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Ahmadu Bukar
Department of Biology
Education, Federal College of
Education (Technical) Potiskum
Yobe State, Nigeria

Muawiyya Haruna
Department of Biology
Education, Federal College of
Education (Technical) Potiskum
Yobe State, Nigeria

Lawan Buba Amshi
Department of Biology, Yobe
State University, Damaturu
Yobe State Nigeria

Corresponding Author:
Ahmadu Bukar
Department of Biology
Education, Federal College of
Education (Technical) Potiskum
Yobe State, Nigeria

Evaluation of adulticidal activities of some selected plant species against *Anopheles gambiae*

Ahmadu Bukar, Muawiyya Haruna and Lawan Buba Amshi

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Abstract

Plants extracts have been used as alternative sources of mosquito control since the ancient time prior to the development of synthetic pesticides due to the presence of repellent properties against insects. *Anopheles gambiae* mosquito is the vectors of Malaria disease in Tropical and Subtropical Africa, and the present practice of using synthetic pesticides to control the vector have results in the development of resistance by the mosquitoes and the pesticides causes environmental pollution and damages the ecosystem. The aim of this study was to evaluate the adulticidal activities of some plant species against *Anopheles gambiae*. The results of the Phytochemical analysis showed that, Anthroquinones, Soluble Steroids and Tannis are present in *Jatropha curcas* while *Adansonia digitata* possesses Saponins, Flavonoids, Anthroquinones and Tannins. *C. citrates* was reported to be possessing Flavonoids, Soluble starch and Tannins and was mostly reported plants to be possessing higher insecticidal properties against mosquitoes. *Zingiber officinale* was found possessing Saponins, Alkaloids and Tannins and finally, the *Lausonia inermis* possessng only Anthroquinones and Alkaloids. The results of the adulticidal activities shows the *Jatropha curcas* causes the highest mortality at 87% of *An. gambiae* among the plant extracts tested in this study at the highest concentrations of 20mg/ml at the lowest LC50 value of 4.217mg/ml and LC90 of 15.76mg/ml respectively and the plant with the lowest was *Z. officinale* with the highest mortality of 21% and the highest LC50 of 6710.051mg/ml respectively. We conclude that, these plants extracts can be used as alternative bioinsecticides for *An. gambiae*, a Malaria vector control to reduce the Malaria burden in the study area that annually causes significant death and overwhelm the health facilities.

Keywords: *Anopheles, gambiae*, plant, extracts, phytochemicals, adulticide

Introduction

Plant extracts have been used as alternative sources of mosquito control since the ancient time prior to the development of synthetic insecticides due to the presence of repellent properties against insects [1]. The presence of plants phytochemicals are responsible factors for their bioactivities against wide range of insects, mites and ticks at variable doses [2]. Plants extracts have been predicted to be the future of mosquito control against various vector borne infectious diseases of public health importance that have become prevalent in Africa, southeast Asia and the Europe [3]. Malaria disease is caused by a protozoan parasite of the genus plasmodium and transmitted through the bites of infected female *Anopheles* mosquitoes causing severe morbidity and mortality. Africa bears the global major burden of the disease accounting for over 80% of the annual cases and more than 90% of the accumulated death [4]. Over one million of cases and estimated death of over 300,000 are reported annually from Africa due to lack of effective and sustainable Malaria control strategy and drug supply [5] *An. gambiae* mosquitoes are the most abundant vectors of malaria occurring in Africa and in Nigeria, this specie is mostly found among the members of the *An. gambiae* complex comprising of *An. gambiae*, *An. arabiensis* and *An. funestus* serving as the major vectors of Malaria transmission [6]. The two species, *An. gambiae* and *An. arabiensis* are the most abundant malaria vector in Northern Nigeria that is annually faced with the challenges of malaria sickness and death particularly during the rainy season due to the poor drainage and abundant breeding grounds for the malaria vectors [7]. The control of Malaria vector was largely due to the application of chemical insecticides approved by World Health Organization for mosquito vector control namely, Organochlorine, Organophosphate, Pyrethroids and Carbamates [8].

However, *An. gambiae* mosquitoes have since developed resistance to these insecticides particularly Organochlorines and Pyrethroids, making it more difficult to control [9] and there accumulation damages the ecosystem affecting the aquatic plants and animals [10] hence, demanding for an alternative means of Malaria vector control using Plants extracts that are environmentally friendly and safe to the ecosystem and the aquatic communities of plants and animals [11]. Previous studies shows that plants extracts have caused inhibition in growth, pupation and adults emergence at low concentrations of methanol extracts of *Azadiracta indica*, *Syzygium aromaticum* and *Rhazya stricta* against *Culex pipiens* [12]. Adulticidal potentials of plants extracts have also been demonstrated in some Malaysian plants and shows higher mortality at LC50 doses after 24hrs [13]. Medicinal plants also shows moderate high insecticidal properties and records higher mortality against mosquitoes in leaf as well as berk extracts from different chemicals [14].

