

Larvicidal potential of some plants from West Africa against *Culex quinquefasciatus* (Say) and *Anopheles gambiae* Giles (Diptera: Culicidae)

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ABSTRACT

Background & objectives: Mosquitoes increased resistance to insecticides, and environmental concerns about the use of insecticides, pose a major challenge in the search for new molecules to deplete and incapacitate mosquito populations. Plants are the valuable source as practices consisting in exploiting plant materials as repellents, and are still in wide use throughout developing countries. The aim of the present study was to screen plants from Côte d'Ivoire for larvicidal activity against mosquitoes.

Methods: Resistant and sensitive larvae (III and IV instar) of *Anopheles gambiae* and *Culex quinquefasciatus* were exposed to crude ethanol extracts (90%) of 45 plants and viability observed after 30 min, 6, 12 and 24 h post-incubation. After partition of active extracts, each fraction (hexane and chloroform washed with NaCl 1%, tannins and aqueous) was tested using the same protocol at various concentrations (1000–31.2 ppm).

Results: Of 49 extracts tested, 7 exhibited high potential ($LC_{50} = 80$ to 370 ppm) against resistant and sensitive III and IV instar larvae of *An. gambiae* and *Cx. quinquefasciatus*. These extracts were from *Cissus populnea*, *Cochlospermum planchonii*, *Heliotropium indicum*, *Phyllanthus amarus*, *Vitex grandifolia* and *Alchornea cordifolia*. However, three most active plant species ($LC_{50} = 80$ –180 ppm) were *Cs. populnea*, *Cm. planchonii* and *P. amarus*. Their hexane and chloroform fractions showed high larvicidal activity.

Conclusion: This study demonstrated that plants from Côte d'Ivoire have a real potential for malaria, yellow fever, filarial and dengue vector control. Those could be used as sources or provide lead compounds for the development of safe plant-based biocides.

Key words *Anopheles gambiae*; *Culex quinquefasciatus*; larvicidal activity; plants; phytochemistry; West Africa

INTRODUCTION

More than two billion people, mostly in tropical countries are at risk from mosquito-borne diseases such as malaria, dengue, haemorrhagic fever and filariasis¹. These infectious diseases mainly impact the tropic's poorest people. An estimated 50 million people are infected with dengue each year². Malaria has a crippling effect on Africa's economic growth and perpetuates vicious cycles of poverty³. Approximately 300–500 million clinical cases and >1 million deaths are recorded every year⁴.

The responsible pathogens are transmitted by bites of blood sucking mosquitoes which are considered to be harmful towards the populations in tropical and subtropical regions⁵. The genera *Culex*, *Aedes* and *Anopheles* are the most important vectors involved in diseases transmission to humans.

Although there are proven strategies to control mosquito-borne diseases, mosquitoes still cause a huge public health problem in Africa. Across African people are

exposed to mosquito bites because the larval habitats are widely distributed in humid areas such as flood areas and rice farms. These sites with larvae might be altered to decrease the mosquito population for the interruption of disease transmission. One of the strategies recommended by WHO is the use of organochlorines (DDT, endosulfan), organophosphates (parathion, temephos) and carbamates. However, these chemical interventions are severely compromised by the development of insecticide resistance in some mosquito vectors and environmental concerns^{6–8}. Also in many African countries the most widely tested interventions based on bednets treated with pyrethroid, have been difficult to implement correctly because of problems related to cost and acceptability⁸.

This situation highlights the need to search for new efficient products with fewer effects on environment⁹. Recently the environmentally safe and biodegradable, natural products of plants have been considered as alternative sources in the control of insects of public health importance¹⁰. Natural products contain a range of bioactive com-

