DOI: https://dx.doi.org/10.4314/gjpas.v29i2.5

GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 29, 2023: 145-152
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EVALUATION OF PHYTOCHEMICAL ACTIVE INGREDIENTS PRESENT IN ORGANIC SOLVENT EXTRACTS AND LARVICIDAL PROPERTIES OF SOME SELECTED PLANTS FROM TARABA STATE AGAINST ANOPHELES LARVAE

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(Received 13 February 2023; Revision Accepted 15 August 2023)

ABSTRACT

The Continuous use of synthetic insecticides and its toxicity problems coupled with the incidence of insect resistance calls for the need for alternative insecticide. Plants extracts a rich source of bioactive compounds can provide an alternative source of insecticide which are ecofriendly. The research evaluated the phytochemical active ingredients present in organic solvent extracts and larvicidal effect of some selected plants from Taraba state against Anopheles larvae**.** World Health Organisation protocol was adopted for the larvicidal bioassay. Twenty group of fourth instar Anopheles larvae were exposed to various concentrations of 200,400,600 and 800ppm, mortality was observed and recorded after 24 hours. The results of preliminary qualitative phytochemical analyses of tested plants revealed the presence of some secondary metabolite that may be responsible for the bio control potentials. Acetone extract of Hyptis suaveoleons against the fourth instant anopheles larval was observed to have the highest percentage mortality among the entire plants extracts than the aqueous. The lowest mortality was observed at 200ppm with 35.0% while the maximum was at 800ppm with 80.0%. Lc_{50} and Lc_{90} values were achieved at 438 and 866ppm with the LCL -UCL 340-540 and 722-1151 respectively. While the biological activity of Hyptis suaveolence aqueous extract had its lowest mortality at 200ppm with 36.67% while the maximum was at 800ppm with 80.0%. Lc_{50} and Lc_{90} values were achieved at 582 and 1225ppm with the LCL -UCL 449-806 and 942-2636 respectively. Ancova results showed no significance difference at $p>0.05$ among the mean percentage mortality of the treated doses.

KEYWORDS: Hyptis suaveoleons, Ocium basicullum, Phytochemica, Lavicidal potential, LC 50. Lc 90

INTRODUCTION

Mosquitoes are perhaps the main group of insects that transmits wide range of diseases which include illnesses like Dengue fever, Yellow fever, Chikungunya, Malaria, Encephalitis and Filariasis [1]. They are major source of concern globally particularly in tropical and sub-tropical nations [2]. Mosquito control has become increasingly difficult due the development of resistance to the available insecticide.

The development of resistance calls for the need for alternative and potent insecticide for effective mosquito control in addition to the utilization of other counter-measures. Studies have shown that even potent insecticide used in vector control over time resistance is developed against such compound which is followed by resurgence of the vectors. [3]. Plants are rich sources of secondary metabolites, with mosquitocidal properties apart from their biodegradable capacity they are viewed as acceptable for the control mosquitoes [4].

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Lymphatic filariasis transmitted by Anopheles and Culex mosquitoes worldwide seventy-six (76) countries are affected by lymphatic filariasis with 51.4 million individuals estimated to be infected with 32 million individual having chronic filariasis, one million are severely disabled [40]. Despite effort at malaria control transmitted by female Anopheles mosquito still 800,000 people are killed worldwide, with over 90% of the death in sub Saharan Africa [5]. More than 90% of the Nigerian population are at risk of stable malaria transmission with huge economic loss [6]. Yellow fever transmitted by Aedes species remains one of the most debilitating diseases of global importance. The disease is endemic in most part of Nigeria and is commonly noticed as devastating epidemics taking its toll on human lives and economy [7].

