}	83.33 ±3.33^{bc}	96.67 ±3.33^{c}
0.3	23.33 ±3.33^{abc}	43.33 ±3.33^{b}	70.00 ±5.77^{c}	83.33 ±6.67^{cd}	96.67 ±3.33^{cd}	100.00 ±0.00^{c}
0.4	33.33 ±3.33^{bc}	50.00 ±5.77^{b}	80.00 ±5.77^{c}	100.00 ±0.00^{d}	100.00 ±0.00^{d}	100.00 ±0.00^{c}
0.5	40.00 ±10.00^{c}	53.33 ±12.02^{b}	83.33 ±3.33^{c}	100.00 ±0.00^{d}	100.00 ±0.00^{d}	100.00 ±0.00^{c}

Mean ± Standard error represents three (3) replicates. Means with the same letter down the column are not significantly different at P > 0.05 using Tukey's HSD (Honest Significant Difference).

Table 3: Larvicidal effect of aqueous extract of *Cleistopholis patens* on *Anopheles gambiae*

Concentration (ml)	Exposure Time (Mins)					
	15	**30**	**45**	**60**	**75**	**90**
0.0	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
0.1	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	10.00 ±0.00^{ab}
0.2	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	3.33 ±3.33^{ab}	10.00 ±0.00^{ab}
0.3	0.00 ±0.00^{a}	0.00 ±0.00^{a}	6.67 ±3.33^{b}	6.67 ±3.33^{b}	10.00 ±0.00^{bc}	13.33 ±3.33^{b}
0.4	0.00 ±0.00^{a}	3.33 ±3.33^{a}	6.67 ±3.33^{b}	10.00 ±0.00^{b}	10.00 ±0.00^{bc}	13.33 ±3.33^{b}
0.5	0.00 ±0.00^{a}	3.33 ±3.33^{a}	6.67 ±3.33^{b}	10.00 ±0.00^{b}	13.33 ±3.33^{c}	16.67 ±3.33^{b}

Mean ± Standard error represents three (3) replicates. Means with the same letter down the column are not significantly different at P > 0.05 using Tukey's HSD (Honest Significant Difference).

Table 4: Adulticidal effect of methanol extract of *Cleistopholis patens* on *Anopheles gambiae*

Concentration (ml)	Exposure Time (Mins)					
	15	**30**	**45**	**60**	**75**	**90**
0.0	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
0.1	0.00 ±0.00^{a}	0.00 ±0.00^{a}	3.33 ±3.33^{ab}	6.67 ±3.33^{ab}	10.00 ±0.00^{b}	20.00 ±0.00^{b}
0.2	10.00 ±0.00^{ab}	10.00 ±0.00^{ab}	10.00 ±0.00^{b}	13.33 ±3.33^{bc}	20.00 ±0.00^{c}	26.67 ±3.33^{bc}
0.3	10.00 ±0.00^{ab}	13.33 ±3.33^{abc}	20.00 ±0.00^{c}	23.33 ±3.33^{cd}	30.00 ±0.00^{d}	33.33 ±3.33^{c}
0.4	20.00 ±0.00^{b}	23.33 ±6.67^{bc}	26.67 ±3.33^{cd}	26.67 ±3.33^{d}	30.00 ±0.00^{d}	33.33 ±3.33^{c}
0.5	20.00 ±0.00^{b}	26.67 ±3.33^{d}	30.00 ±0.00^{d}	30.00 ±0.00^{d}	33.33 ±3.33^{d}	36.67 ±3.33^{c}

Mean ± Standard error represents three (3) replicates. Means with the same letter down the column are not significantly different at P > 0.05 using Tukey's HSD (Honest Significant Difference).

Table 5: Adulticidal effect of aqueous extract of *Cleistopholis patens* on *Anopheles gambiae*

Concentration (ml)	Exposure Time (Mins)					
	15	30	45	60	75	90
0.0	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
0.1	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	6.67 ±3.33^{a}	10.00 ±5.77^{a}	13.33 ±3.33^{b}
0.2	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	6.67 ±3.33^{a}	13.33 ±3.33^{a}	23.33 ±3.33^{bc}
0.3	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	13.33 ±3.33^{a}	16.67 ±3.33^{a}	23.33 ±3.33^{bc}
0.4	0.00 ±0.00^{a}	0.00 ±0.00^{a}	3.33 ±3.33^{a}	13.33 ±3.33^{a}	20.00 ±5.77^{a}	26.67 ±3.33^{c}
0.5	0.00 ±0.00^{a}	3.33 ±3.33^{a}	3.33 ±3.33^{a}	13.33 ±3.33^{a}	20.00 ±5.77^{a}	30.00 ±0.00^{c}

Mean ± Standard error represents three (3) replicates. Means with the same letter down the column are not significantly different at P > 0.05 using Tukey's HSD (Honest Significant Difference).

