



Original Research Article

Larvicidal and synergistic toxicity of *Ficus sycomorus* and *Calotropis procera* leaf extracts against malarial vector *Anopheles gambiae* complex from Kano-Nigeria: A green bio-control approach

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ARTICLE INFORMATION

Received: 20 December 2020

Received in revised: 4 February 2021

Accepted: 9 February 2021

Available online: 25 April 2021

DOI: 10.22034/ajgc.2021.262853.1290

KEYWORDS

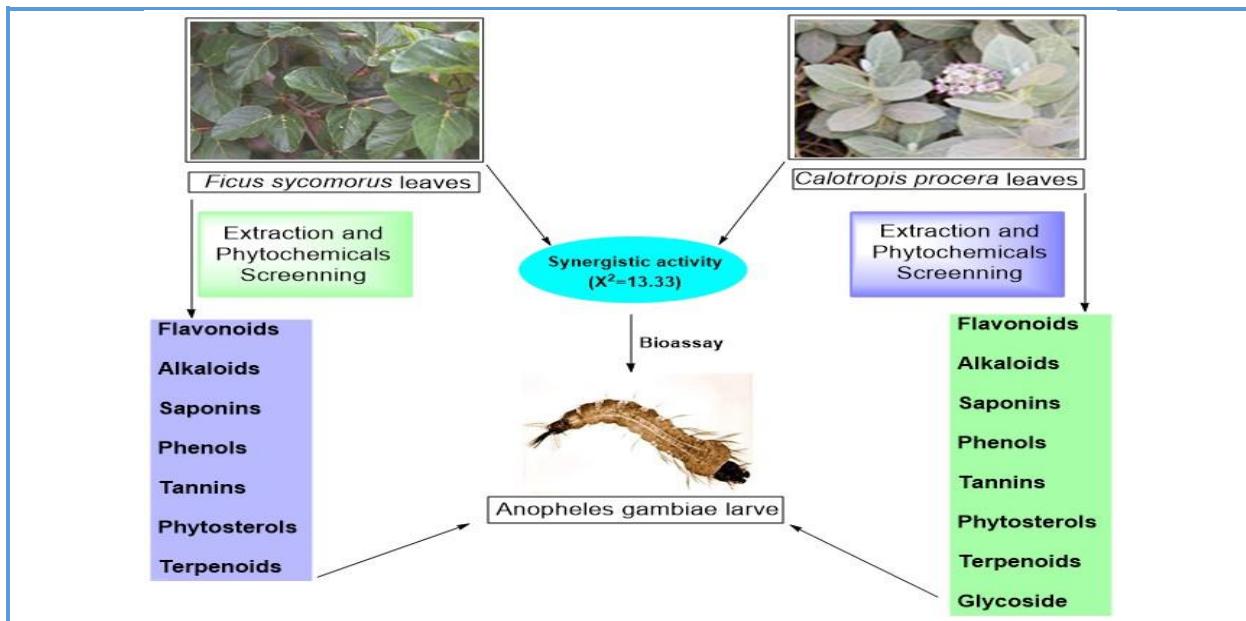
Anopheles
Calotropis procera
Ficus sycomorus
Larvicides
Phytochemicals

ABSTRACT

Plant phytochemicals appeared to be a promising tool to address resistance and environmental problems posed by synthetic insecticides. This study explored the larvicidal effects, and synergistic toxicity of *Ficus sycomorus* and *Calotropis procera* leaves on African malaria vector, *Anopheles* species sourced from agricultural fields in Kano-Nigeria. The qualitative and quantitative phytochemicals were determined using standard methods. Late third Instar larvae (L3) of *Anopheles* mosquitoes were subjected to bioassay at various concentrations (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) of ethanol extracts using the WHO standard protocol with some modifications. The synergistic potential was predicted using mathematical model. The extract of *F. sycomorus* revealed the highest concentration of flavonoids, alkaloids, saponins and phenols, while *C. procera* extract had high concentration of glycosides and tannins. However, only alkaloids concentrations (15.41 mg/mL and 7.7 mg/mL) was statistically significant between the two plants ($p<0.05$). The bioassays show high percentage mortalities in both plants with *C. procera* extract being more toxic ($LC_{50}=0.51$ mg/mL; $\chi^2=0.83$; 95% confidence limits, CI: 0.30-0.84; $p>0.05$) on *Anopheles* larvae than *F. sycomorus* extract ($LC_{50}=1.01$ mg/mL; $\chi^2=0.920$; 95% confidence limit, CI: 0.50-2.05; $p>0.05$). The binary combination (concentration $LC_{25}:LC_{25}$) of the two plants produced promising results of higher mortality than individual highest extract concentrations ($LC_{50}=0.38$ mg/mL; $\chi^2=0.72$; 95% confidence limits, CI: 0.23-0.61; $p>0.05$) due to possibly synergistic effect of the two plants ($X^2=13.33$). The percentage mortalities in all the crude extracts tested were concentration dependent. It is evident from this study that crude extracts of *F. sycomorus* and *C. procera* have promising individual and synergistic larvicidal bioactivities and hence, can be employed in integrated approach for vectors resistance management.