The control of mosquito borne-diseases is very important and control measures can be directed either against the adult mosquito or immature stages [8]. The use of insecticides and many other synthetic agents have been developed and deployed with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to none target organisms in the ecosystem and may be harmful to human health [9]. This has prompted the need to look for alternative insecticides that is biodegradable and ecofriendly in nature. This also involves the use of other management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least toxic mosquito control [41].Extracts or essential oil from plant can serve as alternative sources of mosquito control agents as they constitute a rich source of bioactive compounds that are biodegradable non toxic products and potentially suitable for use in control of mosquito larvae [10]. Thus, botanical larvicides and insecticide are now preferred to synthetic chemicals. Hyptis suaveolens (Bush tea leave) is one of such botanicals. Hyptis suaveolens belongs to the family laminaeceae and is a native of tropical America, but it is wide spread in tropical Africa. Asia and Australia, it grows under a wide variety of soil and climate, mainly in warm area [11]. Basil (Ocimum basicullucum) is an aromatic, low growing herb. The plants have a bright green to purple ovate colour leafs and are grown in warm, tropical climate. It belongs to the botanical family of Ocimum basicullucum, which is commonly known as mint. The basil leaves are known to have many medicinal and other healing properties [12]. The presence of active agent in some herbs used traditionally in the treatment of many diseases affecting majority of African communities, particularly in Nigeria have been documented [13].

MATERIALS AND METHODS

Study Design: Preliminary study was conducted in remote areas and local markets within the study area to consult herbs sellers and elderly people to provide information on plants species frequently used in the control of mosquitoes. Inform consent was obtained orally from all participants. Sample of the plants were therefore, collected and taken to plant science department of MAU, Yola for identification.

Collection of plant materials: Collections of plants was done based on the secondary data collected for plants mention by the communities and was identified using different search engine such as Google scholar and were authenticated by a Taxonomist from the Department of Plant Science MAU, Yola.

Collection and Rearing of Mosquito: Larvae of mosquitoes were obtained from any available stagnant water within the study area using dipper and pipette and transported to the Zoology Department laboratory, Modibbo Adama University of Technology Yola. Larva were reared in a rearing tray containing tap water and covered with fine nylon mesh. The larvae were feed with food containing mixture of cabin biscuit and yeast until 4^{th} instar stage were reached and adult stages as described by Cansado-Utrilla et al. (14).

Preparation of crude extract: Freshly mature green leaves of the entire selected plant samples collected were initially rinsed with distilled water, shade dried, pulverized and sieved to get a fine powder from which the extracts were prepared using acetone and aqueous as the solvent. 200g of the powder in a separate container and 200ml of the solvent were mixed. The cap vial was screwed and shaken vigorously to dissolve or disperse the material in the solvent. The mixture was then filtered through Whatmam filter paper and the filtrate was evaporated under reduced temperature at 50°C on a water bath dryness to obtain the crude extract. The stock solution was diluted separately according to Petrić et al. [15].

Larvicidal Bioassay

Larvicidal Bioassay was done according to a standard procedure provided by World Health Organization; Guideline for laboratory and field testing of mosquito larvicides (WHO, 2010). Twenty (20) fourth instar Anopheles larvae were transferred by means of strainer or droppers into a disposable test cup each containing 200ml of water. The depth of the water in the cups was maintained between 5cm and 10cm. 0.2m1 of the stock was then added to 200m1 in the cups to obtain the desire target dosage stating with the lowest concentration of 200ppm, 400ppm, 600ppm and 800ppm respectively. Three replicates for each concentration and equal number of control were simultaneously set up with tap water, to which 1 ml of the solvent added. Larval food was then added to each test cup. After 24hr exposure, larval mortality was then recorded. Moribund larvae were counted as dead larvae for calculating percentage mortality. The result was then recorded on the data recording forms. Where the control mortality was between 5% and 20%, the mortalities of the treated groups was corrected according to Abbott's formula [16].