Table 1. Plant species selected for larvicidal screening

| Voucher No. | Plant species | Common names (English) | Families | Tested organ |
|-------------|---|---|------------------|------------------------|
| 6470 | <i>Acacia flava</i> Forsk. | Flood-plain acacia | Mimosaceae | Leaves |
| 2308295 | <i>Acacia nilotica</i> L. | Babul acacia | Mimosaceae | Leaves |
| 2308811 | <i>Acacia polyacantha</i> Wild | Catechu tree | Mimosaceae | Stem bark |
| 5867 | <i>Aframomum spectrum</i> Oliv. Hanb | Bear berry | Zingiberaceae | Leaves |
| 8429 | <i>Azelia africana</i> Sn et Perr | Apa, Pod mahogany | Caesalpinaceae | Leaves |
| 19839 | <i>Alchornea cordifolia</i> Muell. Arg | Christmas bush, dovewood | Euphorbiaceae | Leaves |
| 16004 | <i>Allophylus africanus</i> Beauv. | African false currant, African Allophylus | Sapindaceae | Roots |
| 2309690 | <i>Andira inermis</i> Kunth ex DC | Angelin, Dog almond, Bastard mahogany | Fabaceae | Leaves |
| 6138 | <i>Apodostigma pallens</i> Planch. ex Oliv. | Not found | Hyppochrateaceae | Leaves and stem |
| 4650 | <i>Baissea multiflora</i> A. DC | Not found | Apocynaceae | Roots |
| 2308107 | <i>Bobgunnia madagascariensis</i> (Desv.) J.H. Kirkbr & Wiersema | Snake-bean tree | Caesalpinaceae | Roots |
| 19945 | <i>Bridelia ferruginea</i> Benth | Ira | Euphorbiaceae | Roots |
| 2288053 | <i>Cissus populnea</i> Guill. & Perr | Food gum | Vitaceae | Roots |
| 18546 | <i>Cochlospermum planchonii</i> Hook ex Planch | False cotton, Cotton plant | Cochlospermaceae | Roots |
| 2288334 | <i>Cola cordifolia</i> R. Br. | Mandingo kola | Sterculiaceae | Bark |
| 11772 | <i>Combretum molle</i> R. Br ex Don | Velvet-leaved combretum | Combretaceae | Roots, leaves and stem |
| 113612 | <i>Daniellia oliveri</i> Hutch et Dalz | West African copal, African copaiba, balsam tree, nigercopal, maaje | Caesalpinaceae | Young leaves |
| 115451 | <i>Eleusine indica</i> L. | Goose grass, Bermuda grass, wiregrass, fowl foot | Poaceae | Leaves |
| 66252 | <i>Entada africana</i> Guill et Perr | Entada | Mimosaceae | Stem bark |
| 68854 | <i>Erythrina senegalensis</i> DC | Senegal coral tree, Parrot tree, coral tree | Fabaceae | Roots |
| 2314388 | <i>Fadogia erythrophloea</i> Hutch & Dalziel | Not found | Rubiaceae | Leaves |
| 39365 | <i>Ficus congensis</i> Engl | Swamp or hippo fig | Moraceae | Stem bark |
| 2308048 | <i>Heliotropium indicum</i> L. | Indian heliotrope, Heliotrope, cock's comb | Boraginaceae | Leaves |
| 2309989 | <i>Jatropha curcas</i> L. | Jatropha, Physic nu | Euphorbiaceae | Leaves |
| 2303193 | <i>Keetia hispida</i> (Benth.) Bridson | | Rubiaceae | Leaves and stem |
| 16507 | <i>Khaya senegalensis</i> Desr. A. Juss | Dry-zone mahogany | Meliaceae | Stem bark |
| 2309941 | <i>Kigelia africana</i> Lam. Benth | Sausage tree | Bignoniaceae | Roots |
| 2316413 | <i>Landolphia owariensis</i> Smith | White-ball rubber, Vine rubber, rubber vine, ciwo | Apocynaceae | Leaves |
| 113251 | <i>Leptadenia pyrotechica</i> L. | Leptadenia | Asclepiadaceae | Leaves |
| 1774 | <i>Lonchocarpus cyanescens</i> (Schum. & Thonn.) Benth. | West African indigo | Fabaceae | Leaves |
| 63592 | <i>Lophira lanceolata</i> Van Tiegh | Dwarf red ironwood, Ironwood, ekki, meni oil tree, nambanchi | Ochnaceae | Bark |
| 2313051 | <i>Mimusops kummel</i> Bruce | Red milkwood, Bullet wood | Sapotaceae | Roots |
| 115154 | <i>Parkia biglobosa</i> Jacq R. Br | West African locust bean, Dadawa tree | Mimosaceae | Roots and stem bark |
| 2177601 | <i>Phyllanthus amarus</i> Schumach & Thonn | Black catnip, Phyllanthus, amarus plant | Euphorbiaceae | Whole plant |
| 8693 | <i>Phyllanthus muellerianus</i> Kuntze | Myrobalan | Euphorbiaceae | Leaves |
| 2177703 | <i>Premna lucens</i> A. Chev | Not found | Verbenaceae | Roots |
| 2291444 | <i>Pseudocedrela kostchyi</i> Harms | Dry-zone cedar | Meliaceae | Roots |
| 113985 | <i>Sclerocarya birrea</i> A. Rich | Marula | Anacardiaceae | Roots |
| 70889 | <i>Securidaca longepedunculata</i> Fres | Violet-tree | Polygalaceae | Roots |
| 2308860 | <i>Syzygium guineense</i> Willd DC | Water berry, Water Pear | Myrtaceae | Stem bark |
| 2308288 | <i>Tapinanthus dodoneifolius</i> DC | Not found | Loranthaceae | Leaves |
| 20656 | <i>Uapaca togoensis</i> Pax | Charcoal, somon | Euphorbiaceae | Leaves and stem bark |
| 19621 | <i>Vernonia guineensis</i> Benth | Guinean ginseng | Asteraceae | Leaves |
| 2316528 | <i>Vitex grandifolia</i> Gürke | Black plum, Chocolate berry tree | Verbenaceae | Leaves and stem bark |
| 2293337 | <i>Ximenia americana</i> Wild | False sandalwood, Blue Sourplum | Olacaceae | Roots |