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Graphical Abstract



Introduction

The African region is usually inflicted with the burden of malarial infections, vectored by female *Anopheles* mosquitoes [1]. Generally, the malaria vector control against mosquitoes has mainly been made using chemical agents such as organophosphorus (OP) insecticides, insect growth regulators and bacterial larvicides. In sub-saharan Africa and elsewhere, the constant and injudicious applications of insecticides such as pyrethroids and its analogous in domestic and agricultural practices have significantly contributed to the development of resistance in mosquitoes [2, 3]. This practice also caused environmental hazards through persistence and accumulation of non biodegradable toxic components in the ecosystem leading to biological magnifications in the food chain and toxic effects on public health and non-target organisms [4] and eventually high malaria infections. Insecticides resistance in both *Anopheles gambiae* and *Anopheles coluzzii* is wide-spread in Nigeria. Identification of

effective larvicidal compounds from natural sources is therefore, essential to combat increasing mosquitoes' resistance rates, concern for the environment and food safety, the unacceptability of many organophosphates and organochlorines and the high cost of synthetic insecticides. Most of the mosquito control programs targeted the larval stage in their breeding sites with larvicides, because the adulticides may only reduce the adult population temporarily [5]. The use of chemical larvicides is a flourishing way of reducing mosquito densities in their breeding places before they emerge into adult vectors.

Plant kingdom is rich sources of alternative compounds called botanicals, for the control of mosquito larvae, adults and even the malaria infections. They have a reservoir of bioactive secondary metabolites that are highly selective in insecticidal action, easily biodegradable, and have low or no adverse effects on non-target organisms and also the environment, making them the potential candidates for use in integrated pest management control programs.

An estimated 2000 species of terrestrial plants have been reported for their insecticidal potentials [6]. Their insecticidal, fungicidal, bactericidal, antiviral, anti-feedant and insect growth retardant properties are often the result of individual or synergistic interactions among different biologically active phytochemicals such as saponins, tannins, steroids, terpenoids, alkaloids and phenolics [7–9].

The plant family, Moraceae has *Ficus* as one of the main important genus; with many reported biological activities such as antipyretic activity [10], gastro-protective property [11], antioxidant potentials reported by Phan *et al.* [12], anticancer [13], antimicrobial activity [14] and antiulcer property [15]. *Ficus spp* latex was also exploited in South and Central America for its anti-helminthic potential as reported by De-Amorin and colleagues [16]. The parasiticidal property of this genus has been linked to the occurrence of a short peptide called *ficin* [17]. Traditional healers report the use of *F. sycomorus* in the treatment for malaria. The *F. sycomorus* possesses good insect repellent properties and hence reduces the contact of the vector with humans, minimizing incidence of malaria transmission [18]. The insecticidal and acaricidal activities of *F. sycomorus* have also been previously reported by Romeh [19]. This plant has been utilized locally (in combination with other plants) to kill mosquitoes from houses and other public places in some villages in the northern Nigeria. *Calotropis procera*, known as Apple of Sodom, belongs to *Asclepiadaceae* plant family and is found in many countries such as Africa and Western and South Asia, as well as Indo-china. It is reported for its medicinal and pharmacological properties [20]. The milky sap of this plant contains three toxic glycosides: (i) calotropin, (ii) uscharin, and (iii) calotoxin as well as steroidal heart poisons, known as cardiac aglycones [21]. Locally, the plant has

been used as an antifungal, antipyretic and analgesic agent [22, 23]. The coarse shrub possesses acaricidal, schizonticidal, antimicrobial, anti-helminthic, insecticidal, anti-inflammatory, anti-diarrheal, anticancer, and larvicidal activities [24, 25].

The mosquitoes resistant to temephos, the commonly used synthetic larvicide, have been extensively reported [26]. Identification of effective mosquitocidal compounds is therefore essential to combat increasing resistance rates, concern for the environment and food safety, the unacceptability of many organophosphates and organochlorines and the high cost of synthetic insecticides. Therefore, comparative larvicidal properties and synergistic potentials of *C. procera* and *F. sycomorus* leaves will be preliminarily studied against larvae of *An. gambiae* complex, a major malaria vector from Kano-Nigeria as an alternative to synthetic insecticide. The combination may be more effective than individual plant extract, reducing the use of conventional insecticides, protect the environment and combat resistant rate in malaria vectors.

Experiment

Materials and methods

All the chemicals used in this study were of analytical grade procured from BDH, England.

Collection and authentication of plant samples and larvae

The leaves samples of *Ficus sycomorus* and *Calotropis procera* were collected from Bayero universiy botanical garden, Kano (11°98'14"N, 8°48'02"E). The plants were identified by specialist at Department of plant science, Bayero University, Kano and voucher numbers were given as BUKHAN0109 and BUKHAN0132 for *Ficus sycomorus* and *Calotropis procera*, respectively.