Statistical analysis: LC_{50} and LC_{90} value were calculated from a log dosage, probit mortality regression line at 95% CL of upper confidence limit

(UCL) and lower confidence level (LCL). Using SPSS version 26 percentage mortality was calculated and the mean ± standard deviation presented. Analysis of Covariance ANCOVA was conducted to determine the statistical significance difference between concentration and mean mortality of the plants extracts, results at $p<0.05$ was considered statistical significance.

RESULTS

The result of the larvicidal activity of both acetone and aqueous leaf extract of six plants used against Anopheles larvae showed larvicidal potential which led to the investigation of the specific phytochemical ingredient that were responsible for the toxicity effects. The results of preliminary qualitative phytochemical analyses of tested plants revealed the presence of some secondary metabolite that may be responsible for their biocontrol potentiality. The data presented in table (3 and 4), indicated the presence of saponins, flavonoids, terpenoids, alkaloids, phenols tannins and glycosides respectively. Azerderecter indica (Neem) leaves possess the highest amount of bioactive component with saponin 2.4±0.02 followed by tobacco with 2.17± 0.01 while Aescrynomone has the lowest saponin of 0.95±0.02 Hyptis snaveolens poses the highest bioactive component flavonoid of 2.56±0.02 followed by Orange peels with 2.30±0.07 while Tobacco has the lowest flavonoid of 1.56±0.02 respectively. The bioactive component Terponoid found only in Ocium basicullum of 0.19±0.05 while the remaining was negative. The table also revealed that Alkaloids found to be high in Tobacco with 2.38± 0.01 followed by Aescrynomone with 2.25±0.02 while Azerderecta has the lowest alkaloids of 1.43±0.03. Ocium basicullum possess the highest bioactive component phenol of 30.05±0.07 followed by Hyptis suaveolens with 23.97±0.02 while Tobacco has the lowest with 17.49±0.01. Tannin and glycocide were found only in Aescrynomone, Hyptis suaveolens and Neem with 3.76±.03, 0.31±0.001 and 0.17±0.01 respectively. The tables also revealed the number of secondary metabolites extracted by the acetone and aqueous method of phytochemical analysis revealed higher quantity of metabolites in the acetone extract of Ocium basicullum (36.4%) closely followed by Hyptis suaveoleons (30.5%), Neem extract (29.9%), and orange peels (29.0%) Aescrymonene (24.4%) and the acetone extracts of tobacco (23.6%) while aqueous extract showed lowest level of Metabolites with (22.3%) in Tobacco extract. Absence of Terpenoids, Tanins and Glycosides were observed in the Hyptis; Neem, Tobacco Orange peels. Ocium basicullum and aescrynomone while maximum yield of Phenols (30.05%) and flavonoid (2.57%) was shown in acetone extract of Ocium basicullum and Hyptis suaveolens respectively.

Biochemical activity of the various plants extracts after 24 hours exposure of the 4th instar Anopheles larvae to acetone and aqueous extracts of the various plants. Using different concentration (ppm), percentage mortality after 24 hours, Lc_{50} and Lc_{90} were observed, increase in concentration lead to increase in mortality rate, and the maximum mortality was observed at 800 ppm, while the minimum at 200ppm. The biological activity of Hyptis suaveolence acetone extract against the fourth instar Anopheles larval was observed to have the highest percentage mortality among the entire plants extracts than the aqueous. The lowest mortality was observed at 200ppm with 35.0% while the maximum was at 800ppm with 80.0%. Lc_{50} and Lc_{90} values were achieved at 438 and 866ppm with the LCL -UCL 340-540 and 722-1151 respectively (Table I). While the biological activity of Hyptis suaveoleons aqueous extract had its lowest mortality at 200ppm with 36.67% while the maximum was at 800ppm with 80.0%. Lc_{50} and Lc_{90} values were achieved at 582 and 1225ppm with the LCL -UCL 449-806 and 942-2636 recorded respectively, while amongst both the plants extracts, tobacco leaf extract shows the lowest biological activities against Anopheles larvae, the percentage mortality of 50.0% was recorded at the highest concentration of 800ppm and 36.7% and 33.3% at the lowest concentration of 200ppm (Table I and 2). Generally, the result showed that there was no significance difference between the plant extracts as presented in table 1 and 2 ($P>0.05$).

