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Chemical composition and Larvicidal efficacy of *Ficus sycomorus* leaf extract against major malaria vector *Anopheles coluzzii*

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Abstract

Resistance to synthetic insecticides used in control of malaria vectors is a major threat to malaria control globally. Natural insecticides of plant origin provide environmentally safe alternativess. This study characterized the phytochemical contents of the *Ficus sycomorus* leaves and insecticidal activities of its methanolic extract on *Anopheles colluzzii* larvae. The active secondary metabolites from methanol extract of *F. sycomorus* leaves were characterized using gas chromatography - mass spectrometry (GC-MS). A modified WHO standard protocol for larvicidal bioassay was used to determine the activity of methanol extract of the *F. sycomorus*. Molecular species identification showed that 75 % of the larvae were *Anopheles coluzzii* (Coetzee & Wilkerson); 6.25 % *Anopheles arabiensis* and 12.5 % *Anopheles gambiae* s.s. The extract reduced the survival of *An. coluzzii* larvae by approximately 52 % at LC_{50} of 0.225 mg/ml (95% CI: 0.197-0.234; $R^2 = 0.9445$) after 24 h, and the mortality was dose dependent (with highest mortality of 51.67 % at 1 mg/ml). Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phytosterols and phenols, with alkaloid and flavonoids having the highest concentration in the extract. The GC-MS revealed that the extract contained: tetrapentacontane, 66.6 %; stigmasterol, 2.81 %; squalene, 1.01 %; bis(2-ethylhexyl) phthalate, 4.04 %; and 4,8,12,16-tetramethylheptadecan-4-olide, 0.51 %. The toxicity of the extract may be due to different insecticidal phytochemicals, probably working independently, synergistically, or additively. Overall, the *F. sycomorus* leaf extract

is a promising larvicide against malarial vector *An. coluzzii* and should be exploited as an alternative, environmentally safe larvicide.

Key words: *Anopheles coluzzii larvae, botanicals, Ficus sycomorus, GC-MS, insecticide resistance, methanol, extract*

Introduction

The alarming escalation of insecticide resistance in malarial vectors is of major global setback in the successful vector control initiatives (Benelli *et al*., 2018) and eventual malaria eradication. Many research findings confirmed mosquitoes resistant to a class of synthetic insecticides particularly pyrethroids (Ibrahim *et al*., 2016; Babandi *et al*., 2021; Anosike *et al*., 2021) and also other insecticide classes. These resistant results from insecticides target site insensitivity point mutations (Nkya *et al*., 2012), cuticular resistance (Wood *et al*., 2010) and also increased insecticide detoxification capacity of the vector through over-expression of resistant genes (Burton *et al*., 2011; Ibrahim *et al*., 2016). Malaria control programs employed strategies, ranging from simple ecological/environmental/breeding site management strategy, insecticide applications strategy, and complex molecular entomological control approaches. The ecological control management focuses on mosquito's breeding sites eradication, but obviously the mosquitoes can hide and breed in places that cannot be destroyed naturally. Consequently, the control programs are now geared to the larvae control strategy (Mukandiwa *et al*., 2015).

One of the most efficient control strategies to decrease the malarial vector populations is the targeting of the vector larvae stage. Furthermore, the strategic perturbation of physicochemical and biological characteristics of the larval habitat can negatively affect the vectorial capacity and competence of the breeding mosquitoes (Moller-Jacobs *et al*., 2014) and eventually the level of malarial transmission. Presently, the mosquito larvae control depends on chemically synthesized insecticides (Becker *et al*., 2010) such as temophos (organophosphate), bacterial larvicides and growth regulators. However, indiscriminate use of these larvicides has disrupted natural biological control systems and led to resurgence of mosquitoes and has often resulted in widespread development of resistance (Ranson *et al*., 2010; Jacob *et al*., 2013). For examples mosquito larvae resistant to this chemical temophos, the commonly used synthetic larvicide, have been extensively investigated and reported (Grisales *et al*., 2013).

Plant phytochemicals can be profitable as insecticides in comparison with the synthetic insecticides, because of reduced or no undesirable effects on environment and non-target organisms, as well as their inherent biodegradability (Tehri and Singh, 2015) and availability. Botanical insecticides have been considered as eco-friendly and best alternative to chemical insecticides for the control of mosquitoes, the malarial vectors (Rajeswary *et al*., 2014) and other insect pests. Plant phytochemicals with established insecticidal principles have

been used against the major malaria vector *Anopheles gambiae* (Naqqash *et al*., 2016), in developing countries (Hikal *et al*., 2017; Rajasingh *et al*., 2017). These phytochemicals act on insect cellular components inhibiting enzymes, troubling the insects' digestion, evoking hormonal unbalance, disturbance of membrane potential, modulation of ion channels and receptors, neuronal coordination and signaling pathways resulting in either death or impaired development and fitness of the insects (Cavalcante *et al*., 2006; Mithofer and Boland, 2012).