Plant preparation

The *F. sycomorus* and *C. procera* leaves were washed to remove dust and dirt and then drained. The washed leaves were put into the shade for drying for 14 days. The dried leaves were pounded into powdered form. The *F. sycomorus* and *C. procera* powdered leaves were extracted by maceration method. The powdered leaves (200 g) were soaked in 600mL of ethanol for 72 hours with occasional shaking. The extract was then filtered first through a sieving mesh followed by another filtration through Whatman filter paper No. 42 using a vacuum pump. The extracts were then, allowed to stand in a water bath set at 60 °C to one-tenth its original volume and then finally freeze-dried. The dried residue (crude extract) was then stored at 4 °C. One gram of the plant extract was dissolved in 100 mL of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, respectively for larvicidal bioassays [27].

Mosquito culture

The larvae samples were collected from different temporary puddles of water from rainfall and rice paddies in Bichi (12°14'03"N, 8°14'28"E), Kano-Nigeria. From the *Anopheles* larvae collected from the agricultural fields, a colony of mosquitoes was established. The larvae were kept in plastic and enamel trays containing de-chlorinated tap water. They were maintained at 27±2.0 °C and 75-85% relative humidity under 14:10 light and dark cycles.

Phytochemical analyses

The bioactive metabolites such as alkaloids, phenols, terpenoids, phytosterols, flavonoids, glycosides and tannins as well as their

quantities in the extracts were determined using standard phytochemicals identification protocol [28].

Detection of alkaloids (Wagner's test)

Extracts was dissolved in dilute Hydrochloric acid and filtered. Filtrates were treated with Wagner's reagent (1.27 g of Iodine and 2 g Potassium Iodide in 100 mL of water). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of saponins

This was done by using foam test. 0.5 g of extracts was shaken with 2 mL of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols

Extracts was treated with 3-4 drops of 5 % ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

To each of the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Dilute ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 mL) was also added. A yellow colouration that disappears on standing indicates the presence of flavonoids.

Detection of terpenoids (Salkowski's test)

Chloroform (1 mL) was added to 2 mL of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown

precipitate produced immediately indicated the presence of terpenoids.

Larvicidal bioassay

Larvicidal bioassay with extracts was carried out as per the reported guidelines of World Health Organization [27] with some modifications. Firstly, the larvae were exposed to a broad concentrations range with controls to uncover the activity range of each of the plants extract. After evaluating the mortality of larvae at these concentrations, a narrow concentration ranges of 5 different concentrations, yielding between 10% and 95% mortality in 24 hours was prepared, to determine the lethal concentration of 50% (LC_{50}) and the lethal concentration of 95% (LC_{95}) mortality values. The larvicidal activity at test concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL (four replicates each) of each crude leaf extracts were assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of the crude extract. Assay was performed at room temperature ($27 \pm 2^{\circ}\text{C}$) and 70–80% relative humidity and 14:10-h dark and light cycles. Twenty-five (25) late third instar larvae (L3) were introduced into disposable plastic cups by means of the dropper to a distilled water and test concentration. Mortality was observed 24 hours after treatment. Untreated control (distilled water only), treated control (acetone) and positive control (Temephos® at concentrations of 2.5, 5.0, 7.5 and 10.0 mg/mL) were maintained separately and run simultaneously. A total of four replicates per trial for each concentration were done. The percentage larval mortality was calculated using the formula (1) and corrections for control mortality (5 – 20%) when necessary was done using formula (2) of Abbott's formulae [29] after 24h exposure. Moribund and dead larvae were considered affected by the

plant extracts. During the exposure periods, no food was supplied to the larvae and percentage of mortality was calculated:

$$\text{Percentage test mortality (\%)} = (\text{number of larvae dead} / \text{total number of larvae used}) \times 100 \quad (1)$$

$$\text{Corrected Mortality (\%)} = [(\% \text{ test mortality} - \% \text{ control mortality}) / (100 - \text{control mortality})] \times 100 \quad (2) [29].$$

Effect of the binary combination of the plant extracts

Five test groups were run concurrently for each binary combination tested. The two extracts were combined in a 1:1 ratio (Concentration LC_{25}/LC_{25}). The LC_{25} values were mathematically estimated from dose-response curve of each plant extracts. Actual mortalities were compared to expected mortalities based on the model formula:

$$E = OF + OC (1 - OF/100) \quad (3)$$

Where: E is the expected mortality and of and OC are the observed mortalities of crude extracts of the *F. sycomorus* and *C. procera* respectively. The factor of 100 was used to calculate the value of E.

The effects of mixtures were designated as either antagonistic, additive, or synergistic by analysis using χ^2 comparisons:

$$\chi^2 = (OF:C - E)^2/E \quad (4)$$

Where: OF:C is the observed mortality from the binary mixture and E is the expected mortality, χ^2 with $df = 1$, and $p = 0.05$ is 3.84. A pair with χ^2 values > 3.84 and having greater than expected mortality were considered to be synergistic (or antagonistic), with χ^2 values < 3.84 representing additive effects of the extracts [30, 31].