Table 1: Qualitative phytochemical analysis of Acetone and Aqueous extracts of some ethno botanical (Herbs) use as mosquito control.

Plants/solvent	Saponin	Flavonoid	Tapanin	Alkaloid	phenol	Tanin	glyco	Steroid	Total
H..suav Ac	+	+	+	+	+	+	+	+	9
Aq	+	+	+	+	+	-	+	+	7
Az.indi Ac	+	+	+	+	+	-	+	-	7
Aq	+	+	-	+	+	-	+	-	5
Tobac Ac	+	+	-	+	+	-	-	-	5
Aq	+	+	-	+	+	-	-	-	4
O.Peels Ac	+	+	-	+	+	+	-	+	6
Aq	+	+	-	+	-	-	-	+	4
O.basic Ac	+	+	+	+	+	-	-	-	5
Aq	+	+	+	+	+	-	-	-	5
Aescry Ac	+	+	-	+	+	-	-	-	4
Aq	+	+	-	+	+	-	-	-	4

KEY:+ present, - absent AC=Acetone, Aq=Aqueous, H.suav=Hyptis suaveolen,Az.indica=Azadirachtin indica, O.Peel=Orange peels, O=Ocium basicullum, Aesc=Aescrynomone.

Table 2: Quantitative phytochemical analysis of Acetone and Aqueous extracts of some ethno botanical (Herbs) use as mosquito control.

Plants/solvent	Aq	Saponin	Flavonoid	Tapanin	Alkaloid	Phenol	Tanin	glycosides
Hyp.sua	Ac	1.9 ±0.006	2.57±0.02	-	1.66±0.03	23.97±0.02	-	0.31±0.01
	Aq	1.18±0.05	2.48±0.01	-	1.55±0.03	23.14±0.04	-	0.21±0.01
	Ac	2.41	2.12±0.02	-	1.43±0.03	23.77±0.02	-	0.17±0.01
	Aq	±0.02	2.02±0.01	-	1.34±0.03	21.35±0.02	-	0.15±0.00
Az. Ind		2.33 ±0.02						
	Ac	2.17	1.56±0.02	-	2.38±0.01	17.49±0.01	-	-
Toba	Aq	±0.01	1.48±0.01	-	2.34±0.03	16.34±0.02	-	-
		2.10 ±0.02						
	Ac	0.89	23.30	-	168±0.01	24.10±1.40	-	-
O. peels	Aq	±0.02	±0.07	-	1.57±0.01	23.94±0.03	-	-
		0.78 ±0.01	2.14±0.03					
	Ac	1.37	2.39±0.01	0.19±0.05	2.15±0.03	30.05±0.07	-	-
O. basi	Aq	±0.01	1.68±0.02	0.14±0.03	2.07±0.02	28.85±0.01	-	-
		1.30 ±0.02						
Aesc		0.95	1.74±0.03	-	2.25±	15.74±0.03	3.76±0.03	-
Ac	Ac	±0.02	1.65±0.02	-	2.15±0.02	14.58±0.01	14.5±0.01	-
	Aq	0.86 ±0.01						

KEY: AC=Acetone, Aq=Aqueous, H. suav=Hyptis suaveoleons, Az.indica=Azadirachtin indica, O.Peels=Orange peels, O=Ocium basicullum, Aesc=Aescrynomone.

