Original Research Article

Larvicidal efficacy of the combination of *Citrus aurantiifolia* (Rutaceae) and *Lippia chevalieri* (Verbenaceae) methanolic extracts against *Anopheles gambiae* Giles (Diptera culicidae), malaria vector.

Abstract:

The growing resistance of mosquito vectors towards synthetic insecticides constitutes a major challenge in the malaria control and synergistic action of two or more extracts may decrease the risk of insect resistance. For that, singly and combination of *Citrus aurantiifolia* peels and *Lippia chevalieri* leaves methanolic extracts were tested on *Anopheles gambiae* larvae at concentrations of 7.5, 15, 22.5 and 30 mg/mL in the laboratory according to the standard protocol of WHO. Extraction yields were 6.72 and 4.62 for *C. aurantiifolia* and *L. chevalieri* respectively. Saponins, terpenoids, flavonoids, tannins and polyphenols were present in the extracts of the 2 plants . The 2 plant extracts tested singly or in combination caused a significant ($P<0.001$) concentration-dependent larvicidal activity 24 h post-exposure. *C. aurantifolia* peels was revealed as the most potent against the mosquito larvae (LC_{50} = 9.82 mg/mL), while only the binary combination 50%Ca + 50%Lc (CI: 155.22; SF: 1.55) induced a synergistic action against mosquito larvae. Thus, the combination 50%Ca + 50%Lc might be recommended as a natural bio-insecticide in mosquito control program to prevent malaria.

Keywords: Malaria, *Anopheles gambiae*, plant methanolic extracts, combination, larvicide.

1. Introduction

Malaria remains one of the world's leading pandemic and a major public health problem. It is a cosmopolitan disease with approximately 247 million cases worldwide in 2021 and the African region continues to bear the heaviest burden with 234 million cases and 593,000 deaths in 2021 [1]. Malaria is a parasitic disease caused by parasite of the genus *Plasmodium* spp., and is transmitted through the bites of the females *Anopheles* genus [2]. The species *Anopheles gambiae* is the main vector of malaria in Cameroon both in rural and urban areas [3]. The fight against malaria is mainly based on two axes: pest and vector control. Vector control through the deployment of impregnated mosquito nets, indoor residual spraying of insecticides and the application of chemical or biological larvicides is an essential component of strategies to prevent, control and eliminate malaria [4]. However, there are several challenges to the use of these control techniques, such as the high cost of

insecticide treatment, the toxicity of synthetic products and their fraudulent reproduction which limit effective management [5]. Besides, the overuse of synthetic insecticides has led to the development of the resistance in mosquitoes [6]. To face this situation, it is important to look for other less toxic, accessible and natural plant-based products might be the solution. In recent years, plant-derived chemicals have been projected as the key of sustainable mosquito control because they are ecologically safe, highly degradable and less toxic to humans [7]. Indeed, insecticidal effect of several plants against mosquito species has been reported in Cameroon [7,8,9,10]. *Citrus aurantifolia* (Family: Rutaceae) also known as Lime, native to Southeast Asia is a well-known medicinal and food plant widely cultivated around the world [11]. They are rich in secondary metabolites such as alkaloids, flavonoids, saponins, tannins, polyphenols and terpenoids [12,13], its parts are used in traditional medicine as an astringent, diuretic, insect repellent, antiseptic and antimicrobial, for the treatment of gastrointestinal disorders, coughs, colds and sore throats [11]. *C. aurantifolia* has been found to have insecticidal and repellent activity against *Camponotus nearcticus* [14], and also effective against mosquitoes, cockroaches and houseflies in aerosol form [15]. *Lippia chevalieri* Moldenke, is a plant belonging to the Verbenaceae family widely distributed in the world [16]. Flavonoids, saponins, tannins, polyphenols and terpenoids have been identified in the plant [17,16]. *L. chevalieri* is used in traditional medicine for the treatment of respiratory diseases, diarrhea, arterial hypertension, gouty rheumatism, painful and infected wounds, liver pathologies, oral and digestive candidiasis, malaria, painful menstruation and nervousness [18,16]. The plant has also demonstrated its insecticidal efficacy against *Anopheles gambiae* s.l., *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles funestus* [19,20,21]. The synergistic effect between two would probably due to the formation of new additional molecules from the isolated phytocompounds found in different plants. Previous work on synergistic effect research between plants [19,20,10,22,23] has demonstrated that combinations can overcome the side effects associated with high doses of single extracts and decrease the risk of resistance development, by using smaller amounts of each compound and affecting multiple targets simultaneously, making it more difficult to develop resistance. The larvicidal activity of combination of the methanolic extracts of *C. aurantiifolia* peels and *L. chevalieri* leaves were evaluated on 3rd and 4th instar

larvae of *Anopheles gambiae* in order to contribute to this perpetual fight against malaria vectors.