Ficus sycomorus belongs to the plant family of *Moraceae* (Afaf *et al*., 2015; Sheikha *et al*., 2015; Anosike *et al*., 2021) and has about 40 different genera. The family has been reported to have more than 800 plant species. The common name of the *F. sycomorus* is *Fig* and it grows well in tropical climates. The *F. sycomorus* species are traditionally used as anti-venom against snake bites, while some species have been in use as insecticides (Rhome, 2013; Bhalerao and Sharma, 2014). It is also used to treat different human diseases such as cancer, epilepsy, malaria, other microbial infections and cardiovascular diseases (Afaf *et al*., 2015; Sheikha *et al*., 2015; Babandi *et al*., 2019). In general, the differences in ethno-medicinal use of the species vary geographically and with different local populations (Afaf *et al*., 2015; Sheikha *et al*., 2015; Babandi *et al*., 2019; Anosike *et al*., 2021). The other species of the plant such as *F. benghalensis* and *F. sarmentosa var. henryi* have shown positive larvicidal activity against mosquito larvae (Govindarajan, 2010) and thus, proved insecticidal activities.

High insecticide resistance (Ranson and Lissenden, 2016) as well as environmental and behavioral flexibility of *An. gambiae* mosquitoes is threatening to derail malarial control strategy (Sokhna *et al.,* 2013). These characteristics enable them to circumvent the recent vector control strategies. The extended life-span and human-biting behavior of malarial vectors in Africa is the major explanation for high worlds malaria cases (90%) recorded in the African continent (WHO, 2018). It has been suggested that the burden of this disease can be reduced via careful monitoring, timely execution of proper control tools and management of insecticide resistance in the vector's populations (Ibrahim *et al*., 2016). Therefore, the larval control using plant extracts or phytochemicals as larvicides have potential of suppressing the population of malarial vector and hence the subsequent reduction of African malaria disease cases. The development of new, natural, plant-base insecticides is vital in order to counter the emergences of insecticide resistant, long life span and human biting characteristics of the vectors. Despite the various biological properties and many undocumented claims, there is no reported data from northern Nigeria about larvicidal properties of indigenous *F. sycomorus* leaves. This study investigated the chemical composition of the indigenous *F. sycomorus* and evaluate the efficacy of its leaf extract for control of the major malaria vector *An. coluzzii* larvae from Northern Nigeria.

Materials and methods

Anopheles collection, rearing and species identification

The sampling state, Kano is located in the Sudan savannah region of West Africa, about 840 kilometers away from the Sahara Desert. The state has semi-arid savannah vegetation which is flanked by Sahel savannah in the north and the Guinea savannah from the southern part. It has one of the largest irrigation projects in the country (Ibrahim *et al*., 2014). The dams are used for the development of agriculture and provision of foods. The agricultural fields provide suitable breeding places for vectors of diseases such as mosquitoes (Ibrahim *et al*., 2014). The tomatoes, vegetables, and rice paddies have been established along the agricultural irrigation projects and increase the risk of vector-borne diseases such as malaria by providing optimum sites that support vectors growth and development. *Bichi* (12°14′03″N, 8°14′28″E) in Kano have irrigation fields that are mostly used for growing rice and other crops such as green leafy vegetables, tomatoes, pepper and maize, year-round.

The larvae were collected from 37 temporary puddles of water from rainfall in residential sites in *Bichi* (12°14′03″N, 8°14′28″E) in the month of August 2019. Larvae were reared in plastic trays and fed with Tetramin® baby fish food and maintained in the insectary at 28 °C ± 2 ,75% ± 07 relative humidity, and a 12:12 (light: dark) photoperiod. The adults were maintained as above but fed with 10 % sucrose. Morphological identification of the mosquitoes was carried out (Gillies and Coetzee 1987), followed by molecular identification using the SINE200 PCR (Santolamazza *et al*., 2008). The genomic DNA was extracted using the protocol of Livak (1984) and Polymerase Chain Reaction (PCR)-based methods were used for the identification of the member species of *An. gambiae* complex. The *SINE200* primers (SINE200Forward_5'_TCGCCTTAGACCTTGCGTTA_3'and Reverse_5'_CGCTTCAAGAATTCGAGATAC_3') were used to amplify the Short-Interspersed Elements (SINE) from the cDNA of 100 randomly selected individual females. Amplified elements were analyzed by electrophoresis on a 2% ethidium bromide agarose gel and visualized under ultraviolet light. The amplicon size for SINE200 PCR is 479bp for *An. coluzzi*, 240bp for *An. gambiae* s.s and 220bp for *An. arabiensis*.