Statistical analyses

Statistical analysis of all mortality data of larvicidal activities were subjected to Log-

probit analysis [32] to determine lethal concentration causing 50% (LC_{50}) and 95% (LC_{95}) mortality of the larvae, 24 h post exposure, and other statistics at 95% confidence limits (upper confidence limit (UCL) and lower confidence limit (LCL)), R^2 and Chi-square. The differences were considered as significant at $P \leq 0.05$ level. All analyses were performed using SPSS version 21.

Results and Discussion

The preliminary phytochemicals screening of the two plant extracts revealed the presence

of some secondary plant metabolites such as flavonoids, tannins, alkaloids, saponins, phenols, terpenoids, phytosterols with glycosides absent in *F. sycomorus* (Table 1). The larvicidal activities of the plant was concentration dependent and at the highest concentration of 1.0 mg/mL (Table 2, 3 and 4), all extracts exhibited a significant percentage mortalities and synergistic property.

The results in the Table above shows the phytochemicals presence in ethanol extracts of *Ficus sycomorus* and *Calotropis procera*.

Table 1. Qualitative phytochemicals analysis of *Ficus sycomorus* and *C. procera* ethanol extracts

S/N	Phytochemical constituent.	<i>F. sycomorus</i>	<i>C. procera</i>
1	Flavonoids	++	++
2	Alkaloids	++	+
3	Saponins	+	+
4	Phenols	+	+
5	Tannins	++	+
6	Phytosterols	+	+
7	Terpenoids	+	+
8	Glycosides	-	++

Key: + =present; - =absent

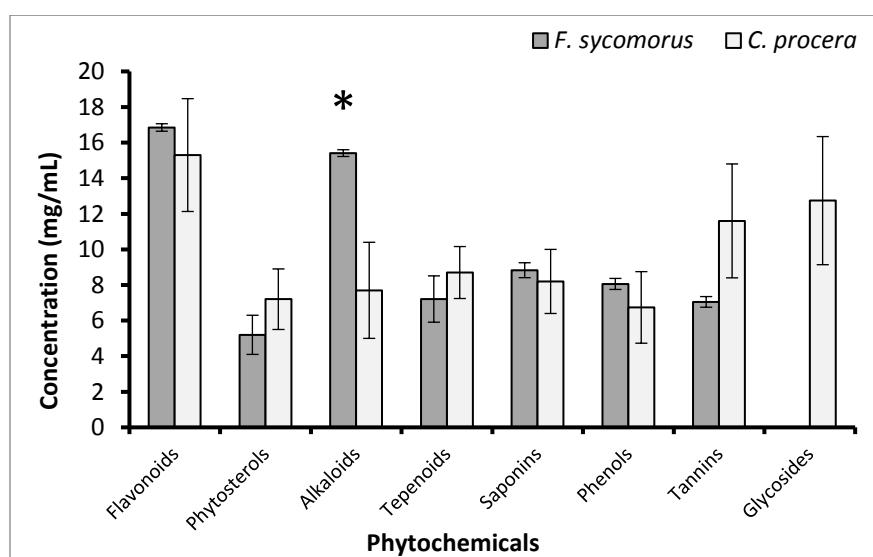


Figure 1. Quantitative analysis of ethanol Extracts of *Ficus sycomorus* and *Calotropis procera*

The two plants differ in the concentration of the phytochemical constituents in which *F. sycomorus* extract have the highest concentration of flavonoids, alkaloids, saponins and phenols (16.85, 15.41, 8.83 and 8.06 mg/mL respectively) while *C. procera* have large concentration of glycosides and tannins (12.74 and 11.60 mg/mL respectively) (Figure 1), while *F. sycomorus* showed absent of glycosides. Statistically, only alkaloids 15.41 mg/mL and 7.7 mg/mL showed a significant variation between the *F. sycomorus* and *C. procera* leaves extracts respectively ($p<0.05$).

The larval exposure to different concentrations of *F. sycomorus* extract shows percentage mortality in concentration dependent manner. The highest concentration used, 1.0 mg/mL had mortality of 50.72 % while the 0.1 mg/mL concentration produces 15 % larval mortality after 24 h exposure (Table 2). The *F. sycomorus* extract also showed good linear relationship with insecticidal toxicity

($R^2=0.9608$) against *Anopheles gambiae* complex (Figure 2).

The *C. procera* leaves extract exposure at 1 mg/mL concentration shows highest larval mortality of 63% and while only about 11% mortality of the exposed larvae in the 0.1 mg/mL concentration was recorded after 24 h (Table 3). The larval mortality was also concentrations dependent (Figure 3) with R^2 value of 0.958.

The 1:1 combination of *F. sycomorus* and *C. procera* ($L_{25}:LC_{25}$) produces high larval mortality (82%) at highest concentration (1 mg/mL) than the individual plant after 24 h (Table 4). This combination was computed to be synergistic effect of the two plants with coefficient of synergy been positive ($X^2=13.33$). The extracts combination also showed linear relationship with toxicity ($R^2=0.9068$) against the mosquito larvae (Figure 4).