pounds⁶ and related commercial insecticides are commonly perceived as “safe” in comparison to synthetic repellents¹⁰. Traditionally plant based repellents have been used for generations as protection measures against mosquitoes. These are still extensively used throughout rural communities in Benin¹¹, Tanzania¹² and Côte d’Ivoire. These plants are burned overnight in rooms to drive away nuisance mosquitoes. Some of these African plants have been shown to be larvicides^{13, 14}.

The present study investigated 45 plants from West Africa for larvicidal activity against mosquitoes as safer natural alternatives to synthetic molecules. Most of the selected plants have been used for medicinal purposes for a long time, because these are not harmful to either humans or domestic animals.

MATERIAL & METHODS

Preparation of extracts

The plant species studied were selected on the basis of criteria (Table 1), such as lack of information on activity against *Anopheles* and *Culex* larvae, botanical families (Euphorbiaceae, Verbenaceae, and Meliaceae) from which number of larvicidal plant were reported and large distribution in West Africa. Voucher specimens are deposited at the herbarium (Base ivoire) of Centre Suisse de Recherches Scientifiques en Côte d’Ivoire, Adiopodoumé.

A quantity of the different plant parts were collected from April to October 2005 in the region of Ferkessedougou (northern Côte d’Ivoire), located in the Savanna area (9–11°N, 4–7°W) of the Côte d’Ivoire. The roots, leaves and stem bark were dried in an air-conditioned room (22°C) and pounded by hand in a mortar. A quantity of 10 g of the powder was extracted with 100 ml of 90% ethanol under mechanical stirring (150 rpm) during 24 h and then filtered. The extracts were concentrated in a rotary evaporator (Rotavapor) at 40°C and lyophilized. In all, 49 extracts have been prepared for *in vitro* larvicidal screening.

Mosquito larvae tested

The larvae included wild resistant *An. gambiae* strains, resistant *Cx. quinquefasciatus* strains, and sensitive Kisumu strain (from Kenya). The resistant strains were collected from breeding sites around the village of Adiopodoumé, located in the northern peri-urban part of Abidjan. These sites were selected because of their proximity to Centre Suisse de Recherches Scientifiques en Côte d’Ivoire (CSRS). Breeding sites located in this village were around crop farms. After collection, the III and

IV instar larvae were transferred in plastic bottles and maintained at the laboratory.

The susceptible strain (Kisumu) was provided by the insectarium of CSRS. The eggs were put in distilled water maintained at 21–22°C and safe from contaminations. Eggs hatched after 24 h and larvae were fed with powdered cat kibble.

Larval bioassays

Larvicidal activity was assessed as per the protocol previously described by WHO with slight modification¹⁵. The assays were performed in two steps: (i) Detection of susceptibility of larvae to extracts; and (ii) Determination of larvicidal concentration (LC₉₅). The sensitivity of the larvae to the extracts was determined at single concentration (1000 ppm). In 220 µl of distilled water or dimethylsulfoxide (DMSO) 220 mg of extract was dissolved. Then 100 µl of extract solution was added to 5 ml of water from breeding site (wild strain) or distilled water (Kisumu strain). The final volume was adjusted to 10 ml and 20 larvae were added to each tube. A control tube containing only distilled water or 0.1% DMSO was prepared. Mortality is assessed by direct observation of larvae movements. An extract is active if 100% of larvae died between 30 min and 24 h¹⁶. The tests were repeated three times.