Ficus sycomorus collection and identification

Ficus sycomorus leaves was collected from botanical garden of Bayero university, old campus (11°98′14″N, 8°48′02″E), Kano-Nigeria. Plant leaves was authenticated by a botanist from the department of Plant Science, Bayero University, and the accession number *BUKHAN 109* was assigned.

Extraction of Ficus sycomorus leaves

Extraction of *F. sycomorus* leaves was made according to method of Gueye *et al*. (2011) with slight modifications. A 600 g of shade-dried *F. sycomorus leaves were weighed using weighing balance (Scout Pro Spu 402 model). It was then* powdered, sieved and extracted with methanol (3L) and re-extracted twice at

room temperature for 4 days, filtered and combined. The combined filtrate was concentrated to dryness by rotary evaporation at 40 °C and freeze-dried at 4°C until used for bioassays.

***Phytochemicals analyses*:**
The crude extract was subjected to both qualitative and quantitative phytochemical analyses (Tiwari *et al.,* 2011).

Test for tannins (Gelatin test)
To one (1) g of crude *F. sycomorus*, 1% gelatin solution containing the 10% sodium chloride (NaCl) was poured in a test tube. Formation of white precipitate indicates the tannins presence (Tiwari *et al.,* 2011).

Test for Phenolic compounds (Ferric chloride test)
To one (1) ml of *F. sycomorus* solution, 2ml of distilled water was added followed by few drops of 10% ferric chloride. Blue or green coloration shows the presence of phenolics in the *F. sycomorus* leaves (Tiwari *et al.,* 2011).

Test for Alkaloids
Five (5) grams of *F. sycomorus* was stirred with 5 ml of 1% aqueous hydrochloric acid (HCl) on water bath and then filtered. One (1) ml of the filtrates was taken in test tubes and used for the test of alkaloids (Tiwari *et al.,* 2011).

Dragendroff's test: To 1 ml of the filtrate, 1 ml of Dragendroff's reagent (potassium bismuth iodide solution) was added. The appearance of orange-red precipitate indicated the presence of alkaloids in *F. sycomorus* leaves.

Wagner's test: To 1 ml of the filtrate, 2 ml of Wagner 's reagent (iodine in potassium iodide) was added. A reddish-brown precipitate indicates alkaloids are presence (Tiwari *et al.,* 2011).

Test for Phytosterols (Salkowski's test)
One (1 g) gram of *F. sycomorus* was dissolved in 10 ml of chloroform and filtered. The filtrate was treated with few drops of concentrated sulphuric acid (H_2SO_4), the mixture was shaken and allowed to stand. Golden-yellow colour appearance indicates the presence of triterpenes in the *F. sycomorus* leaves (Tiwari *et al.,* 2011).

Test for Flavonoids (Lead acetate test)
Few (3-4) drops of lead acetate solution were added to a test tube containing *F. sycomorus* extract. A yellow precipitate indicates the presence of flavonoids in the extract (Tiwari *et al.,* 2011).

Test for Saponins (Froth test)
One (1 g) of *F. sycomorus* was diluted with distilled water to 20 ml in graduated cylinder and this was shaken vigorously for a period of 15 minutes. Appearance

of about one (1 cm) centimeter layer of foam in the cylinder shows the presence of saponins in the extract (Tiwari *et al.,* 2011).

Larval rearing and bioassays

The WHO standard procedure (2005) with modification was used to determine the toxicity of the crude plant extract and fractions against *An. coluzzii* 4th instar larvae. Larvae were maintained in the insectary with a mean room temperature of 27 ±2 °C and a relative humidity of 70-80%. The stock solutions (1%) of the extract were prepared using acetone by dissolving appropriate amount (1g in 100ml of solvent) of *F. sycomorus* extract. From the stock solution, various concentrations ranging from 0.1-1.0 mg/mL of crude *F. sycomorus* leaves were made. 1 ml of DSMO/Acetone in 99 ml of distilled water was used as negative control while temophos served as standard positive control. Twenty-five (25) 4th instar larvae were released into each cup containing 100 ml of test solution and mortality were recorded after 24h post-exposure. No food was provided either to the tests or controls during the experiments. The lethal concentration (LC_{50}) was calculated using probit analysis. Where mortality is up to 5% in the control; Abbott formula was used for correction. The percentage mortalities after the extract exposure were calculated using WHO (2005) formula:

Percentage test mortality (%) = (number of larvae dead / total number of larvae used) ×100----------(1)

Corrected mortality (%) = [(% test mortality - %control mortality) / (100-control mortality)] ×100 ---(2) (Abbott, 1925).