Table 2. Percentage Mortality of *Anopheles gambiae* complex larvae exposed to different concentration of *F. sycomorus* ethanol extract

Concentration (mg/mL)	% mortality
Normal control (water)	1.70
Negative control (acetone)	3.10
0.1	15.41
0.2	21.00
0.4	27.79
0.6	42.25
0.8	47.51
1.0	50.72

The third instar stage larvae (L3) of *An. gambiae* complex from the agricultural fields were subjected to larvicidal bioassay at various concentrations of the ethanol extracts of the plants using WHO [27] bioassay protocol with some modifications. The result demonstrated high percentage mortalities in both plants with *C. procera* ($LC_{50}=0.51$ mg/mL; $\square^2=0.83$; CI: 0.30-0.84; $p>0.05$) more active in *An. gambiae*

larvae than *F. sycomorus* extract ($LC_{50}=1.01$ mg/mL; $\square^2=0.92$; CI: 0.50-2.05; $p>0.05$). The binary combination (concentrations- $LC_{25}:LC_{25}$) of the two plants produced promising results of high mortality than individual plants highest concentrations ($LC_{50}=0.38$ mg/mL; $\square^2=0.72$; CI: 0.23-0.61; $p>0.05$) (Table 5). In general, the percentage mortalities in all the extracts tested were concentration dependent.

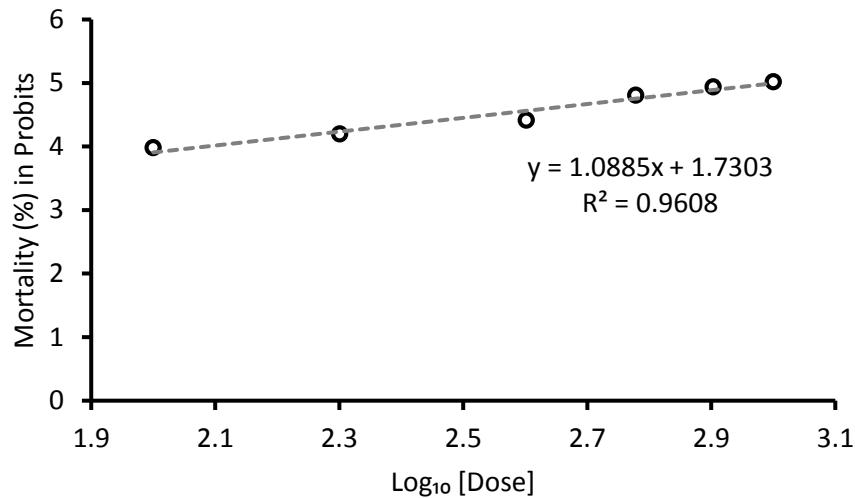


Figure 2. The Log-Probit Curve of *F. sycomorus* leaves ethanol extract against *Anopheles gambiae* complex larvae

Table 3. Percentage mortality of *Anopheles gambiae* complex larvae exposed to different concentration of ethanol extract of *C. procera*

Concentration(mg/mL)	% mortality
Normal control (water)	0.33
Negative control (acetone)	3.10
0.1	11.31
0.2	31.62
0.4	47.00
0.6	58.71
0.8	59.22
1.0	63.10

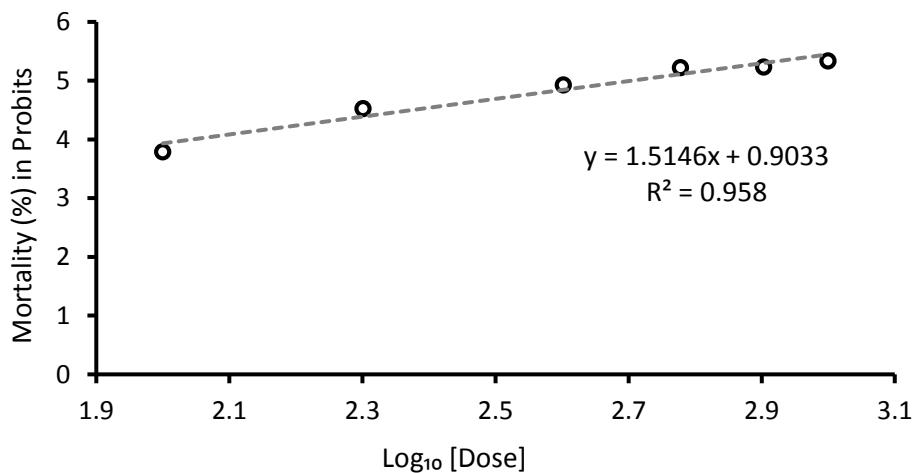
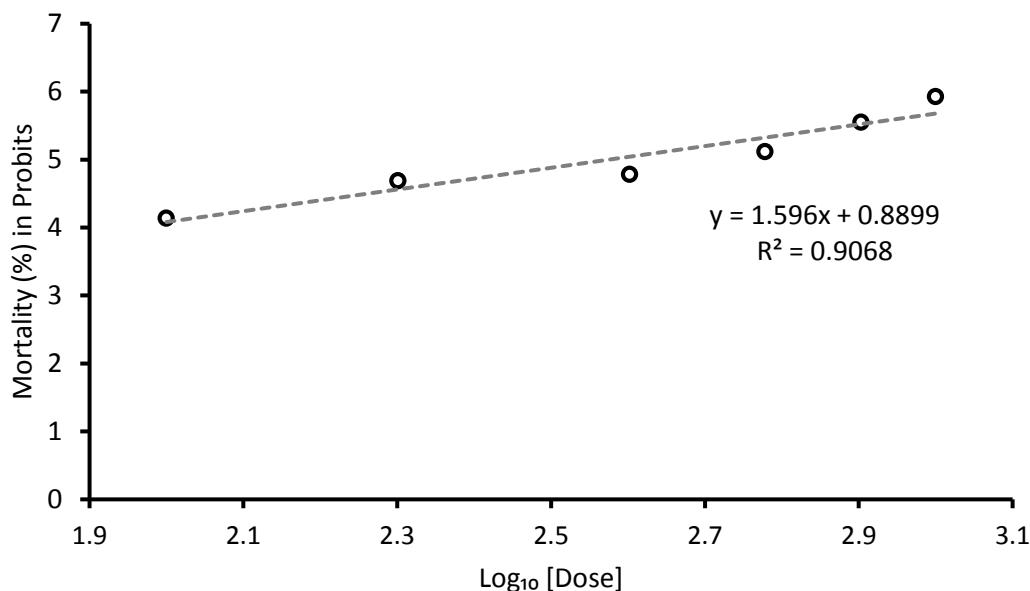