2. Materials and methods

2.1. Plant material

2.1.1. Harvest and processing of the plants

Green leaves of *Lippia chevalieri* Mold. were collected at Wack (Adamaoua-Cameroon: latitude 7°40'43''; longitude 13°33'20'') in November 2021 and identified at National Herbarium of Cameroon in comparison with the material of R. Letouzey n° 7349 of Herbarium collection n° 9057/SFR/Cam. Lime peels were removed with knife from the riped fruits of *Citrus aurantiifolia* were collected at ''Ngaoundai'' (Ngaoundéré; Adamaoua-Cameroon: latitude 7°27'95''; longitude 13°61'10'') at the same period and identified in comparison with Essombe material n°1 of Herbarium collection specimen n° 67471/HNC. Each plant parts collected was washed with tap water, shade dried at room temperature (24 ± 2°C; 76 ± 4% HR) during 10 days. The dried plant material was then grinded using an electric grinder equipped with an integrated sieve with a diameter of 1 mm mesh size. Each plant powder was kept in the dark bottles at the ambient temperature until their extraction.

2.1.2. Extraction of plants

The methanolic extracts of the different plants were obtained by macerating 200g of each powder in 2 L of methanol (95%) for 72 h at room temperature (Temperature = 22 ± 2°C; Relative Humidity = 45 ± 4%%), the mixture was then centrifuged at 3500 rpm for 10 min. The supernatant obtained was filtered using Whatman No. 1 paper and concentrated using a rotavapor. The concentrated solution obtained was kept in an oven set at 40°C for 5 days for a complete drying. The extracts obtained were weighed and stored in dark glass bottles in the refrigerator at 4°C for our various tests.

The extraction yield of each plant was calculated according to the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{weight (g) of extract obtained}}{\text{weight (g) of plant powder used}} \text{x}100$$

2.1.3 Qualitative Phytochemical screening of the extracts

The qualitative phytochemical analysis of the methanolic extracts of *L. chevalieri* and *C. aurantiifolia* was carried out according to the standard methods of colorimetric characterization in tubes described by Harbone [24] in order to identify the major phytochemical groups present in the extracts including alkaloids, flavonoids, saponins, tannins, polyphenols

and terpenoids which, according to the literature, possess an insecticidal action [25].

2.2. Mosquito strain

Anopheles gambiae eggs were obtained at OCEAC (Organization for Coordinating the figth against Endemics in Central Africa; Yaounde, Cameroon) and reared in insectarium of the zoology laboratory of the Higher Teacher's Training College of Yaoundé under the ambient conditions of the insectarium (27 ± 2°C; 74 ± 4 % r.h.) according to the standard WHO protocol [26]. Indeed, the mature eggs were transferred into a tray containing 1L of tap water for hatching. After 24 h, the larvae obtained were divided into several batches to avoid overpopulation and were fed with TetraMin®baby food (3mg per 100 larvae per day) for about 5 days until mature larval stages (L3 and L4) were obtained, which were used for larvicidal tests [7].

2.3. Larvicidal activity of methanolic extracts

The larvicidal activity of the methanolic extracts of the leaves of *L. chevalieri* and *C. aurantiifolia* peels was evaluated on the blend of 3rd and 4th instar larvae of *An. gambiae* according to [26] standard protocol. Efficacy was evaluated individually and in binary combination in the proportions 100%A + 0%B, 25%A + 75%B, 50%A + 50%B, 75%A + 25%B and 0%A + 100%B; where A and B represent the different plant extracts [10]. After the preliminary tests, a stock solution of each plant was prepared at the concentration of 50 mg/mL by dissolving in a glass flask 1000 mg of each extract in 20 mL of methanol. Tests concentrations of 7.5; 15; 22.5 and 30 mg/mL were then prepared by diluting each appropriate volume of the stock solution of each plant in methanol. Twenty-five (25) third and fourth instar larvae were transferred into 250 mL plastic cups containing tap water to which 1 mL of test solution of different concentrations was added and the volume was made up to 100 mL per cup. The negative control consisted of adding 1 mL of methanol to 99 mL of tap water, while the positive control was a solution of Dichlorvos DDVP (1 mg/mL). Each concentration was repeated 4 times and larval mortality was recorded after 24 h post-exposure.