GC-MS analysis of the most active fraction

The GC-MS analysis was carried out to identify the chemical constituents of the effective plant fractions and methanol extract (Ferrer and Thurman 2003). The compounds were characterized and quantified based on GC retention time on the capillary column compared to reference compounds. The name, molecular weight, and structure of the components of the test material were ascertained by correlating with the EI-MS library of NIST/EPA/NIH. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas (NIST 2005; Rautela *et al*., 2018).

The active fraction of *F. sycomorus* analysis was done using a GC-MS (model: QP2010 PLUS SHIMADZU JAPAN) equipped with GC-2010 capillary column with Viscosity Comp Time: 0.2 sec, the pumping time: 5 sec, Injection. Port Dwell Time: 0.3 sec, Washing volume: 8 uL, Column oven temperature: 100.0°C, Injection Temp: 275.00°C, Flow control Mode: Linear velocity, Pressure: 100.0 KPa, Total flow: 50.0 mL/min, Column flow; 1.33 mL/min, Linear velocity: 43.0 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 1.0. The oven temperature was set between 1000°C - 280.0°C, Hold time between 4.00-20.00 min at a rate of 10.00. The equilibrium time was 3.0 min, Ion source temperature: 230.00 °C, Interface temp: 250.00°C, Solvent

Cut Time: 2 mins, Threshold: 1000, Start time: 3.00 min, End time: 28.00 min, Event Time: 0.50 sec, Scan speed: 1250, Sample Inlet Unit: GC.

Statistical analyses

Statistical analysis of all mortality data of larvicidal activity was subjected to probit analysis (Finney 1971) to determine lethal dosages causing 50 % (LD_{50}) mortality of larvae 24 h post exposure, and other statistics at 95% confidence limits (upper confidence limit (UCL) and lower confidence limit (LCL). The differences were considered as significant at $P \leq 0.05$ level. All analyses were performed using SPSS version 21 and R-software package 2017.

Results

Mosquito species

Mosquitoes were identified as belonging to *An. gambiae* Complex, from their features. The SINE200 PCR revealed that the major larval species were *An. coluzzii* (corresponding to 75 % of all larvae collected), followed by 25 % *An. arabiensis* and 12.5 % *An.gambiae s.s* (Fig 1).