Figure 3. The Log-Probit Curve of *C. procera* leaves ethanol extract against *Anopheles gambiae* larvae

Table 4. Percentage Mortality of *Anopheles gambiae* larvae exposed to different concentration of ethanol extract of *C. procera* and *F. sycomorus* (Combined)

Concentration(mg/mL)	% mortality
Normal control(water)	0.33
Negative control(acetone)	3.10
0.1	19.46
0.2	37.81
0.4	41.42
0.6	54.76
0.8	71.00
1.0	82.33
Expected mortality	55.2
X ²	13.33

**Figure 4.** The Log-Probit of *F. sycomorus : C. procera* leaves ethanol extract against *Anopheles gambiae* complex larvae

The combined extracts showed more potency (low LC₅₀) compared to individual plant (Table 5), possibly indicating synergistic effect ($X^2=13.33$) in the observed larval mortalities. According to WHO recommendation made in 2013 [33], 98–100 mosquitoes' mortality indicates susceptibility, 90–97% suggests a suspected resistance that needs to be confirmed, <90% mortality indicates resistance. The highest mortality observed is 82.33% when both plants were

combined indicating possible resistance to the extracts (Table 4).

Mosquito larval control using various chemical larvicides is an indispensable component in the strategic control of mosquitoes borne diseases. Plant is naturally considered as viable and preferred alternative in the control of the mosquito vector species at the community level due to obviously their availability, easy access and potential to reduce environmental pollution and mosquitos'

resistance. The mosquito larval control is simpler and highly effective compared to other conventional methods of mosquito control which have serious public and environment limitations. The larval stage or immature forms are one of the striking targets for insecticides/pesticides because their

developmental stages is usually confined in water and thus, very simple to handle in this atmosphere [34]. The results from this study show promising larvicidal and synergistic activities of the *F. sycomorus* and *C. procera*.

Table 5. The LC₅₀ and LC₉₅ of *F. sycomorus*, *C. procera* and their combination of crude ethanol extracts against *Anopheles gambiae* complex larvae

Plant	LC ₅₀ mg/mL	LCL	UCL	LC ₉₅ mg/mL	LCL	UCL	R ²	X ²	SD	SE	Fit	Significance
Fs	1.01	0.50	2.05	32.96	16.20	67.06	0.96	0.92	0.00	0.00	Good Fit	NS
Cp	0.51	0.30	0.84	6.30	3.79	10.47	0.96	0.83	0.00	0.00	Good Fit	NS
Fs:Cp	0.38	0.23	0.61	4.15	2.56	6.74	0.91	0.72	0.00	0.00	Good Fit	NS

Key: Fs - *Anopheles gambiae* complex larvae expose to different concentration of *F. sycomorus*

Cp- *Anopheles gambiae* complex larvae expose to different concentration of ethanol extract of *C. procera*

An-Cp-Fs- *Anopheles gambiae* complex larvae expose to different concentration of ethanol extract of *C. procera* and *F. sycomorus* (1:1combined)

95% UCL- upper confidence limit, 95% LCL-lower confidence limit, SE- standard error, SD- standard deviation, NS- Not significant

The *C. procera* possessed high activities (lower LC₅₀) against *An. gambiae* complex larvae compared to *F. sycomorus* (higher LC₅₀) leaves (Table 5). The larval mortality generally may depend on time of exposure, plant species and chemical composition or phytochemicals presence. The variation of phytochemicals presence and quantitative chemical composition (Figure 1) of the plants could probably explain the differences in LC₅₀ values obtained. In general, the higher the time and mortalities of mosquitoes after exposure to the insecticidal extract, the lesser the lethal concentrations (LC) (Table 5).