The percentage of larval mortality was calculated according to the following formula:

$$\text{Mortality percent (\%)} = \frac{\text{number of dead larvae}}{\text{total number of larvae used}} \times 100$$

And corrected using Abbott's formula [27] if the percentage of larval mortality in the negative control was between 5% and 20% according to the following formula:

Corrected mortality (%)

$$= \frac{\%\ \text{of Test Dead} - \%\ \text{of Control Dead}}{100 - \%\ 0\text{f Control Dead}} \times 100$$

The LC_{50} values of the methanolic extracts of the plants were then determined as well as those of the various combinations tested, which allowed us to calculate the cotoxicity index and the synergistic factor of each of the mixtures as described by Lame *et al*. [10]:

For the binary combination *Citrus aurantiifolia* (Ca) + *Lippia chevalieri* (Lc) we have:

Toxicity index (TI) of Ca = 100 and

$$\text{(TI) of Lc} = \left[\frac{\text{LC50 Ca}}{\text{LC50 Lc}}\right] \times 100$$

$$\text{Observed TI of the mixture (Ca + Lc)} = \left[\frac{\text{LC50 Ca}}{\text{LC50 (Ca + Lc)}}\right] \times 100$$

Theoretical TI of the mixture (Ca + Lc) = TI Ca × % Ca in the mixture + TI Lc × %Lc in the mixture.

$$\text{Cotoxicity index} = \left[\frac{\text{Observed TI of the mixture}}{\text{Théoretical TI of the mixture}}\right] \times 100$$

When one component of the mixture causes a low mortality (<20%) at all doses tested, cotoxicity index of the combination would be calculated as follows:

$$\left[\qquad\qquad\right]$$

Cotoxicity index

$$= \frac{\text{LC50 Ca}}{\text{LC50 (Ca + Lc)}} \times 100$$

According to Sun and Johnson [28]:

- If cotoxicity index is less than 80, it is considered an antagonistic action;
- If cotoxicity index is between 80 and 120, it is considered an additive action;
- If cotoxicity is greater than 120, it is considered a synergistic action.

Synergistic factors were also calculated according to Kalyanasundaram and Das [29] method as follows:

Synergistic factor (SF)

$$= \frac{\text{LC50 of the plant extract alone}}{\text{LC50 of the mixture}}$$

Value of SF > 1 indicates synergistic action and SF < 1 indicates the antagonistic action.

2.4. Statistical analysis

Statistical analysis of the data was performed using SPSS (Statistical Package for the Social Sciences) software version 16.0. The corrected mortality percentages were submitted to an analysis of variance (ANOVA) to obtain the average of the mortality percentages and the standard error between these different means. The

separation of means was performed using Tukey's comparison test at 5% (P = 0.05). Probit analysis was performed to determine the lethal concentrations that caused 50% (LC_{50}) and 95% (LC_{95}) mortality of mosquito larvae.

3.Results

Extraction yields

Maceration of plant powders gave the extraction yield of 6.73% for *C. aurantifolia* and a low yield of 4.62% was obtained with *L. chevalieri* as shown in table 1.

Phytochemical screening

Phytochemical analysis of methanolic extracts of *C. aurantifolia* peels and *L. chevalieri* leaves as presented in table 2 revealed the presence of saponins, terpenoids, flavonoids, tannins and polyphenols in variable concentrations in the *C. aurantifolia* peels and *L. chevalieri* leaves.

Table 1: Extraction yield of methanolic extracts of *Citrus aurantifolia* and *Lippia chevalieri*.

Plant species	Powder used (g)	Extract obtained (g)	Extraction yield (%)
Citrus aurantifolia	200	13.45	6.73
Lippia chevalieri	200	9.24	4.62

Table 2: Phytochemical composition of methanolic extracts of *Citrus aurantifolia* and *Lippia chevalieri*.

Extracts	Saponins	Terpenoids	Flavonoids	Tannins	Polyphenols	Alkaloids
Citrus aurantifolia	+++	+	++	++	+++	_
Lippia chevalieri	+++	++	+	+++	+++	_

+ = present at low concentration, ++ = present at moderate concentration, +++ = present at high concentration and - = absent.

Effect of the combination of *C. aurantifolia* and *L. chevalieri against An. gambiae larvae.*