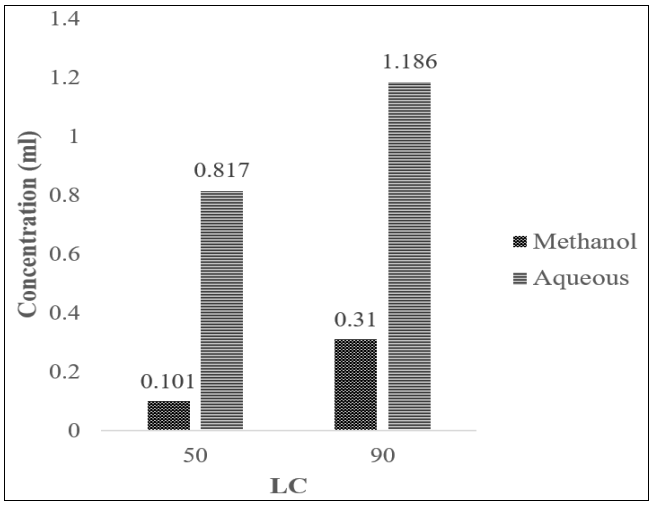

Fig 1: LC_{50} and LC_{90} of *Cleistopholis patens* on *Anopheles gambiae*'s larvae

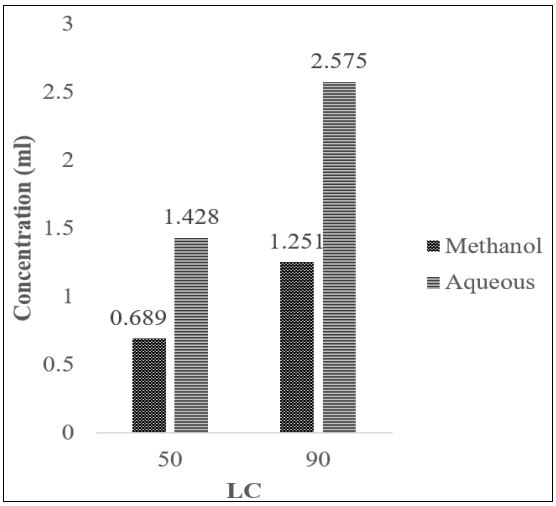

Fig 2: LC_{50} and LC_{90} of *Cleistopholis patens* on *Anopheles gambiae*'s adult

Discussion

In this study, *Cleistopholis patens* has also shown potential to serve as botanicidal in controlling *Anopheles gambiae*. This concise with other researches that suggest phytochemicals as an alternative to conventional insecticides in mosquito control. In 2018, Ileke and Adesina [18] reported the bioefficacy of *Clerodendrum capitatum* and *Bridelia machrantha* leaves extracts against *Anopheles gambiae* giles, recording high mortality rate with *C. capitatum*. Also, Afolabi *et al.* [29] succeeded in examine *Ocimum caninum*, *Ocimum gratissimum*, *Chromolaena odorata* and *Datura stramonium* against adult *Anopheles gambiae* and reported that essential oils from the plant extracts have adulticidal and repellent effects on the *An. gambiae* mosquitoes. Furthermore, *C. patens* is available locally, not expensive to get, easy to cultivate and relatively safe. *C. patens* was reported by Ojo *et al*. [30] to be safe for humans, as its toxicological and histopathological effects were considered, and it proved not to be toxic to the liver and kidney of albino rats at the highest dose of 10%.