Therefore, this research was conducted due to the annual reported cases of Malaria transmitted by *An. gambiae* in the study area that annually causes significant death and overwhelm the health facilities.

Justification

Anopheles gambiae is one of the vectors of malaria in Tropical and Subtropical Africa, and the present practice of using synthetic chemical pesticide to control the vector have results in developing a serious resistance to chemicals such as Organochlorines and Pyrethroids causing environmental pollution and damaging the ecosystem. Hence, there is need for provision of an alternative means of controlling the population of *Anopheles gambiae* that will be environmentally safe and support the ecosystem. Therefore, this research is a step forward in providing alternative ways in the control of the population of *Anopheles gambiae* using World Health Organisation protocol for Malaria vector susceptibility and base line testing.

Aim

The aim of this study was to evaluate the adulticidal activities of some selected plant species against *Anopheles gambiae*.

Objectives

1. To identify and extract the selected plant species.
2. To determine the adulticidal activities of the selected plant species.

Materials and Methods

Plants Samples

The selected plants are *Jatropha curcas* (Bini dazugu), *Adansonia digitata* (Kuka), *Cymbopogon citrus* (Tsabarin kamshi) and *Zingiber officinale* (Citta) *Lawsonia inermis* (Lalle) against *Anopheles gambiae*. *Jatropha curcas*.

Plants Sample Preparation and Study Location

The samples of the study were purchased from the commercial sellers at the Potiskum Central Market, except *Jatropha curcas* which was supplied from outside Potiskum. The plants were shed-dried and grinds to powder using electric blender in Potiskum. Potiskum is the commercial Local Governments of Yobe State and is located at 11° 43'N 11° 04'E and 11.717° N and 11.067°E. It has an area of 559 square kilometer in Yobe State, it is also located in the Northeastern part of Nigeria at 12°

00N, 11°3'E and 12.00° N, 11.500° E of the meridian. (216 sqmi).

WHO Bioassay Kit supply

The World Health Organization kit for susceptibility of insecticides testing was purchased, supplied at the Vector Control Research Unit of Universiti Sains Malaysia dated 13th August, 2018 with the Reference number WHO/VCRU/179/2018-R1 and GST No. CBP: 001408204800.

Table 1: Plants samples, their common names and the parts used

S/N	Plants Name	Common Name	Part used
1	<i>Jatropha curcas</i>	Bini da zugu	Leaf
2	<i>Adansonia digitata</i>	Kuka	Leaf
3	<i>Cymbopogon citratus</i>	Tsabarin kamshi	Leaf
4	<i>Zingiber officinale</i>	Citta	Leaf
5	<i>Lawsonia inermis</i>	Lalle	Leaf

Plant Extraction and Phytochemical Screening

The plant leaves extraction was conducted using Soxhlet extractor by sonification and screened for the presence of phytochemicals and secondary metabolites at the Biochemistry Department Laboratory Bayero University Kano, Nigeria. The crude extracts were also concentrated using Rotary Evaporator. Each plant was extracted using Ethanol as a solvent according to the method adopted by [15].

Anopheles gambiae mosquito

An. gambiae mosquito larvae were collected from various breeding grounds in Potiskum Local Govt. Area, Yobe Sate Nigeria and environs and were reared to the adults for bioassay. Other species such as *Culex quinquefasciatus* found breeding together with *An. gambiae* were discarded after collection.

Adulticidal Bioassay

The adult mosquito adulticidal bioassay using plant extracts were conducted following the procedure [8] Plants extracts were mixed and dissolved with acetone at various concentrations of 10mg/ml, 15mg/ml and 20mg/ml. 2.5ml of test concentrations were impregnated to No.1 Whatman filter paper sized (12 x 15cm). Ethanol was also impregnated to No.1 Whatman filter paper as a negative control in each plant bioassay. The extracts impregnated in the papers were allowed to air dried for 5 minutes before putting into the exposure tube. Twenty 2-5day old, female mosquitoes starved from blood were introduced to the holding tube and allowed for 1 hour. The testing mosquitoes were later moved by gentle blowing back to the holding tube to recover after 1 hour exposure period. 10% glucose solution soaked in cotton were placed on the mesh of each tube to feed the surviving mosquitoes. At the end of the 24 hours period of recovery, the death mosquitoes were recorded and the percentage mortality were calculated for each extract tested.