Table 3 presents the percentage of mortality of *An. gambiae* larvae treated with methanolic extracts singly and in combinations (75% + 25%; 50% + 50%; 25% + 75%) of *C. aurantifolia* and *L. chevalieri* and the LC_{50} and LC_{95} values (mg/mL) after 24h of exposure. The methanolic extracts of the two plants induced a significant ($P < .001$) concentration-dependent larvicidal activity both individually and in binary combinations. Mortality rate of *C. aurantifolia* methanolic extract ranging from 38% at 7.5 mg/mL to 99% at 30 mg/mL with a LC_{50} of 9.82

mg/mL and LC_{95} of 27.09 mg/mL. Mortality rate of *L. chevalieri* methanolic extract ranging from 39.34% at 7.5 mg/mL to 100% at 30 mg/mL with a LC_{50} of 10.05 mg/mL and LC_{95} of 29.42 mg/mL. Combinations of the two plants caused high activity with larval mortality percentages ranging from 39% at 7.5 mg/mL to 98% at 30 mg/mL with a LC_{50} of 9.53 mg/mL and LC_{95} of 25.99 mg/mL for the 75%Ca + 25%Lc mixture. Mortality rate of the mixture 50%Ca + 50%Lc ranging from 46% at 7.5 mg/mL to 100% at 30mg/mL with a LC_{50} of 8.50 mg/mL and LC_{95} of 21.60 mg/mL. Mortality rate of the mixture 25%Ca + 75%Lc ranging from 40% at 7.5 mg/mL to 100% at 30 mg/mL with a LC_{50} of 10.04 mg/mL and LC_{95} of 29.65 mg/mL. A mortality rate of 100% was also registered in the positive control (Dichlorvos) at the recommended single concentration of 1 mg/mL (1000 ppm) and a mortality rate of less than 5% in the negative control.

Table 4 below presents the cotoxicity index and the synergistic factors of the binary combinations of the methanolic extracts of *C. aurantifolia* and *L. chevalieri* on the larvae of *An. gambiae* 24h post-exposure; the combinations 75%Ca + 25%Lc (CI: 103.68; SF: 1.03) and 25%Ca + 75%Lc (CI: 99.51; SF: 0.99) each presented an additive action while the combination 50%Ca + 50%Lc (CI: 155.22; SF: 1.55) exhibited a synergistic action on *An. gambiae* larvae 24h post exposure.

4. Discussion

Vector control using medicinal plants has longtime demonstrated its effectiveness on the developmental stages of mosquitoes [30,31]. However, several studies have shown that the combination of natural molecules would better overcome the increasing resistance mechanisms developed by mosquitoes rather than with isolated compounds [32]. The combined insecticide approach is encouraged to optimize the efficacy of insecticide products and could apparently preserve the effectiveness of the insecticide product for many years [10]. The extraction yield of *C. aurantiifolia* peels corroborate those of Monica *et al*. [33] in which, methanol extraction yields ranging from 1.9 to 7.6% for peels and from 13.0 to 20.8% for the leaves were recorded with *C. aurantifolia* collected in three different regions of Calabria, Italy. However, the methanolic extraction yield of *L. chevalieri* is lower than those obtained by Mindiediba [17], and Nacoulma [16].

Table 3: Percentage of mortality, LC_{50} and LC_{95} of *An. gambiae* larvae treated with methanolic extracts and binary combinations of *C. aurantifolia* and *L. chevalieri* 24h post exposure.

Combinations	Con(mg/mL)	Mortality (M ± SE)	Slope ± SE	R^2	LC_{50} (CI) (mg/ml)	LC_{95} (CI) (mg/ml)	χ^2
100%Ca+0%LC	0	1±1.00E	3.73±0.18	0.94	9.82(8.62-10.90)	27.09(23.41-33.16)	48.91***
	7.5	38±1.15D					
	15	65±1.91C					
	22.5	93±1.91B					
	30	99±1.00A					
	DDVP	100±0.00A					
	F(5,18)	868.19***					
75%Ca+25%Lc	0	1±1.00D	3.77±0.18	0.93	9.53(8.37-10.58)	25.99(22.56-31.58)	47.54***
	7.5	39±1.00C					
	15	67±1.00B					
	22.5	96±1.63A					
	30	98±1.15A					
	DDVP	100±0.00A					
	F(5,18)	1423***					
50%Ca+50%Lc	0	1±1.00D	4.06±0.21	0.90	8.50(7.21-9.61)	21.60(18.54-27.03)	64.44***
	7.5	46±3.83C					
	15	74±2.00B					
	22.5	99±1.00A					
	30	100±0.00A					
	DDVP	100±0.00A					
	F(5,18)	447.50***					
25%Ca+75%Lc	0	4±2.83E	3.49±0.17	0.96	10.04(8.38-11.50)	29.65(24.40-40.16)	79.54***
	7.5	40±1.63D					
	15	58.50±1.50C					
	22.5	90±2.00B					
	30	100±0.00A					
	DDVP	100±0.00A					
	F(5,18)	907.82***					
0%Ca+100%Lc	0	0±0.00E	3.52±0.17	0.96	10.05(8.47-11.45)	29.42(24.41-39.12)	73.74***
	7.5	39.34±2.21D					
	15	59.64±1.94C					
	22.5	89.72±1.93B					
	30	100±0.00A					
	DDVP	100±0.00A					
	F(5,18)	656.67***					

Mean of mortality ± Standard Error within a column followed by the same letter did not differ significantly according to Tukey test (P=0.05); ^{ns}P>0,05; *P<0,05; **P<0,01; ***P<0,001; CI= Confidence Interval; LC= Lethal Concentration; R^2= coefficient of determination; χ^2= chi square.