The extracts showing larvicidal activity at 1000 ppm were further diluted from 1000 to 31.2 ppm. The viability of larvae was observed after 30 min, 6, 12 and 24 h and scored according to larvae movements and physiological state: 0 = Dead larvae; 1 = Low or almost absence of movement; 2 = Activity; and 3 = Hyperactivity. The number of dead larvae was counted to determine the mortality rate and monitored for determination of KT₅₀, the time required to kill 50% of the larvae.

Partition of active extracts

The three most active extracts were subjected to a liquid-liquid partition with different solvents of increasing polarity. In whole 10 g of plant powder was extracted with ethanol 90% using 10-fold solvent under mechanical stirring (150 rpm) during 14 h. The filtrate was successively partitioned with hexane, chloroform and water. The chloroform fraction was washed with NaCl 1% (1 g/100 ml water) in order to remove tannins. All fractions were evaporated in a rotary evaporator to dryness at 40°C and lyophilized.

Larvicidal test with fractions prepared from active extracts

The fractions obtained from active extracts were tested

against III and IV instar larvae of *An. gambiae* and *Cx. quinquefasciatus* where 11 mg of each fraction was dissolved in 110 µl of DMSO. The test was performed as mentioned above. Mortality was assessed visually by direct observation of larvae movements. A fraction is active if 100% of larvae died between 30 min and 24 h of exposure.

TLC phytochemical analysis

Plant extracts (hexane, and chloroform) showing larvicidal activity were investigated by thin layer chromatography (TLC). TLC plates were prepared from 10 µl of extract solution (10 mg/ml in methanol) on silicagel 60 F₂₅₄ plates (aluminum), developed in hexane-ethyl acetate (1:1) as mobile phase. After drying, the chromatograms were analyzed at 254 and 366 nm, pre- and post-spraying with specific reagents according to the nature of chemi-

cals¹⁷⁻¹⁹. The retention factor (Rf) values were calculated, using the following formula:

$$R_f = \frac{\text{Distance moved by the compound}}{\text{Distance moved by the solvent front}}$$

RESULTS

In this study, we investigated the larvicidal activities of 45 plants, traditionally used in Côte d'Ivoire. Of the 49 ethanol crude extracts 7 (14.29%) showed high activity against III and IV instar larvae of *Anopheles* and *Culex* at 1000 ppm 24 h post-exposure. These seven extracts were obtained from six plant species: *A. cordifolia*, *P. amarus*, *H. indicum*, *C. populnea*, *V. grandifolia* and *Cm. planchonii*. Six of the extracts had effect on viability of susceptible and resistant larvae of *Anopheles*, resulting

Table 2. Mortality rates of resistant larvae of *Anopheles gambiae* and *Culex quinquefasciatus* in the presence of active plant species

| Mosquito species | Concentrations (ppm) | Mortality ± S.D. | | | | |
|-------------------------------|---------------------------------|------------------|--------------|--------------|---------------|--------------|
| | | 0.5 h | 1 h | 6 h | 12 h | 24 h |
| | Control (DMSO) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| <i>Anopheles gambiae</i> | <i>Phyllanthus amarus</i> | | | | | |
| | 1000 | 28.33 ± 7.64 | 83.33 ± 7.64 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| | 500 | 23.33 ± 7.64 | 70 ± 1 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| | 250 | 16.67 ± 7.67 | 18.33 ± 7.64 | 66.67 ± 7.64 | 100 ± 0 | 100 ± 0 |
| | 125 | 0 ± 0 | 0 ± 0 | 5 ± 0 | 15 ± 0 | 35 ± 0 |
| | 62.5 | 0 ± 0 | 0 ± 0 | 6.67 ± 2.89 | 8.33 ± 1 | 33.33 ± 2.89 |
| | 31.2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| | <i>Cissus populnea</i> | | | | | |
| | 1000 | 33.33 ± 7.64 | 78 ± 7.64 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| | 500 | 28.33 ± 7.64 | 73 ± 1.00 | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| | 250 | 0 ± 0 | 11.67 ± 2.89 | 11.67 ± 2.89 | 81.67 ± 10.41 | 100 ± 0 |
| | 125 | 0 ± 0 | 0 ± 0.00 | 1.67 ± 2.89 | 3.33 ± 5.77 | 6.67 ± 7.64 |
| | 62.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| | 31.2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| | <i>Cochlospermum planchonii</i> | | | | | |
| | 1000 | 0 ± 0 | 0 ± 0 | 11.67 ± 7.64 | 76.67 ± 2.89 | 100 ± 0 |
| | 500 | 0 ± 0 | 0 ± 0 | 8.33 ± 2.89 | 71.67 ± 2.89 | 100 ± 0 |
| | 250 | 0 ± 0 | 0 ± 0 | 3.33 ± 2.89 | 66.67 ± 2.89 | 100 ± 0 |
| | 125 | 0 ± 0 | 0 ± 0 | 3.33 ± 2.89 | 18.33 ± 2.89 | 23.33 ± 2.89 |
| 62.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| 31.2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| <i>Culex quinquefasciatus</i> | <i>Cochlospermum planchonii</i> | | | | | |
| | 1000 | 0 ± 0 | 0 ± 0 | 25 ± 0 | 83.33 ± 7.64 | 100 ± 0 |
| | 500 | 0 ± 0 | 0 ± 0 | 16.67 ± 2.89 | 75 ± 1 | 100 ± 0 |
| | 250 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 20 ± 1 | 25 ± 1 |
| | 125 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| | 62.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| | 31.2 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |

S.D. : Standard deviation.

in death of larvae.

Alchornea cordifolia extract exhibited activity only against Kisumu strain. The extract of *Cm. planchonii* was the only active against larvae of *Culex*. Mere weak or no effect on larvae was observed following exposure to the remaining 42 extracts.

The decrease in viability is more pronounced at high concentrations from 1000 to 250 ppm at all examination points. At the lowest concentrations, no effect on larvae was observed with any of the extracts tested. These results show a dose response activity.

Phyllanthus amarus and *Cs. populnea* caused 100% mortality of resistant larvae of *Anopheles* after 6 h contact (Table 2). The mortality rates range between 6.67 and 100% for *Anopheles* and 25–100% for *Culex*.

The most active extracts causing 100% mortality of larvae were *Cm. planchonii*, *P. amarus* and *Cs. populnea* 24 h post-incubation. For these extracts, the LC₅₀ were 80–180 ppm against *Anopheles* and 370 ppm against *Culex* (Table 3). *Phyllanthus amarus* and *Cs. populnea* killed resistant *An. gambiae*, with KT₅₀ ranged between 41 and 42 min. *Cochlospermum planchonii* caused death of *Anopheles* and *Culex* with KT₅₀ values of 125 and 145 min respectively.

Incubation with extract of *Cm. planchonii* (Table 3) and related fractions (hexane, and chloroform) resulted in death of larvae of both *Anopheles* and *Culex*, at LC₅₀ values ranging between 80 and 370 ppm (Table 4).

Following exposure to *Cs. populnea* extract, sensitive and resistant larvae of *An. gambiae* died at LC₅₀ values of 80 and 180 ppm respectively. Its hexane fraction was more active (LC₅₀ = 180 ppm) than the chloroform fraction, LC₅₀ = 370 ppm (Table 4). The TLC phytochemical analysis revealed at least trace amount of monoterpenoids, polyphenols and alkaloids (Table 5).

In this study *P. amarus* exhibited high larvicidal potential against *An. gambiae* (LC₅₀ = 80–180 ppm). Incubation with derivatives (hexane and chloroform) caused death of larvae at LC₅₀ = 180–370 ppm between 12–24 h (Table 4). No effect was observed with aqueous and tannin fractions. Preliminary phytochemical studies have shown presence of monoterpenoids, flavonoids, anthrones and anthraquinones (Table 5).

DISCUSSION

Plant phytochemicals have more specific effects and could be usefully integrated with other control measures to design comprehensive, appropriate and effective management protocols with less collateral harm to the environment and non-target species²⁰.