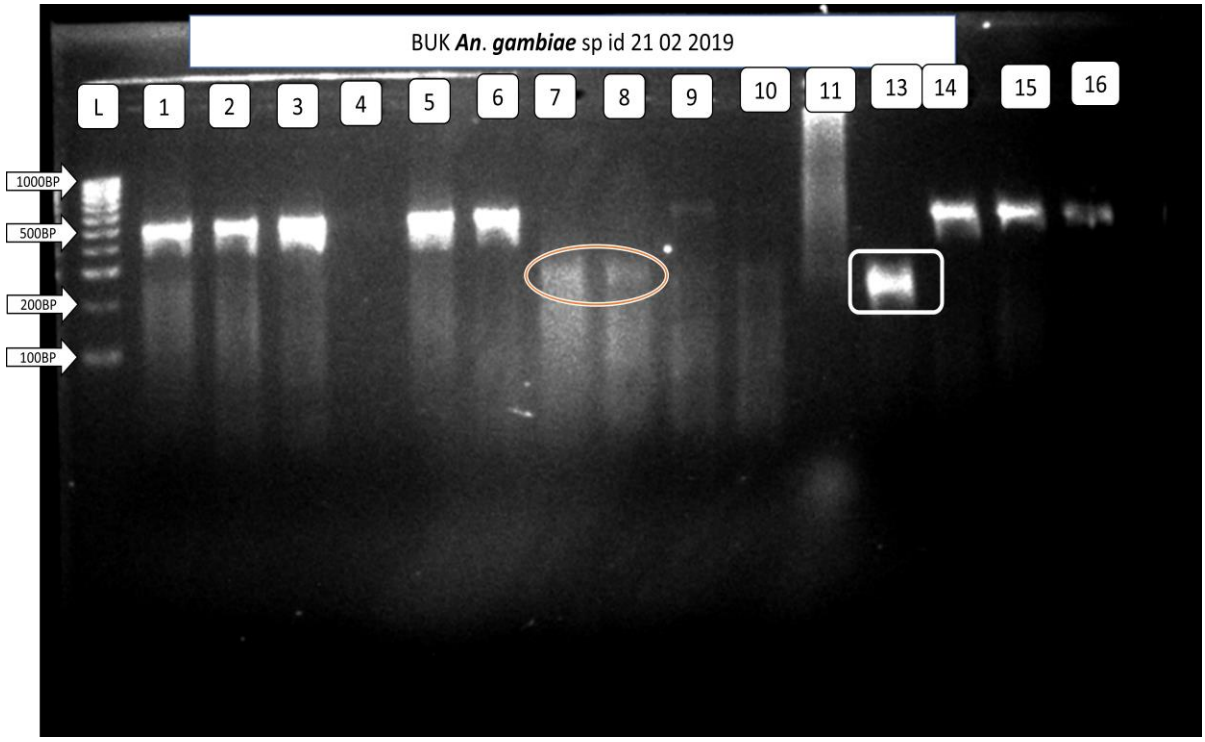

Figure 1: Molecular identification of the Anopheles mosquitoes.

Phytochemical screening

The methanol extraction yielded about 7 g of dark-greenish, sticky extract. Phytochemical screening revealed the presence of different classes of phytochemicals, including alkaloids, flavonoids, tannins, saponins, terpenoids, phytosterols and phenols. Alkaloids and flavonoids had highest concentrations compared to other detected phytochemicals (Fig 2).

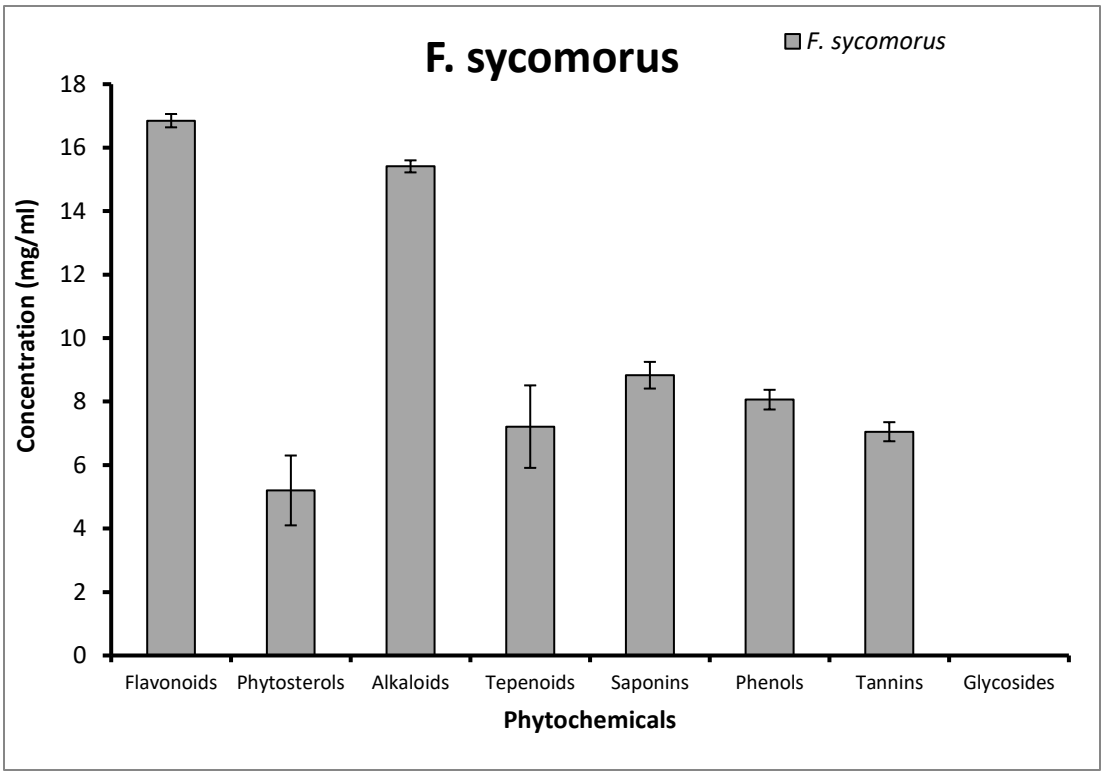

Figure 2: Concentration of phytochemicals in methanol extract of *F. sycomorus*.