Statistical analysis

The adult percentage (%) mortality was calculated using the standard formular as

$$\% \text{ mortality} = \frac{\text{Total number of death mosquitoes}}{\text{Total Number of all mosquitoes}} \times 100$$

and the test with more 20% control mortality were discarded while those with less than 20% control mortality were corrected

using Abbott formular according to [8]:

$$\text{Corrected mortality} = \frac{\% \text{ mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Statistical package, SPSS version 20.4 software was used, LC50 and the LC90 together with the upper, lower confidence limits and the chi-square (X^2) were also calculated. Results with $P < 0.05$ were considered statistically significant.

Results

The results of the Phytochemical screening of the adulticidal

activities of the selected plants extracts were presented in (Table 2) below. The phytochemicals present in *Jatropha curcas* were Anthroquinones, Soluble Steroids, and Tannis while *Adansonia digitata* possesses Saponins, Flavonoids, Anthroquinones and Tannins. *C. citrates* was reported to be possessing Flavonoids, Soluble starch and Tannins and was mostly reported plants to be possessing higher insecticidal properties against mosquitoes. *Zingiber officinale* was found possessing Saponins, Alkaloids and Tannins and finally, the *Lausonia nermis* was reported possessing only Anthroquinones and Alkaloids respectively.

Table 2: Various Phytochemicals presents in the selected plants species

Plant extracts	Phytochemicals							
	Saponins	Flavonoids	Terpenoid	Anthroquinone	Soluble starch	Steroids	Alkaloids	Tannins
<i>Jatropha curcas</i>	-	-	-	+	-	+	-	+
<i>Adansonia digitata</i>	+	+	-	+	-	-	-	+
<i>C. citrates</i>	-	+	-	-	+	-	-	+
<i>Zingiber officinale</i>	+	-	-	-	-	-	+	+
<i>Lausonia nermis</i>	-	-	-	+	-	-	+	-

(+) = present, (-) = Negative

The results of the adulticidal activities of the plants extracts presented (Table 3). *Jatropha curcas* shows the highest adulticidal activities at 87% mortality of *An. gambiae* among the plant extracts tested in this study at the highest concentrations of 20mg/ml. it also shows a good activity of 64% mortality at 10mg/ml and at the least concentration of 5mg/ml it also shows a significant activity of 53% mortality. The estimated LC50 was 4.217mg/ml and LC90 of 15.76mg/ml respectively. *C. citratus* extract also demonstrated the mortality of *An. gambiae* at 66% at the concentration highest of 20mg/ml at 10mg/ml, it also shows mortality of *An. gambiae* at 42%

while the mortality recorded at the least concentration of 5mg/ml was very low compared with *Jatropha curcus* which recorded 53% at the lowest concentration. *Adansonia digitata* also shows activity towards *An. gambiae* at the highest mortality of 54% at the highest concentration and the least at 23%. The remaining *Z. officinale* and *Lausonia inermis* were having the least activity at 21% and 43% at the highest concentrations of 20mg/ml. However, *Lausonia inermis* have a good activity of 34% and 15% mortality at 15mg/ml and 10mg/ml respectively.

Table 3: The Adulticidal activities of plants extracts against *An. gambiae* after 24hrs exposure period

S/N	Plant extract	Concentration (mg/ml)	Mean \pm S.E	(%) Mortality	LC ₅₀	LC ₉₀	X ²
1	<i>Jatropha curcas</i>	Control	0.0 \pm 0.0	0	4.217	15.76	33.372
		5	15.6 \pm 5.17	53			
		10	21.3 \pm 1.81	64			
		20	23.7 \pm 4.62	87			
2	<i>Adansonia digitata</i>	Control	0.0 \pm 0.0	0	18.432	45.32	8.356
		5	13.0 \pm 4.12	23			
		10	18.7 \pm 3.52	32			
		20	25.3 \pm 6.51	54			
3	<i>C. citratus</i>	Control	0.0 \pm 0.0	0	28.074	88.067	7.645
		5	3.0 \pm 0.60	8			
		10	5.0 \pm 0.87	42			
		20	11.5 \pm 0.64	66			
4	<i>Z. officinale</i>	Control	0.0 \pm 0.0	0	6710.051	4.2421+111	6.835
		5	5.2 \pm 0.71	13			
		10	6.4 \pm 0.57	18			
		20	9.0 \pm 1.29	21			
5	<i>Lausonia inermis</i>	Control	0.0 \pm 0.0	0	46.389	98.383	5.018
		5	4.7 \pm 0.25	15			
		10	9.5 \pm 0.84	34			
		20	21.5 \pm 2.65	43			