Table 3: Mean % mortality and estimate of LC_{50} and LC_{90} values of acetone leaf extract against Anopheles larvae exposed for 24 hours

plant/solvent Acetone	Mean % mortality after 24 hours ± SD at varying concentration					Lc50(ppm) LCL-UCL	Lc90(ppm) LCL-UCL	ANCOVA (F.val) P-val
	0	200	400	600	800			
H. suaveolence	0.00±0.00	35.00±1.00	40.00±0.00	56.67±0.58	80.00±1.00	438 340-540	866 722-1151	(2.065)0.21 NS
Az, Indica	0.00±0.00	31.67±1.15	36.67±2.52	50.00±1.00	65.00±1.00	571 448-764	1162 911-1820	
Tobacco	0.00±0.00	36.67±0.58	40.00±1.00	46.67±0.58	50.00±0.00	686 538-1153	1362 1025-2451	
O. peels	0.00±0.00	33.33±0.58	46.67±0.58	53.33±0.58	75.00±1.00	504 396-637	1006 818-1427	
O. basicullum	0.00±0.00	35.00±1.00	40.00±1.00	43.00±1.00	53.33±1.53	650 508-9943	1318 998-2311	
Aescrynomone	0.00±0.00	31.67±0.58	45.00±1.00	50.00±1.00	56.57±1.15	589 463-800	1197 932-1919	

KEY: H.suav=Hyptis suaveolen, Az.indica=Azadirachtin indica, O.Pill=Orange peels, O=Ocium basicullum, Aesc=Aescrynomone.
NS=Not significant

Table 4: Mean % mortality and estimate of LC_{50} and LC_{90} values of aqueous leaf extract against Anopheline larvae exposed for 24 hours

plant/solvent Aqueous	Mean % mortality after 24 hours ± SD at varying concentration					Lc_{50}(ppm) LCL-UCL	Lc_{90}(ppm) LCL-UCL	ANCOVA (f.val) sd
	0	200	400	600	800			
H. suave	0.00±0.00	36.67±0.58	45.00±0.00	50.00±0.00	61.67±1.58	582 449-806	1225 942-2036	(1.99) 0.19 NS
Az, Indic	0.00±0.00	33.33±0.58	38.33±0.58	45.00±1.00	55.00±1.00	663 521-962	1322 1004-2304	
Tobacco	0.00±0.00	33.33±0.58	46.67±0.58	48.33±1.15	50.00±1.73	776 601-1292	1509 1100-3088	
O. peels	0.00±0.00	25.00±0.00	36.67±1.15	46.67±0.58	58.33±0.58	634 504-877	1245 964-2033	
O. basic	0.00±0.00	35.00±1.00	45.00±1.00	50.00±1.00	51.67±1.15	676 535-975	1321 1007-2279	
Aescry	0.00±0.00	38.35±0.58	45.00±1.00	51.67±1.53	53.35±1.53	723 558-1164	1461 1070-2900	

KEY: H. suav=Hyptis suaveole, Az.indica=Azadirachtin indica, O.Peel=Orange peels, O=Ocium basicullum, Aesc=Aescrynomone.