Larvicidal activities of F. *sycomorus extract*

The larval mortality rate increases with increased concentrations of *F. sycomorus* extract. The lethal dose (LD_{50}) of the crude extract that kills 50% of the larvae was estimated to be 0.225 mg/ml (95% CI: 0.19-0.23) after 24 h (Fig 3a). The regression analysis of the dose-response curve showed a concentration-dependent significant correlation of the larval mortality with *F. sycomorus* extract (Fig 3b). Strong correlation was observed between the concentrations and the percentage mortality (R^2 = 0.9449), with mortalities increasing with increased extract concentrations (with highest mortality of 51.67% at highest concentration of 1 mg/ml). The positive control temephos killed 100 % of the larvae.

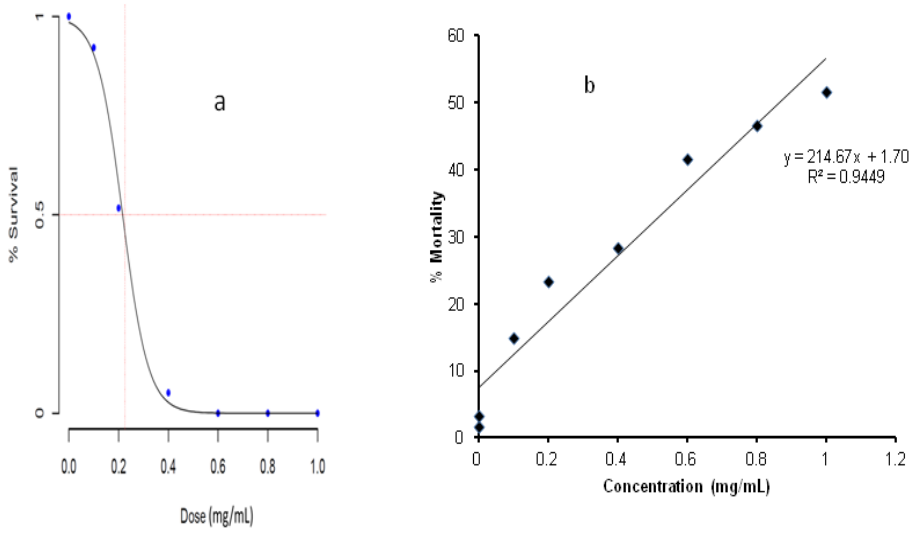

Figure 3: a) The LD_{50} of methanol extract of *F. sycomorus* calculated by Probit analysis, using a Generalized Linear Model (GLM) functions with mass package of R-software (R Core Team, 2017). b) Dose-response curve (Average % mortality ± SD) for different concentration of *F. sycomorus* leaves against *Anopheles coluzzii* from Kano

GC-MS analysis

GC-MS analysis of *F. sycomorus* methanol extract revealed 9-12 active compounds, with tetrapentacontane predominant (66.62 %), followed by tetracontane (15.24 %), bis(2-ethylhexyl) phthalate (4.04 %), sigmasterol (2.81 %), Heptadecane (1.65 %), squalene (1.01 %), Eicosane (0.84 %) and 4,8,12,16-Tetramethylheptadecan-4-olide (0.51 %) (Table 1).

Table 1: GC-MS chemical profile of *Ficus sycomorus* leaves extract

S/N	Chemical Name	Classification	Retention Time	Molecular formula	Molecular weight	% Area
1	Dodecane, 4,6-dimethyl-	Alkane	6.930	$C_{14}H_{30}$	198	1.29
2	Heptadecane	Alkane	9.736	$C_{17}H_{36}$	240	1.65
3	Eicosane		14.137	$C_{20}H_{42}$	286	0.84
4	4,8,12,16-Tetramethylheptadecan-4-olide	-	28.786	$C_{21}H_{40}O_2$	324	0.51
5	Bis(2-ethylhexyl) phthalate	-	31.220	$C_{24}H_{38}O_4$	390	4.04
6	Tetracontane	Alkane	32.747	$C_{40}H_{82}$	562	1.28
7	Tetracontane	Alkane	33.972			0.92
8	Squalene	Terpenoids/alkene	34.512	$C_{30}H_{50}$	410	1.01
9	Tetrapentacontane		35.441	$C_{54}H_{110}$	758	66.62
10	Tetracontane	Alkane	39.069	$C_{40}H_{82}$	562	15.24
11	Sigmasterol	Phytoecdysteroid/ triterpene	44.263	$C_{29}H_{48}O$	412	2.81
12	Tetrapentacontane	alkane	44.666	$C_{40}H_{82}$	562	1.96