It has been reported that plant containing alkaloids, coumarins, flavonoids, quinines, saponins, steroids and terpenoids may be toxic to mosquitoes' larvae [35, 36]. These botanicals and their derivatives possessed mosquito larvicidal properties in general, which directly attack on the insects' nervous system and destroy it, affecting the midgut epithelium primarily and secondarily affect the gastric caeca and the malpighian tubules in mosquito

larvae [37]. It act as mitochondrial poison [38] and work by interacting with cuticle membrane of the larvae ultimately disarranging the membrane which is the most probable reason for larval death [39]. The larval mortalities observed could probably be due to the presence of alkaloids, flavonoids, steroids, tannins, terpenes and terpenoids (Table 1) and it is said that several groups of the above mentioned phytochemicals from different plants have been reported for their insecticidal activities [40]. All the detected phytochemicals from this study may perhaps act in a concerted way to non-specifically induce toxicity the *An. gambiae* complex larvae (Table 5). The insect control ability of those plants may possibly varies with age of the plant, species of the plant, part extracted, collection site, and solvent used for extraction [41, 42, 43]. Different plant parts have different phytochemicals compounds which have different toxicities to target species [44].

However, the difference in toxicity expressed by the different plant species may be

due to the quantitative and qualitative variation in the chemical composition of the plant extracts (Figure 1). The *F. sycomorus* extract have the highest concentration of flavonoids, alkaloids, saponins and phenols while *C. procera* have high concentration of glycosides and tannins (Figure 1). The alkaloids concentration is significantly higher in *F. sycomorus* than *C. procera* (Figure 1) but *F. sycomorus* showed absent of glycosides and may account for the observed high toxicity of *C. procera* (Table 5). The mode of action of most of the plant extracts on mosquito larvae is still unknown. However, previous research documented that some phytochemicals could interfere with the proper functioning of the insects' mitochondria particularly at the proton transferring site [45]. Some other bioactive molecules of plant extracts have been found to primarily affect the mid-gut epithelial surface and secondarily the gastric caeca and the malpighian tubules of the mosquito larvae [37].

The *Ficus benghalensis* and *Ficus sarmentosa* var. *henryi* were previously proved larvicidal against different larval stages of both *Culex* and *Anopheles* mosquitoes [46], thus, corroborating the findings of this study that *F. sycomorus* leaves larvicing against L3 stage of *An. gambiae* complex (Table 5). Treatment of *An. gambiae* larvae with the methanolic extract of *Ageratum conyzoides* depicted dose-dependent effects with highest mortality percentages of $\geq 69\%$ ($LC_{50} = 84.71 - 232.70$ ppm) observed when exposed with 250 ppm and 500 ppm for 48 h against *Anopheles gambiae* s.s.[47]. This also largely agreed with the findings of this study which showed highest percentage mortality of 52% ($LC_{50}=1.01$ mg/mL) *F. sycomorus* exposed (Table 2), 63% ($LC_{50}=0.51$ mg/mL) *C. procera* exposed (Table 3) and 82% ($LC_{50}= 0.38$ mg/mL) when the two plants synergistically combined after 24 h (Table 4). Ethanol extracts *P. dodecandra* killed

more of the exposed *An. gambiae* larvae than water extracts and the recorded mortalities due to exposure to the extracts were less than the WHO threshold of $> 80\%$ [48]. Also, another similar study suggested that *An. gambiae* s.s. and *An. arabiensis* were highly susceptible to 0.5% pyriproxyfen granule at very low dosages as suggested by Mbare *et al.* [49]. The combination of the two plants generally shows a synergistic activity (Table 4) against *An. gambiae* complex larvae ($X^2=13.33$). However, the synergistic effect of two insecticide mixtures is infrequently identified [50]. The potential reason for the synergistic effect of the two extracts could be as a result of larval susceptibility and different toxic phytochemical compositions [51]. Thus; the mixtures of these phytochemicals act individually on and disrupt different target sites in the larvae, leading to high mortality (Table 4) which is greater than 80% as suggested by the WHO [48].

A wide range of chemical compounds including cardiac glycosides, flavonoids, phenolic compounds, terpenoids have been previously isolated from *C. procera* [52]. Thus, the observed larvicidal effects of *F. sycomorus* and *C. procera* may also be attributed to these phytochemicals. The saponins from ethyl acetate extract of *Achyranthes aspera* was found effective against the larvae of *Aedes aegypti* and *Culex quinquefasciatus* with LC_{50} value of 18.20 and 27.24 ppm, respectively [53]. The tannins, alkaloids, steroids, glycosides, triterpenoids and saponins have also been reported to be responsible for larval toxicity of *A. aegypti*, *C. quinquefasciatus* and *Anopheles* species [54, 55]. The alkaloids extracted from papaya peels and seeds have been reported to have lethal effect on mosquitoes larvae [56].