It was observed in the result that mortality increases as the concentration increases and as well as exposure time increases. This is a positive indicator that *C. patens* can remain effective for more than one and half hours in eliminating mosquitoes. This observation was similar with the report of Akinneye and Afolabi [12] as ethanolic extract of *C. patens*'s stem-bark (Benth) were tested against the larvae and adults of *Anopheles gambiae*. Likewise, on *Sitotroga cerealella* infesting rice grains, Akinneye and Oyeniyi [31] reported insecticidal efficacy of *C. patens* powder to be even effective at 96 hours, achieving 78 – 100% mortality with the highest concentration used. Absolute (100%) mortality was also recorded with *C. patens* stem bark powder against both larvae and adults of *P. interpunctella* at 96 hours by Adeyera *et al.* [32]. The ability of *C. patens* to persist for a longer period may be due to the presence of some phytochemicals that have high insecticidal effects, parts of the plant used and solvents used to extract the active ingredients. Plant extracts usually contain alkaloids, saponins, terpenoids and tanins which have insecticidal properties on insect vectors. In relation to Table 1, the availability of Juvenile hormone III through the application of *C. patens* extract, might have inhibited *A. gambiae* metamorphosis and then terminated their development to pupal or adult. Juvenile hormone (JH) regulates post-embryonic development of an insect. At the end of larva stage, the level of JH needs to be lowered to permit ecdysteroid to process for pupal and adult characteristics. Research has also indicated that both α and β-pinene have numerous insecticide effects [33, 34]. It has also been demonstrated that phellandrene has insecticide ability to inhibit AChE [35]. Germacrene D is also a volatile terpene that can repel insects.

Using methanol extract on the larvae stage, absolute mortality (100 %) was started with 0.4 ml concentration at one hour exposure and the least concentration to this, was 0.3 ml concentration at 90 minutes of exposure. It took more exposure time for the least concentration to achieve the highest mortality. The increase in the mortality of the plant extract at higher concentrations may be due to the increase in the concentration of the active ingredient present in the extracts. Also, the lower concentrations may later attain 100 % mortality, as it has been predicted by Kasim *et al.* [36] that most plants induced 50% larval mortality after some hours of exposure to the extract. Meanwhile, absolute mortality was not achieved on the adult mosquito, the highest mortality recorded was 36.67 % at 0.5 ml concentration for 90 minutes exposure. This means that larvae are more susceptible to this plant than adults and this could be attributed to the active feeding stage of the larvae [37].

This study has also revealed the efficiency of organic based solvent in extracting the active ingredients from the plants. The concentration requires to achieve LC_{50} and LC_{90} (0.101 ml and 0.31 ml respectively) of *A. gambiae*'s larvae by methanol extract of *C. patens* was far lesser to that of the aqueous extract (0.817 ml and 1.186 ml respectively). This was also observed with the adult and this may be due to the fact that the active ingredients in the plant are more polar in organic solvent than aqueous solvent. There is a fact that the extraction yield and biological activity of the resulting extract is not only affected by the extraction technique but also by the extraction solvent [38, 39]. Many solvents, including methanol, ethanol, acetone, and water, have been used for extracting bioactive compounds from the plant material. Due to the variety of bioactive compounds contained in plant materials and their differing solubility properties in different solvents, the optimal solvent for extraction depends on the particular plant materials, and the compounds that are to be isolated. In 2019, Dieu-Hien *et al.* [40] reported significant difference in the extraction yield of

S. buxifolia using different solvents, where methanol resulted in the highest extraction yield, followed by distilled water, ethanol, acetone, chloroform, and dichloromethane, indicating that the extraction efficiency favors the highly polar solvents. According to Harborne [41], a good solvent is characterized by its optimal extraction and its capacity in conserving the stability of the chemical structure of desired compounds.

Conclusion

This study has shown that the plant extracts of *C. patens* used for this research have good larvicidal properties and also adulticidal properties at higher concentration against malaria mosquitoes. The result also suggest that methanol is a good solvent for bio-active compounds extraction for this plant. The use of this potential plant is ecofriendly with no mammalian toxicity and as such can be included in vector management to reduce the morbidity and mortality caused by mosquito-borne diseases in tropical countries where mosquito-borne diseases are endemic.

Acknowledgements

My sincere appreciation goes to Prof. J.O. Akinneye, Prof. M.O. Oniya, lecturers and staff of Biology Department, Federal University of Technology Akure for the successful completion of this research work.