Exposures to studied plants resulted in death of susceptible and resistant larvae of *An. gambiae*. For the active plant species, the mortality rates range between 6.67 and 100% for *Anopheles* and 25–100% for *Culex* after 24

Table 3. LC₅₀ and LC₉₅ (ppm) of ethanol extracts of active plant species on III and IV instar larvae of *Anopheles gambiae* and *Culex quinquefasciatus*

| Plant species | Plant parts | <i>Anopheles gambiae</i> | | | | <i>Culex quinquefasciatus</i> | |
|---------------------------------|-------------|--------------------------|------------------|------------------|------------------|-------------------------------|------------------|
| | | Sensitive strain Kisumu | | Resistant strain | | Resistant strain | |
| | | LC ₅₀ | LC ₉₅ | LC ₅₀ | LC ₉₅ | LC ₅₀ | LC ₉₅ |
| <i>Cochlospermum planchonii</i> | Roots | 80 | 22.22 | 180 | 342 | 370 | 703 |
| <i>Phyllanthus amarus</i> | Whole plant | 80 | 22.22 | 180 | 342 | ND | ND |
| <i>Heliotropium indicum</i> | Leaves | 180 | 342 | 370 | 703 | ND | ND |
| <i>Cissus populnea</i> | Roots | 80 | 22.22 | 180 | 342 | ND | ND |
| <i>Vitex grandifolia</i> | Leaves | 180 | 22.22 | 370 | 703 | ND | ND |
| <i>Vitex grandifolia</i> | Stem bark | 180 | 342 | 370 | 703 | ND | ND |

ND = Not determined.

Table 4. LC₉₅ and LC₅₀ (ppm) of chloroform and hexane fractions

| Plant species | Mosquitos species | Hexane fraction | | Chloroform fraction | |
|---------------------------------|-------------------------------|------------------|------------------|---------------------|------------------|
| | | LC ₉₅ | LC ₅₀ | LC ₉₅ | LC ₅₀ |
| <i>Cochlospermum planchonii</i> | <i>Anopheles gambiae</i> | 342 | 180 | 703 | 370 |
| <i>Cochlospermum planchonii</i> | <i>Culex quinquefasciatus</i> | 342 | 180 | 703 | 370 |
| <i>Cissus populnea</i> | <i>Anopheles gambiae</i> | 342 | 180 | 703 | 370 |
| <i>Phyllanthus amarus</i> | <i>Anopheles gambiae</i> | 342 | 180 | 703 | 370 |

Table 5. Possible compounds present in fractions of the most active plants

| Plant fractions | Pre-derivatization | | | | Post-derivatization | | | | | | | | | Possible types of compounds | | | |
|--|----------------------|---------|----------------------------|------------------|---------------------|--------|-----------------|---------|-------------|---------|--------|---------|--------|-----------------------------|----|--|---|
| | Rf | Visible | 254 nm | 366 nm | Godin | | Folin-Ciocalteu | | Dragendorff | | KOH | | | | | | |
| | | | | | Visible | 366 nm | Rf | Visible | Rf | Visible | Rf | Visible | 366 nm | | Rf | | |
| <i>Cissus populnea</i> (Chloroform) | 0.65 | Visible | Blue | | Blue | 0.53 | | | | | | | | | | | ND Monoterpenoids Monoterpenoids Polyphenols Anthrones |
| <i>Cissus populnea</i> (Hexane) | 0.65 | Visible | Blue | | Violet | 0.8 | | | | | | | Yellow | 0.0 | | | ND Monoterpenoids Monoterpenoids Monoterpenoids Polyphenols Polyphenols Alkaloids |
| <i>Phyllanthus amarus</i> (Chloroform) | 0.65 0.70 0.08 | Visible | Violet Orange Orange | | Blue | 0.56 | | | | | Orange | 0.59 | | | | | ND ND ND Monoterpenoids Polyphenols Anthraquinones Anthrones |
| <i>Phyllanthus amarus</i> (Hexane) | 0.65 | Green | Visible | Orange Orange | Violet | 0.79 | | | | | | | | | | | ND ND Monoterpenoids Flavonoids Flavonoids Polyphenols Polyphenols Polyphenols Anthraquinones |
| | | | | | | Orange | 0.69 | | | | | | | | | | |
| | | | | | | Yellow | 0.56 | | | | | | | | | | |
| | | | | | | | | Blue | 0.80 | | | | | | | | |
| | | | | | | | | Blue | 0.59 | | | | | | | | |
| | | | | | | | | Blue | 0.53 | | | | | | | | |
| | | | | | | | | | | | | | Red | 0.71 | | | |
| | | | | | | | | | | | | | Yellow | 0.0 | | | |

ND : Not determined.

h exposure. In a previous study, *Schinus terebinthifolia* essential oil displayed activity after 72 h, the mean mortality percentage ranged from 0.5 to 96.75% for *Cx. quinquefasciatus* and 13.75 to 97.91% for *An. gambiae*¹².