Phytochemicals detected using GC-MS were compared with National Institute of Standard and Technology (NIST)

Discussion

Molecular analysis revealed *An. coluzzii* as the major malaria vector in the population collected, followed by *An. gambie* s.s. Only small percentage of the larval population were *An. arabiensis*. Indeed, several studies have shown that in the last few years *An. coluzzii* is fast becoming a dominant malaria vector in the Sudan and the Sahel savannah regions of northern Nigeria (Ibrahim *et al*., 2014; Ibrahim *et al*., 2016; Anosike *et al*., 2021), as well as even in southern Nigeria (Babandi *et al*., 2021; Muhammad *et al*., 2021). This is possibly due to the ability of this species to exploit sites with anthropogenic activities, such as irrigation sites as explained in several studies (Ibrahim *et al*., 2014).

This study showed the presence of various secondary metabolites at different concentrations in *F. sycomorus* leaves. In the present study, the methanol extract of *F. sycomorus* was found to have larvicidal activity probably due to the presence of higher amount of bioactive phytocompounds (e.g., alkaloids, flavonoids, phenols, tannins) that might be responsible for mosquitocidal activity against immature stages of the vectors (Rajasingh *et al*., 2017; Kilickaya *et al*., 2019). The extract showed larvicidal activities in concentration dependent manner. Previous studies by Jang *et al*. (2002) and Cavalcanti *et al*. (2004) suggested that crude or partially purified fractions are highly efficacious, less expensive and eco-friendly for mosquitoes' control than single or pure compounds. Plant extracts are a good alternative to chemical insecticides in the control of the mosquito species at the local community level in many parts of the world (Hikal *et al*., 2017; Rajasingh *et al*., 2017). A study on the anti-plasmodial and phytochemical analysis of fruit extract from *F. sycomorus* (Babandi *et al*., 2019) has established presence of phytochemicals with saponins absent in the fruit extract of the plant. This is contrary to leaves of the plant which contain saponins but showed absence of glycosides.

Several studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae and perform their action by attacking the cuticular membrane of the larvae, eventually disturbing the membrane, which is the main cause for larval death (Hostettmann and Marston, 2005). Thus, the larvicidal activities observed in this study may be due to complex mixtures of phytochemicals in *F. sycomorus* methanol extract which affect different targets in the larvae, inducing larval mortality.Various phytochemicals were reported to have insecticidal properties. Alkaloids form DNA adduct (Babandi *et al*., 2021), saponins interfere with protein synthesis, disrupt membrane and proton motive force or inhibits enzymes, decrease nutrients absorption and modulate gene expression (Bagavan *et al*., 2008; Kotzekidou *et al*., 2008). Flavonoids were reported as insecticides (Kotkar *et al.,* 2002; Anosike *et al*., 2020; Babandi *et al*., 2021). Tannins were implicated in insect feeding deterrence forming enzyme-tannin complex making it difficult during digestion (Cavalcante *et al*., 2006; Barbehenn and Constabel, 2011). It is also suggested that tannins are highly susceptible to molecular oxidation at a pH of the insect guts leading to

production of reactive oxygen species (ROS) and semi-quinones radicals, and initiate toxicity via lipid peroxidation mechanism (Barbehenn and Constabel, 2011). This concurrently decreases the development and the overall larval survival rate (Procopio *et al*., 2015). Polyphenols has proapoptotic and pro-oxidants activities and can precipitate insects' proteins (Frazier *et al*., 2010). Timmel and colleagues (2013) also suggested that these compounds can bind to DNA/RNA increasing the activity of topoisomerase II and eventually inducing DNA breakage and decrease cellular survival and thus, increases mortality. The terpenoids binds to cell membrane components leading to calcium ion channels modifications (Svoboda and Hampson, 1999). Reports have indicated that plant monoterpenoids cause insect mortality by inhibiting the enzymes, acetylcholinesterase activity (Houghton *et al*., 2006).

Lugunes (1994) stated that plant extracts are promising insecticides when they cause mortality equal to or greater than 40 %. Thus, *F. sycomorus* methanol extract exposure has mortality of 51.67 % mortality at 1 mg/ml after 24 h. The LC_{50} of *Ficus benghalensis* methanol extract against *Anopheles stephensis* was 89.55 ppm (Govindarajan, 2010) which is higher than what was obtained in this study (0.225 mg/mL). Insecticidal effect of *Moringa oleifera* extract on termites has been reported by Paiva *et al.* (2010) and Muhammad (2012). The *An. arabiensis* mortality after exposure to *Ocimum basillicum* was reported to be exposure time and concentration dependent (Mahmoud *et al*. 2017). This study is in consonance with these findings were *F. sycomorus* methanolic extract was found to have insecticidal effect against *Anopheles coluzzii* mosquitoes in concentration dependent manner (Fig. 3).