DISCUSSIONS

Different plant species have been identified to contain various phytochemical constituents which are in form of secondary metabolites mostly for the protection of the plants [35]. In this study, the qualitative and quantitative biochemical in the extracted samples obtained by the use of acetone and aqueous solvents of Hyptis suaveoleons, Azerderecta indica, Ocium basicullum, Orange peels, Tobacco and Aescrynomone, showed the presence of saponin, flavonid, tapanin, alkaloid, phenol, tannin, glycocide, steroid and anthraquinone in acetone extracts of the various plants. While in aqueous, terpenoid, tannins were absent. It was observed that the presence and absence of phytochemical constituents depend on the solvent medium used for the extraction and the plant in question, this was also postulated by Komalamisra, et al (32). The phytochemical contents and larval efficacy of Flavonoids, saponins, phenol and alkaloids were evidently present in all the plant extracts tested. This however differs from the findings of a similar research [39]. Previous studies have reported that these phytochemicals are present in Hyptis suaveolens [17], Azerderecter indica [17], Ocium basicullum [17], Orange peels [19], Tobacco [20] and Aescrynomone [18]. However, in a similar study Akinmoladun et al., [37] reported the absence of alkanoids in aqueous extract of leaf. The presence of flavonoids, alkaloids, saponins, tannins, glycosides were revealed in the plant extracts could have accounted for the larvicidal actions against Anopheles larvae which agrees with the report of Gupta et al. [21], Veniprasad et al. [22] and Alexander [23]. The finding in this study also agrees with Talabi et al. [24] who reported alkaloids and saponins in appreciable quantity, moderate quantity of glycosides. Saponin's larvicidal potential has also been reported in many previous literatures [25, 26, 36]. Meanwhile, saponins can react with larval cuticles by reconfiguring the cuticular membrane a probable reason for larval death [27]. It should, however, be noted that most importantly, the phytochemicals can exhibit synergistic effects in their crude form (Mohammed et al., [28]. The result also revealed that maximum occurrence of phytochemical constituent is in acetone extracts than the aqueous extracts in all the sample plants. The differences in the amount of active ingredients extracted by the two solvents used may be attributed to their differences in polarities which play a vital role in increasing the amount and the type of phytochemical constituents as reported by Naima et al., (32).

The percentage of larvicidal effects of these plants extracts against 4^{th} instar larvae showed that acetone extracts of Hyptis surveolen was the most effective agent used with 80% mortalities observed at 800ppm against Anopheles larvae which was similar to what Murugan et al. [34] reported. Orange peels extract prove to be the next most effective after Hyptis suaveolens with 75% mortality against the Anopheles larvae than A. indica and Ocium basicullum. This finding is in agreement with some recent findings Elango et al., [29] and Marcombe et al., [30] who reported that larvicidal activity of ethanoic and hexane leaf extracts of Lantana camara displayed larvicidal activities towards Anopheles stephensi and Aedes aegypti larvae. The findings however differs from that of Okigbo et al. [17] who reported that Petroleum ether leaf extracts of H. suaveolens did not compare effectively as larvicide with O. gratissimum and A. indica. The variation in the mortality amongst the plant extracts may be attributed to the variation in the concentration of saponins, tannins, phenol, alkaloids and terpenoids as observed in table 3. This agreed with the findings of El-Shekh et al. [24] and Mambe et al. [38]. The probit analyses revealed the acetone extracts of Hyptis suaveolens, Azachareta indica, tobacco, Orange peels, Ocium basicullum and Aercyrynomone have high larvicidal effect against the 4^{th} instar Anopheles larvae with LC_{50} and LC_{90} (438,571,686, 584, 650, and 589ppm) and (1225, 1322, 1509, 1245, 1321 and 1461ppm) respectively. This finding is in line with the finding of Sharma et al. [31] who reported that the acetone extracts of Nerium indicum and Thuja oriertellis has been studied with lc_{50} value of (200.87, 127.53, 2009. 00, and 150.97 ppm against 3^{rd} instar larvae of Anopheles stephensi and Culex quinquetaciates respectively. Ancova results showed no significance difference ($p>0.05$) among the mean percentage mortality of the treated doses.

CONCLUSIONS

In this study, different organic solvent extracts have been identified to contain various phytochemical constituents and larval efficacy of Flavonoids, saponins, phenols and alkaloids were evidently present in all the plant extracts tested. The percentage of larvicidal effects of these plants extracts against 4^{th} instar larvae showed that acetone extracts of Hyptis surveolen supported by evidence to be the most effective agent used with 80% mortalities observed at 800ppm against Anopheles larvae. The difference in the mortality amongst the plant extracts may be attributed to the variation in the concentration of saponins, tannins, phenol, alkaloids and terpenoids as observed in this present study. Moreover, this study revealed that organic solvent extracts used are rich source of botanical insecticides which may play an important role on the ongoing effort to reduce the mosquito population and the transmission of mosquito-borne diseases to humans.

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