Table 4: Synergistic factor and cotoxicity index of the combination of the methanol extracts of *C. aurantiifolia* and *L. chevalieri* against *An. gambiae* 24h post exposure.

Combinations	LC_{50} (mg/mL)	SF	CI	Type of action
100%Ca: 0%Lc	9.82	-	-	-
75%Ca: 25%Lc	9.53	1.036	103.63	Additive
50%Ca: 50%Lc	8.50	1.552	155.22	Synergistic
25%Ca: 75%Lc	10.04	0.995	99.51	Additive
100%Lc: 0%Ca	10.05	-	-	-

SF=Synergistic factor; CI=Cotoxicity index; LC=Lethal Concentration.

The solubility of endogenous compounds present in the extracted materials might be a factor affecting the efficiency of the extraction yield [34].

Monica *et al*. [33] reported the presence of a significant amount of phenols, total soluble flavonoids and terpenes in extracts of *C. aurantifolia* peels in agreement with previous work [35] on the chemical composition of peels of 13 Citrus species. Abdallah [12] and Reddy *et al*. [13] also reported a variable presence of saponins, phenolic compounds, tannins, flavonoids and alkaloids in the methanolic extracts of *C. aurantifolia* leaves. Phytochemical composition of methanolic extracts of *L. chevalieri* leaves is slighly close to that obtained by Mindiediba [17] except for the absence of polyphenols in his samples and Nacoulma [16] indicated furthermore the presence of alkaloids. Qualitative and quantitative variations in phytochemical components between and within plant species have been attributed to variation in maturity, genetic variation, growth stages, drying and post-harvest storage [36,37]. They can also be due to the geographical location of the plants harvested, period when these plants were harvested, the extraction methods, the polarity of the solvent used for the extraction and the parts of the plants used [38].

Larvicidal activity is considered an ideal approach for mosquito control as it contributes to the reduction of larval density before their emergence where they are harmful and can transmit diseases. The methanolic extracts of the 2 plants caused a significant ($P < .001$) concentration-dependent larvicidal activity on the 3rd and 4th stage larvae of *An. gambiae* 24h post exposure both individually and in binary combination. Individually, the methanolic extract of *C. aurantifolia* peels was more larvicidal. The binary combination 50%Ca + 50%Lc induced a synergistic action on *An. gambiae* larvae 24h post-exposure and was found to be the best combination because it optimized the efficacy of the combination by considerably lowering the LC_{50} value. The efficacy of plant extract mixtures would depend on the type of mixture produced (proportional or balanced) and the species considered [22]. Previous works revealed that several varieties of *Citrus* contained ingredients that can be used as a natural insecticide in mosquitoes [39]. Fajar *et al*. [40] obtained significant larvicidal activity of *C. aurantifolia* peels extracts on 3rd and 4th stage larvae of *Aedes aegypti*. Adina *et al*. [41-42] demonstrated that the combination of ethanolic extracts of *C. aurantifolia* and doxorubin produced a synergistic effect on the inhibition of the growth of MCF-7 cancer cells. Other works also corroborate our results by highlighting the larvicidal and adulticidal power of *L. chevalieri* both

individually and in binary combinations with other plants on *An. gambiae* s.l., *Ae. aegypti*, *Cx. quinquefasciatus*, *An. Funestus* [19-20, 21]. The combination of *Cymbopogon schoenanthus* and *L. chevalieri* has an antagonistic effect on *An. gambiae* larvae [19], while the binary combinations of *L. chevalieri* and *Lantana camara*, *Lippia multiflora*, *Cymbopogon schoenanthus* were synergistic for *Cx. quinquefasciatus* [20]. The mixture of plants extract does not necessarily give an additive effect and this hypothesis is supported by Ayiki *et al*. [22] and those of Ngatanko and Ngamo [23]. The differences between the insecticidal activities of the extracts individually and in binary combinations might be due to the composition and chemical nature of the active compounds acting jointly or independently on mosquitoes. These phytocompounds are ingested orally or through cuticule, which could affect the physiological balance of the insect and lead to death [43]. phytochemicals present in many plants act on the digestive system of the insect; after ingestion, they will be absorbed by the intestinal wall and then circulate with the blood which will disturb the energy metabolism of the organism so that it will lack energy for its vital activities, resulting in larval spasms and possibly death [44]. Terpenic compounds act on neurons by inhibiting the activity of acetylcholinesterase, an enzyme which destroys acetylcholine after transmission of nerve impulses [45]. Saponins have the effect of disrupting the developmental stage by blocking the evolution of the larval cuticle [46]. The synergy of the extracts is likely due to the formation of additional new molecules from the phytocompounds found in the mixture and may act synergistically as neurotoxic insecticides interfering in the ligand-gated chloride channel of the mosquito nervous system by blocking octopamine or cholinergic receptors, important target sites for insect pest control [47]. The increased activity may also be due to the simultaneous effects on different targets, increasing the insecticidal effect of the tested extracts up to ten times, severely impacting the survival of the insect [48].