The alkaloids are nitrogenous compounds that show insecticidal properties at low concentration and the mode of action on insect vectors varies with the structure of their

molecules, but many are reported to affect acetylcholinesterase (AChE) or sodium channels as inhibition of acetylcholinesterase activity is responsible for terminating the nerve impulse transmission through synaptic pathway [57]. The class includes *sabadilla* obtained from *Schoenocaulon officinale* seeds, whose mode of action is similar to that of the pyrethrins. Nicotine, nornicotine and anabasine, are synaptic poisons that mimic the neurotransmitter acetylcholine. They cause symptoms of poisoning to the insects similar to organophosphate and carbamate insecticides [58]. Liu *et al.* [59] suggested alkaloids among the active plant metabolites to be toxic to mosquito larvae. The alkaloids work by constricting blood vessels and depressing autonomic nervous system activity, thereby contributing to the insecticide's effectiveness in killing the larvae of mosquitoes and disrupting the life cycle of the mosquito [60]. Similarly, the alkaloids reported to be present in the latex of *C. procera* have been shown to contain insecticidal properties [23]. Thus, the *An. gambiae* larval mortality observed in this study may have been contributed largely by alkaloids detected in both plants (Figure 1).

Several studies indicated that tannins, another bioactive compound, possess the capacity to bind free protein present in the tubes for larval nutrition that can lead to death [61]. It is also reported to possess insecticidal properties and act as mitochondrial poisons for insect vectors [38]. The finding of acute larvicidal effects of polyphenols against certain larval *Culicidae*, *Chironomidae* and *Simuliidae* has already suggested the prospect of using these polyphenols in dipteran pest control [62, 63]. Mann and Kaufman [38] proven that a typical lipophiles, such as terpenoids and the essential oils passed through the cell wall and cytoplasmic membrane, disrupt the structure of different membrane polysaccharides, fatty

acids and phospholipids and perforated them. Cytotoxicity of these lipophilic compounds appeared to include membrane disruption and eventual death of the insects. Also, plant terpenoids are suggested to possess insecticidal properties (acute toxicity) [38]. The precocenes (terpenoids) specifically, have been reported to be anti-juvenile hormone, accelerating the development of insects and inducing dwarfness associated with low survival rates [5]. Triterpenoids were generally credited with mosquito larvicidal activities according to Gbolade [64]. Phytochemicals that agonize or antagonize the effects of insect development hormones have been reported to be good biopesticides [65]. These compounds disrupt the normal metabolism of the insects' hormones during the development of the juveniles leading to failure of emergence of the adults and lethality [66, 67].

The flavonoid (rotenone) has insecticidal properties acting as a mitochondrial poison, which blocks the electron transport chain and prevents energy production in insects [68]. Other secondary metabolites which have been previously studied and found to have larvicidal activity include saponins [69] and tannins [70] whose presence in the study plants could have contributed to larvicidal activities. Saponins were found to interact with the cuticle membrane in a way causing its disarrangement, which was considered as the most probable reason for larval death [70, 71]. Thus, the presence of alkaloids, saponins, tannins, phenols and flavonoids in all the plants studied and glycosides in *C. procera* only could have contributed to their larvicidal activity observed against *An. gambiae* larvae complex.

Conclusions

Phytochemicals from some tropical plants may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe,

inexpensive, and are readily available throughout the world. Natural insecticides, especially those derived from plants that are more selective, easily degradable, and are more promising in this aspect. The results of this study suggest that the leaf extract of *C. procera*, *F. sycomorus* and their combination are remarkable larvicides against *An. gambiae* larvae due to the presence of some phytochemicals (alkaloids, flavonoids, tannins, phenols, and glycosides). The extract of *F. sycomorus* revealed significant concentration of alkaloids (15.41 mg/mL) compared to *C. procera* (7.7 mg/mL). The larvicidal bioassays after 24 h showed high percentage mortalities of 50.72% ($LC_{50} = 1.01$ mg/mL), 63.10% ($LC_{50} = 0.51$ mg/mL) and 82.33% ($LC_{50} = 0.38$ mg/mL) for *F. sycomorus*, *C. procera* and the synergistic combination ($X^2=13.33$) respectively. These plants may be used as natural biocides for mosquito bio-control. The bioactive phytochemicals from these plants could probably be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes, the malaria vectors. It is evident from this study that these crude extracts have promising individual and synergistic larvicidal bioactivity due to the phytochemicals detected.

Acknowledgements

The authors would like to appreciate the logistic and technical supports granted by *Dr. S.S Ibrahim*, Fellow, Wellcome Trust, UK, LSTM, *Dr. H.M Yakasai*, *Kamaluddeen Babagana* and *Muhd Mahe Mukhtar* of Vector control unit, Department of Biochemistry, BUK. We also appreciated the support granted by *Tetfund*, Nigeria.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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How to cite this manuscript: Babandi Abba*, Anosike Chioma Assumpta, Lawrence US Ezeanyika. Larvicidal and synergistic toxicity of *Ficus sycomorus* and *Calotropis procera* leaf extracts against malarial vector *Anopheles gambiae* complex from Kano-Nigeria: A green bio-control approach. *Asian Journal of Green Chemistry*, 5(3) 2021, 248-262. DOI: 10.22034/ajgc.2021.262853.1290