LC= Lethal Concentration, SE= Standard Error, X² =Chi square

Discussion

The findings of this study reveals that the extract of *Jatropha curcas* showed the highest adulticidal activity of 87% of mortality and LC50 value of 4.2mg/ml against *An. gambiae* tested, The result was in agreement with the findings of the

study by [16] with a methanol extract of *Jatropha curcus* against *Culex quinquefasciatus* showing highest mortality at the LC50 value of 1.2%. This broad activity of *J. curcas* against various mosquito vectors could be attributed to the presence of Anthroquinone, steroids and ternins when extracted with

ethanol. It is also in close agreement with findings of the study on the adulticide activity of *J. curcas* against adult house flies, *Musca domestica* at LC50 of 4.5mg/cm³ with the higher percentage of trans-phytol as the major component of the phytochemicals and secondary metabolites responsible for its insecticidal properties [17]. The ethanol extract of *J. curcas* was also effective against tick of the genus *Rhipicephalus annulatus* showing inhibition in the hatching of the laid egg of the treated tick [18].

Adansonia digitata shows potency towards *An. gambiae* with an estimated LC50 of 18.4mg/ml and LC90 of 45.3mg/ml in this study. These findings are in agreement with findings of the study conducted using n-hexane extract of *A. digitata* against the *Culex quinquefasciatus* larvae. The study further founds the presence of all the phytochemicals obtained in this study as could be responsible for the higher mortality of the mosquitoes [19]. The same phytochemicals also obtained in the other study using three different plants extracts including *A. digitata* against the larval stage of *Ae. aegypti* and causes 100% mortality of the larvae [20]. The insecticidal activities of *A. digitata* was not surprising as the plant have been used for medicinal, chemo preventive, antiviral and preventives for human and various animal models using some parts of the plants mostly leaves and barks [21, 23].

C. citratus was one of the plants earlier explored for insecticidal activities against mosquitoes due to its scents and was previously used in the olden ages as insects repellent [24]. The activity of *C. citratus* at the estimated LC50 28.074mg/ml in this study matched with the findings of the study of [25] who synthesized nanoparticles of gold from the leaves of *A. digitata* against the larvae of *An. stephensi* and *Ae. aegypti* and found it much effective against *Ae. aegypti* at percentage mortality of almost the same with this study. In a recent study, the essential oils of *C. citratus* was very effective against the larvae of *Ae. aegypti* in Vietnam at higher LC50 of 38.8mg/L, almost the same found in this study even though the study did not shows the screened phytochemicals presence in the anhydrous sodium sulfate extracts, as the presence of the phytochemicals depends on the extracting solvent [15] which could be responsible for the efficacy of the *C. citratus* extract against *Aedes aegypti* [26]. Several studies have reported the insecticidal activities of this plant extracts on insects such as *Musca domestica* and different mosquitoes and several other flying arthropods and found it effective on adulticides, repellent and inhibition of life cycle developmental stages [27].

Z. officinale is an active insecticide repellent according to the several studies particularly in stored products study due to the presence of heptanol, heptyl acetate and linalool, it was also effective against the Maize known pest, a Coleoptera, *Prostephanus truncatus* that causes much damage to the cereal. It repels and causes adults emergence inhibition in treated Maize, it also cause oviposition deterrent in *An. stephensi*, *Culex quinquefasciatus* and *Ae. aegypti* but not effective in adulticidal activity as shown in this study [28]-[30]. That also could be the cause of its higher estimate LC50 value in this study. Moreover, it was also reported to be effective against the larval stage of the *Ae. aegypti* causing higher mortality at the same concentration used in the study [31].

Lausonia inermis is also used for treatment as antimicrobial and anticancer when formulated with ZnO as nanoparticle according to the recent study [32] but it is an effective larvicide against Malaria vector, *An. stephensi* [33]. It shows adulticidal activity in this study at the LC50 of 46.389mg/ml which was in

agreement with findings of its activity against Red flour beetle causing higher mortality [34]. It was also in agreement with the study by [35] which reported the significant mortality of *Culex quinquefasciatus* larvae treated by *L. inermis* as a source of biocontrol of the filarial vector in Nigeria.

Conclusion

All the plants used in this study showed adulticidal activities at various concentrations and at various LC50 and LC90 with *J. curcas* showing the highest mortality at a very low Lethal Concentration doses as opposed to *Z. officinale* having the least efficacy against *An. gambiae*. The findings of this study also support the previous findings showing the presence of various phytochemical in the different plants extracted with ethanol. Hence, these plants extracts can be used as alternative insecticides for *An. gambiae*, a Malaria vector control to further reduced the Malaria burden in the study area that annually causes significant death and overwhelm the health facilities.

Conflict of interest

The authors have agreed and declared that there were no conflicts of interest among them.

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