Conclusion

Methanolic extracts of the 2 plants demonstrated a significant larvicidal effect on *An. gambiae* both individually and in combination. Individually, the methanolic extract of *C. aurantiifolia* was more active on mosquito larvae than other extract. The binary combinations produced a synergistic larvicidal effect for the balanced mixtures (50 :50) between *C. aurantiifolia* and *L. chevalieri.* Thus, the combination of methanolic extracts of *C. aurantiifolia*, and *L. chevalieri* in ratio

50:50 may constitute an exploitable source for the production of a bio-larvicide to trontrol An. gambiae, vector of malaria.

References

[1] World malaria report. (2022). Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO.

[2] ANOFEL. (2014). Polycopie Nationale. Association Française des Enseignants de parasitologie et Mycologie. Université Médicale Virtuelle Francophone. 441p.

[3] Antonio-Nkondjio C., Fossog B.T., Ndo C., Djantio B.M., Togouet S.Z., Awono-Ambene P., Costantini C., Wondji C.S. and Ranson Hilary. (2011). *Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaounde (Cameroon). *Influence of urban agriculture and pollution malaria Journal*, 10:154p.

[4] WHO. (2019). Geneva: World malaria report; World Health Organization. 22-142p.

[5] Fokam Tagne M.A., Dainone I.D., Noubissi P.A., Adamou A., Mouraissou J.M and Ndjonka D. (2021). *In vitro* anthelmintic properties of hydroethanolic extracts of *Terminalia macroptera* (Combretaceae) on gastrointestinal strongyles, *Haemonchus contortus* (Trichostrongyloidae)., Journal of Experimental and Applied Tropical Biology. 1(2): 24-36p.

[6] Antonio-Nkondjio C., Ndo C., Njiokou F., Bigoga J.D., Awono-Ambene P., Etang J., Ekobo A.S. and Wondji C.S. (2019). Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination. *Parasites Vectors*, 12: 501 https://doi.org/10.1186/s13071-019-3753-8

[7] Foko Dadji G.A., Tamesse J.L. and Messi J. (2007). Insecticidal effects of *Capsicum annuum* on *Anopheles gambiae* Gilles under laboratory conditions. *Journal of Entomology*. 4(4): 299-307p.

[8] Foko Dadji G.A., Tamesse J.L. and Fekam Boyom F. (2011). Adulticidal effects of essential oils extracts from *Capsicum annuum* (Solanaceae), *Piper nigrum* (Piperaceae) and *Zingiber officinale* (Zingiberaceae) on *Anopheles gambiae* (Diptera-Culicidea), vector of malaria. Journal of Entomology. 8(2): 152–163p.

[9] Foko Dadji G.A., Nyegue M., Tsila G., Awono Ambene P.H., Ndong

Massouka P. and Tamesse J. (2016). Chemical Composition and Ovicidal, Larvicidal and Pupicidal Activity of *Ocimum basilicum* Essential Oil against *Anopheles gambiae*. (Diptera: Culicidae). *European Journal of Medicinal Plants*. 16(3): 1-13p.

[10] Lame Y., Francine K., Mallam K.O., Aurore C.S.B., Joseph L.T. and Elias N.N. (2020). Combined Effect of Methanol Extracts and Essential Oils of *Callistemon rigidus* (Myrtaceae) and *Eucalyptus camaldulensis* (Myrtaceae) against *Anopheles gambiae* Giles larvae (Diptera: Culicidae). International Journal of Zoology. 2020(4952041): 9p.

[11] Enejoh O.S., Ogunyemi I.O., Bala M.S., Oruene I.S., Suleiman M.M. and Ambali S.F. (2015). Ethnomedical Importance of *Citrus aurantifolia* (Christm) Swingle. The Pharma Innovation Journal 4(8), 1-6p.

[12] Abdallah EM. (2016). Preliminary Phytochemical and Antibacterial Screening of Methanolic Leaf Extract of *Citrus aurantifolia*. Pharm Biotechnol Curr Res. 1:1.