References

1. Smith RC, Vega-Rodríguez J, Jacobs-Lorena M. The *Plasmodium* bottleneck: Malaria parasite losses in the mosquito vector. Mem Inst Oswaldo Cruz. 2014; 109:644-661.
2. Akpan GE, Adepoju KA, Oladosu OR, Adelabu SA. Dominant malaria vector species in Nigeria: Modelling potential distribution of *Anopheles gambiae* sensu lato and its siblings with MaxEnt. PLoS ONE. 2018; 13(10):e0204233.
3. Talapko J, Škrlec I, Alebić T, Jukić M, Včev A. Malaria: The Past and the Present. Microorganisms. 2019; 7(6):179.
4. Mapossa AB, Focke WW, Tewo RK, Androsch R, Kruger T. Mosquito-repellent controlled-release formulations for fighting infectious diseases. Mal. J. 2021; 20:165.
5. World Health Organisation. Malaria, 2021. https://www.who.int/news-room/fact-sheets/detail/malaria
6. Walker TW, Robert LL, Copeland RA. Field evaluation of arthropod repellents, DEET and piperidine compound against *Anopheles arabiensis* in Western Kenya. J. Am. Mosq. Cont. Assoc. 1996; 12:172-176.
7. Debboun M, Frances S, Strickman D. Insect repellents handbook. 2nd ed. Boca Raton: CRC Press, 2014.
8. Afify A, Potter CJ. Insect repellents mediate species-specific olfactory behaviours in mosquitoes. Mal. J. 2020; 19(127):1-10.
9. Sudakin DL, Trevathan WR. DEET: A Review and Update of Safety and Risk in the General Population. J. toxicol. y: Clin. Toxicol. 2003; 41(6):831-839.
10. Dechovskaia A, Abou-Donia MB, Goldstein LB. Effect of daily dermal application of DEET and permethrin, alone and in combination, on sensorimotor performance, blood brain barrier and blood testes barrier in rats. J. Toxicol. & Environ. Healt. Part A. 2001; 62:523-541.
11. Sekar M, Abd Rahim FN. Formulation and Evaluation of Novel and Natural Mosquito Repellent Liquid to Prevent Dengue Mosquitoes. Ann. Res. & Rev. Biol. 2017; 18(1):1-6.
12. Akinneye JO, Afolabi OJ. Toxicity and Fumigant Effect of Powder and Oil Extracts of *Cleisthopholis patens* (Benth) against Larvae and Adults Anopheles Mosquito, J. Mosq. Res. 2014; 4(11):1-6.
13. Chang ST, Chen PF, Chang SC. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*, J. Essen. Oil Res. 2001; 77:123-127.
14. Gurdip S, Sumitra M. Antimicrobial, antifungal and insecticidal investigations on essential oils óAn overview. Nat. Prod. Rad. 2005; 4(3):179-192.
15. Osuntokun OT. Evaluation of Inhibitory Zone Diameter (IZD), Phytochemical Screening, Elemental Composition and Proximate Analysis of Crude *Cleistopholis patens* (Benth) on Infectious Clinical Isolates. J. Mol. Biom. & Diagn. 2018; 9(2):1-9.
16. Rajkumar S, Jebanesan A. Mosquitocidal activities of Octacosane from *Moschosma polystachyum* Linn. (Lamiaceae). J Ethnopharma-cology. 2004; 90:87-89.
17. Rajkumar S, Jebanesan A. Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi*. J. Insect Sci. 2005; 5(15):1-3.
18. Ileke KD, Adesina FP. Bioefficacy of Larvicidal and Pupicidal properties of *Clerodendrum capitatum* and *Bridelia machrantha* leaves extracts against Malaria Vector, *Anopheles gambiae* giles [Diptera: Culicidae]. J. Bio. & Med. 2018; 2(1):007-011.
19. Awosolu O, Adesina F, Iweagu M. Larvicidal effects of croton (*Codiaeum variegatum*) and Neem (*Azadirachta indica*) aqueous extract against Culex mosquito. Int. J. Mosq. Res. 2018; 5(2):15-18.
20. Wongsrichanalai C, Pickard AL, Wernsdorfer WH, Meshnick SR. Epidemiology of drug-resistant malaria. Lancet Infect. Dis. 2002; 2:209-218.
21. Udem SC, Ezeonuegbu UC, Obidike RI. Experimental Studies on the Hypolipidemic and Haematological Properties of Aqueous Leaf Extract of *Cleistopholis Patens* Benth. & Diels. (Annonacae) in Hypercholesterolemic Rats. Ann. Med. & Healt. Sci. Res. 2011; 1(1):115-121.
22. Ngnokam D, Tsopmo A, Ayafor JF, Nuzillard J-M, Sterner O. 4,5-Epoxide-1,6-dimethyl-1-vinylhexyl p-coumarate: A novel monoterpene derivative from *Cleistopholis patens*. Bull. Chem. Soc. Ethi. 2003; 17(2):177-180.
23. Ouattara ZA, Boti JB, Attioua KB, Ahibo AC, Casanova J, Tomi F, *et al*. Chemical Variability of *Cleistopholis patens* (Benth.) Engl. et Diels Leaf Oil from Ivory Coast. Chem. & Biodiv. 2013; 10(2013):2053-2060.
24. Ouattara ZA, Boti JB, Ahibo CA, Bekro YA, Janat M, Casanova J, *et al*. Composition and chemical variability of *Cleistopholis patens* trunk back oil from Cote d'Ivoire. Chemistry and Biodiversity. 2017; 14(2):1-9.
25. Ouattara ZA, Yapi TA, Békron Y-A, Mamyrbékova-Békro AJ, Paoli M, Tomi P, *et al*. Composition and Chemical Variability of Root Bark oil from Ivoirian *Cleistopholis patens*. Nat. Prod. Comm. 2018;