Cochlospermum planchonii, *Cs. populnea* and *P. amarus* extracts caused cent percent mortality of larvae 24 h post-incubation, with LC₅₀ of 80–180 ppm and LC₉₅ values of 22.22–342 ppm. Other active species such as *A. cordifolia*, *H. indicum* and *V. grandifolia* exhibited activity with LC₅₀ and LC₉₅ values ranging between 180–370 and 342–703 ppm respectively. Following partition of crude extracts and larvicidal assays, only hexane and chloroform fractions exhibited activity against larvae, with LC₅₀ and LC₉₅ values of 180 and 342 ppm respectively for hexane. Chloroform fraction showed LC₅₀ and LC₉₅ values of 370 and 703 ppm respectively. The results revealed that increased larval mortality was observed with increased concentration of the extracts tested against *An. gambiae* and *Cx. quinquefasciatus*. Similar

finding was obtained against *An. stephensi* with the leaf of *Adansonia digitata*⁴. Chloroform extract of the plant showed LC₅₀ and LC₉₀ values of 88.55 and 168.14 ppm respectively, while its hexane extract showed LC₅₀ and LC₉₀ of 111.32 and 178.63 ppm respectively in 24 h. However, in the present study, hexane fractions displayed stronger potential than chloroform fractions against *An. gambiae* and *Cx. quinquefasciatus*.

The KT₅₀ values ranged between 41–125 and 145 min against resistant *An. gambiae* and *Cx. quinquefasciatus* respectively. Previous study demonstrated that the time required to knock down 50% of the wild adult *An. gambiae* in Tanzania was 11.29 min for *S. terebinthifolia* essential oil¹².

The larvicidal activity of some studied plants such as *Cm. planchonii*, *H. indicum* and *A. cordifolia* was reported against *Ae. aegypti*¹⁴. The ethanolic extracts of these species caused death of larvae 30 min and 24 h post-incubation respectively at single concentration tested of

500 µg/ml. The present study gave further data on the potential use of these plants against malaria, yellow fever, filarial and dengue vector control.

The active plants contain phytochemicals such as monoterpenoids and flavonoids. The fresh rhizomes of *Cm. planchonii* yield essential oils, with a high rate of oxygenated compounds (86.4% of ketones and esters)²¹. *Cissus populnea* and *P. amarus* also contained essential oils^{22, 23}. Several authors have demonstrated strong responses of mosquito odour receptors to volatiles produced by plants. Essential oils were found to be larvicidal against *Anopheles* and *Culex*^{17, 24, 25}. The finding of the present study is in line with the high potential of non-polar (dichloromethane, chloroform and hexane) extracts^{4, 19} demonstrated against mosquito larvae.

The selection of plants based on their botanical family or genus can be valuable criteria for identifying high larvicidals. Of the 7 active species, 2 were Euphorbiaceae. Several species of this family were reported to be larvicidal against mosquitos. *Ricinus communis*²⁶, *Acalypha indica*²⁷, and *Acalypha alnifolia*¹ have shown activity against resistant and susceptible *Anopheles* and *Culex* larvae. *Alchornea cordifolia*, *Bridelia aubrevillei* and *B. grandis* caused death of *Ae. aegypti*¹⁸. Thus, Euphorbiaceae is a promising family for vector control.

Vitex grandifolia displayed activity on resistant and susceptible larvae of *Anopheles*; disappointingly in this study, the species lacked activity against *Cx. quinquefasciatus*. Other species of the same genus, *V. trifolia*, *V. peduncularis* and *V. altissima* exhibited activity on IV instar larvae of *Cx. quinquefasciatus*²⁸. The extract of *V. negundo* was repellent against adult mosquitoes²⁹. Therefore, there is no doubt that *Vitex* spp are of great interest in control of mosquitoes.

This is the first hand report of the larvicidal activity of studied plants discussing whether some are well-known for treating malaria. Phytochemical investigations, repellent study and field evaluation are ongoing.

CONCLUSION

In the present study, the larvicidal potential of 45 plants from West Africa was evaluated against sensitive and resistant *An. gambiae* and *Cx. quinquefasciatus*. Some of these plants exhibited high larvicidal activity. The results show that some of plants traditionally used in West Africa could gain place in control of African malaria vectors. The efficacy exhibited by these plants has given an opportunity for further investigation on eggs and adult mosquitoes and to evaluate them in small-scale field trials.

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