[13] Reddy L.J., Jalli R.D., Jose B. et Gopu S. (2012). Evaluation of Antibacterial and Antioxidant Activities of the Leaf Essential Oil & Leaf Extracts of *Citrus Aurantifolia*. Asian Journal of Biochemical and Pharmaceutical Research 2: 346-354p.

[14] Mensah F.A., Inkum I.E., Agbale C.M. and Eric A. (2014). Comparative Evaluation of the Insecticidal and Insect Repellent Properties of the Volatile Oils of *Citrus Aurantifolia* (Lime), *Citrus Sinensis* (Sweet Orange) and *Citrus Limon* (Lemon) On *Camponotus Nearcticus* (Carpenter Ants). International Journal of Novel Research in Interdisciplinary Studies 1(2): 19-25p.

[15] Ezeonu F.C., Chidume G.I. and Udedi S.C. (2001). Insecticidal properties of volatile extracts of orange peels. Science Direct 76(3), 273-274p.

[16] Nacoulma O.G. (1996). Plantes médicinales et Pratiques médicales Traditionnelles au Burkina Faso : cas du plateau central T1 et T2. Thèse Doctorat d'Etat ès Sciences Naturelles. Université de Ouagadougou, 242, 285p.

[17] Mindiediba J.B. (2012). Etude phytochimique et activites biologiques des tiges feuillées de *Lantana camara* L. et de *Lippia chevalieri* Moldenke: deux

Verbenaceae du BURKINA FASO. Thèse unique, Université de Ouagadougou. 77-78p.

[18] Bassolé I.N.H., Ouattara A.S., Nebie R., Ouattara A.T., Kabore Z.I. and Traore S.A. (2003). Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from. Burkina Faso. Phytochemistry, 62: 209-212p.

[19] Wangrawa D.W., Ochomo E., Upshur F., Zanré N., Borovsky D., Lahondère C., Vinauger C., Badolo A. and A. Sanon. (2022a). Essential oils and their binary combinations have synergistic and antagonistic insecticidal properties against *Anopheles gambiae* s. l. (Diptera: Culicidae). Biocatalysis and Agricultural Biotechnology. 42(102347).

[20] Wangrawa D.W., Kosgei J., Machani Maxwell., Opala James., Agumba S., Yameéogo F., Borovsky D. and Ochomo E. (2022b). Larvicidal Activities and Synergistic Effects of Essential Oils against *Anopheles funestus* and *Culex quinquefasciatus* (Diptera: Culicidae) from Kisumu, Kenya. A Journal of Entomology. 2022(8302696): 13p.

[21] Muturi E.J., Ramirez J. L., Doll K. M., and Bowman M. J. (2017). "Combined toxicity of three essential oils against *Aedes aegypti* (Diptera: Culicidae) larvae," Journal of Medical Entomology.vol. 54, 1684–1691p.

[22] Ayiki E.N., Tamgno B.R., Madou C., Vatsou J., Ngassoum M.B and Ngamo Tinkeu.L.S. (2019). Toxicity of the powdery formulation of *Hyptis spicigera* (Lamiaceae), *Azadirachta indica* (Meliaceae) and *Vepris heterophylla* (Rutaceae) Single and in combination on *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae). Int. J. Biol. Chem. Sci. 13(3): 1613-1620p.

[23] Ngatanko I. et Ngamo Tinkeu L. (2015). Optimization of the insecticidal effect of essential oils of three medicinal plants against rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). International Journal of Agronomy and Agricultural Research. 7(2): 55-63p.

[24] Harborne J. B. (1998). A guide to modern techniques of plant analysis. Springer, 3rd Edn, India (New Delhi). 32p.

[25] Raymond V., Barbehenn C and Constabel P. (2011). Tannins in

plant– herbivore interactions. Phytochemistry, 72: 1551–1565p.

[26] WHO. (2005). Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization. Geneva, Switzerland.

[27] Abbott W.S. (1925). "A method of computing the effectiveness of an insecticide," Journal of Economic Entomology.18(2): 265–267p.

[28] Sun Y.P. and Johnson E. R. (1960). "Synergistic and antagonistic actions of insecticide-synergist combinations and their mode of action," Journal of Agricultural and Food Chemistry. 8(4): 261–266p.

[29] Kalyanasundaram M. and Das P. K. (1985). "Larvicidal and synergistic activity of plant extracts for mosquito control," Indian Journal of Medical Research. 82, 19–23p.