13(6):767-770.
26. Gillies MT, De-Meillon B. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region) 2nd. South African Institute for Medical Research; Johannesburg, 1968, 1-343.
27. Alouani A, Rehimi N, Soltani N. Larvicider of a Neem tree extract (Azadiractin) against mosquito larvae in the republic of Algeria. Jordan J. Bio. Sci. 2009; 2(1):15-22.
28. Abbot WS. A method of computing the effectiveness of an insecticide. J Econ. Entomol. 1925; 18(2):265-267.
29. Afolabi OJ, Simon-Oke IA, Elufisan OO, Oniya MO. Adulticidal and repellent activities of some botanical oils against malaria mosquito: *Anopheles gambiae* (Diptera: Culicidae). Beni-Suef Uni. J. Basic & Appl. Sci. 2018; 7:135-138.
30. Ojo DO, Omotoso OT, Obembe OM, Odetoye AA. Toxicological and histopathological effects of *Cleistopholis patens* Benth root bark powder used as cowpea protectant and medicine. Asian J. Biol. Sci. 2019; 12:565-571.
31. Akinneye JO, Oyeniyi EA. Insecticidal efficacy of *Cleistopholis patens* (Benth) against *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) infesting rice grains in Nigeria. J. Crop Prot. 2016; 5(1):1-10.
32. Adeyera OJ, Akinneye JO, Odeyemi OO. Studies on *Cleistopholis patens* (benth.) [Magnoliales: Annonaceae] powders as fumigant and contact insecticides to *Plodia interpunctella* (Hübner) [Lepidoptera: Pyralidae]. African J. Agric. Res. 2021; 17(3):425-431.
33. Kounink H, Hance T, Noudjou FA, Lognay G, Malaisse F, Ngassoum MB, *et al*. Toxicity of some terpenoids of essential oils of Xylopia aethiopica from Cameroon against *Sitophilus zeamais* Motschulsky. J. Appl. Entomol. 2007; 131:269-274.
34. Asgar E. Iranian plant essential oils as sources of natural insecticide agents. Int. J. Bio. Chem. 2011; 5(5):266-290.
35. Liu J, Hua J, Qu B, Guo X, Wang Y, Shao M, *et al*. Insecticidal Terpenes from the Essential Oils of *Artemisia nakaii* and their Inhibitory effects on Acetylcholinesterase. Front. Plant Sci. 2021; 12(720816):1-9.
36. Kasim M, Ann O, Yahaya MM. Larvicidal activity of Garlic (*Allium Sativum*) on Anopheles and Culex mosquito Larvae. Pharmacologyonline. 2019; 2:130-138.
37. Ileke KD, Ogungbite OC. *Alstonia boonei* De Wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease. J. Coast Life Med. 2015; 3:557-563.
38. Ajanal M, Gundkalle M, Nayak S. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient Sci. Life. 2012; 31(4):198-201.
39. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pacific J. Trop. Biomed. 2012; 2(12):960-965.
40. Dieu-Hien T, Dinh HN, Nhat TAT, Anh VB, Tuong HD, Hoang CN. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and *In Vitro* Anti-Inflammatory Activities of *Severinia buxifolia*. J Food Qual, 2019. Article ID 8178294, 9 pages.
41. Harborne JB. Phytochemical methods a guide to modern techniques of plant analysis. Plant Pathol. J. 1999; 48:146.