The GC-MS peaks of crude *F. sycomorus* extract revealed the presence of alcohol, alkanes, aldehydes and terpenoids. Many of the chemical components present in the promising extracts were known to be mosquitocidal agents (Shaalan *et al*., 2005; Govindarajan, 2010; Rajasingh *et al*., 2017; Kilickaya *et al*., 2019; Anosike *et al*., 2021) and other biological activities which strongly support the larvicidal properties illustrated by the results of this study. The analysis revealed the presence of 12 active compounds. Compounds such as β-sitosterol, stigmasterol, tannins and hentriacontane have been previously reported in *F. racemosa* bark extract (Ayyanar and Ignacimuthu, 2009). Dias and Moraes (2014) recognized various structural characteristics of chemical compounds that influence larvicidal potential, one of which is lipophilicity of the compound. The hydrophobic properties of chemical compounds are associated with larvicidal activities of the compounds (Scotti *et al*., 2014). Hydrocarbons and terpenoids compounds (e.g., stigmasterol and squalene) detected in this study (Table 2) have lipophilic tendency, thus, the observed larvicidal properties of *F. sycomorus*. In another study, Santos and colleagues (2011) reported some properties linked to enhanced or decreased larvicidal actions of monoterpenes. They observed that presence of heteroatoms in the structure of the hydrocarbon majorly decrease larviciding potential of monoterpenes. Also, a conjugated and exo-

double bond(s) in the chemical structure increases the larvicidal activities. Substitution of double bond in the structure with reactive epoxides decreases the efficacy in larvicidal properties. These chemical characteristics were found with the detected compounds in the methanol extract of *F. sycomorus* and may be responsible for the observed lethality towards larvae.

The GC-MS analyses of the *F. sycomorus* methanol extract indicated the presence of polar (e.g., stigmasterol) and nonpolar (e.g., squalene) terpenes, which were also reported for larvicidal activities. This could probably be proposed as mechanism of *F. sycomorus* leaves extract larviciding i.e. enhancing the membrane fluidity of cuticular resistance strain of *An. coluzzii* by stigmasterol and squalene as such ease penetration of other chemical compounds that exert different physiological effects that characteristically killed the larvae (Ahmad *et al.*, 2006; Lin *et al.*, 2012). The steroidal terpenoid ring structures (stigmasterol) strongly meddle with the insects molting process and reproduction capacity of the mosquitoes by mimicking the juvenile hormones and/or inhibiting synthesis of other developmental hormones (Wang *et al.,* 2014). Stigmasterol acetate, another compound has been suggested to have mosquito repellent property (Chogo and Crank, 1981). Ihsan and colleaques (2014) have suggested the dose-dependent insecticidal behavior of Pthalic acid against *G. mellonella*. This compound and its esters might have originated from the chemical fertilizers and pesticides used by the farmers in the sampling sites (Wang *et al.*, 2013). It may also be partly accountable for the observed insecticidal property of the *F. sycomorus* plant. To the best of our knowledge, this is the first report of a compound, 4, 8, 12, 16-tetramethylheptadecan-4-olide in the genus *Ficus* (Table 2). These compounds have been discovered by GC-MS analysis in this study and most of them are physiologically beneficial and harmless to mankind and the environment.

Conclusion

Malaria vector control targeting larvae can contribute to reduce malaria burden and indices in Nigeria and Africa. The *F. sycomorus* crude extract showed larvicidal activity against natural populations of the major malaria vector *An. coluzzii*. Various phytochemicals detected as well as GC-MS revealed compounds may be responsible for the bioactivities. The insecticidal activity of the plant may be because of synergistic interactions among different biologically active components such as terpenoids, alkaloids, flavonoids, saponins and polyphenolics. The terpenoids may act to reduce cuticular type resistance by increasing membrane permeability enhancing the penetration of the extract in the field-collected populations of the mosquito larvae exposed to the extract and thus other phytochemicals exerted their biochemical effects at different target sites leading to eventual death of the larvae. Overall, the *F. sycomorus* is a promising source of larvicides against *An. coluzzii* larvae, and should be investigated further for use as eco-friendly, larviciding agent.

Conflict of interest
The authors declared no conflict of interest.

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