[30] Issoufou Y., Djibo A. K., Idrissa M., Abdoulaye T., Inoussa M. M., Karim S., Jean-Luc P., Tilman M. et Jean Maurille O. (2019). Caractérisation Phytochimique et Activité Larvicide d'extraits bruts de plantes issues de la pharmacopée traditionnelle du Niger sur les Larves d'*Anopheles gambiae* S.L. European Scientific Journal. 15(12): 1857 – 7881p.

[31] Wangrawa D. W., Badolo A., Guenne S., and Sanon A. (2016). "Larvicidal and oviposition-deterrence activities of four local plant extracts from Burkina Faso against *Anopheles gambiae* s.l .(Diptera: Culicidae)," Int J Mosq Res. 3, 11–19p.

[32] Williamson E. M. (2009). "Synergy and other interactions in phytomedicines," in Trease and Evans Pharmacognosy. Elsevier, Amsterdam, Netherlands, 16th. 53–61p.

[33] Monica R.L., Rosa T., Marco B., Federica M., Damiano DL., Carmela C et Francesco M. (2012). Evaluation of *Citrus aurantifolia* peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. *Sci Food Agric*. 10:1002

[34] Sultana, B., Anwar F. and Przybylski R. (2007). Antioxidant activity of phenolic components present in barks of barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica,* and *Eugenia jambolana* Lam. trees. Food Chemistry. 104: 1106-1114p.

[35] Ghasemi K., Ghasemi Y. and Ebrahimzadeh MA. (2009). Antioxidant activity, phenol and flavonoid contents of 13 Citrus

species peels and tissues. Pakistan J Pharm Sci 22 :277–281p.

[36] Hussain A.I., Anwar F., Sherazi S.T.H and Przybylski R. (2008). Chemical composition. Antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry. 108 : 986-995p.

[37] Anwar F., Hussain A.I., Sherazi S.T.H and Bhanger M.I. (2009). Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel (*Foeniculum vulgare* Mill.) fruit at different stages of maturity. Journal of Herbs Spices Medicinal Plants. 15: 1-16p.

[38] Pavela R. (2015). "Essential oils for the development of eco-friendly mosquito larvicides: a review," Industrial Crops and Products. 76, 174–187p.

[39] Ferguson J.J and Spann T.M. (2010). Medicinal Use of Citrus. HS892, Institute of Food and Agricultural Sciences, University of Florida 1:1–3p.

[40] Fajar A. and Fahrul I. (2018). Effectiveness of Pomelo (*Citrus maxima*) and Lime (*Citrus aurantifolia*) orange peel extract as *Aedes aegypti* biolavisida. Mamuju Health Polyclinic, Department of Environmental Health.376-380p.

[41] Adina A.B., Handoko F.F., Setyarini I.I., Septisetyani E.P., Riyanto S and Meiyanto E. (2008). Ekstrak Etanolik Kulit Jeruk Nipis. (*Citrus aurantifolia* (Cristm.) Swingle) Meningkatkan Sensitivitas Sel MCF-7 terhadap Doxorubicin, Proceding, Kongres Ilmiah ISFI ke-16, Yogyakarta, Indonesian. 55-62p.

[42] Adina A.B., Goenadi F.A., Handoko F.F., Nawangsari D.A., Hermawan A., Jenie R.I and Meiyanto E. (2014). Combination of Ethanolic Extract of *Citrus aurantifolia* Peels with Doxorubicin Modulate Cell Cycle and Increase Apoptosis Induction on MCF-7 Cells. Iranian Journal of Pharmaceutical Research. 13 (3): 919-926p.

[43] Rattan R.S. (2010). "Mechanism of action of insecticidal secondary metabolites of plant origin," Crop Protection. 29(9) 913–920p.

[44] Murdani R. (2014). Effectiveness of Lime Leaf Extract (*Citrus aurantifolia*) on the Death of Instar III *Aedes aegypti* Mosquito Larvae, Thesis, Universitas Muhammadiyah, Surakarta. 290p.

[45] Ryan M.F. and Byrne D.O. (1988). Plant insect coevolution and inhibition of acetylcholinesterase. J. Chem. Ecol., 40: 1965-1975p.

[46] Chaieb I. (2010). Protection L De. Saponins as Insecticides: a Review.5(1).

[47] Priestley C. M., Williamson E. M., Wafford K. A., and Sattelle D. B. (2003). Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABAA receptors and a homo-oligomeric GABA receptor from Drosophila melanogaster," British Journal of Pharmacology. 140(8): 1363–1372p.

[48] Youssefi M. R., Tabari M. A. and Esfandiari A. (2019). "Efficacy of two monoterpenoids, carvacrol and thymol, and their combinations against eggs and larvae ofthe West Nile vector *Culex pipiens*," Molecules, vol. 24, Article ID 1867.