WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 1, 151-169 Research Article ISSN 2278 – 4357

IN VITRO EVALUATION OF THE BIOCIDAL EFFECTS OF PLANT EXTRACTS FROM KINSHASA (DR-CONGO) ON MOSQUITO LARVAE

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Article Received on
02 Nov. 2018,
Revised on 23 Nov. 2018,
Accepted on 13 Dec. 2018
DOI: 10.20959/wjpps20191-12901

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ABSTRACT

In the course of our studies on the valorization of plants used by the Nkundo people in Democratic Republic of Congo (DRCongo), we have carried out a preliminary screening of 50 aqueous extracts (at a standard concentration of 1%) from 35 plants for their larvicidal potential on late third or early fourth instar larvae of *Culex quinquefasciatus*. It resulted that 7 ethanol extracts from 4 plants including *Crossopteryx febrifuga* (Cf) root bark, *Penianthus longifolius* root bark, *Piper guineense* fruit, root and stem barks (PG) and *Quassia africana* stem and root bark (QA) were found to be most active with larval mortality rates ranging from 85-100% after 24 h exposure. Piper guinsense leaves produced 58.33±2.89% mortality. In addition to these 4 most active samples, 7 corresponding 80% ethanol extracts were

prepared by maceration. In total, 14 ethanol extracts were tested at 10 concentrations (20 - 0.4 mg/ mL) on late third or early fourth instar larvae of 3 types of larvae [*Aedes aegypti (AA), Anopheles gambiae (AG)* and *Culex quinquefasciatus (CQ)*]. Based on the 24 h LC_{50} values, the ethanol extracts were found more active than their aqueous counterparts; *AG* and *CQ* were more sensitive. Most interesting extracts displayed LC_{50} values < 0.039 mg/mL. They included ethanolic extracts from fruits, root bark and stem bark of *PG* towards both *AG* and *CQ* as well as from root bark and stem bark of *QA* towards *CQ*. In conclusion, the most active extracts may constitute a basis for the production of eco-friendly and biodegradable plant-based insecticides. They can be used solely or in potentially synergistic combinations with other known plant-based insecticides.

KEYWORDS: Nkundo people, larvicidal activity, plant extracts, Bandundu.

1. INTRODUCTION

Mosquitoes (about 3,500 species) are the most important group of bloodsucking arthropods from both medically and ecological perspectives. They cause not only nuisance to humans but in addition they are vectors of pathogens infecting more than 700 million people annually and worldwide through diverse diseases including malaria and others, mostly classified as Neglected Tropical Diseases (NTD), with many socioeconomic consequences (Becker et al. 2010).

The most incriminated mosquito species belong to the genera *Aedes*, *Anopheles* and *Culex*. *Aedes aegypti* Linnaeus (1762) is a day biting mosquito which is the disease vector for many important viral human diseases such as dengue, zika, chikungunya and yellow fever (Gunaratne et al. 2016; Hay et al. 2013; Juliano et al. 2005; Morrison et al. 2008).

At least 8 species belonging to the *Anopheles gambiae* Giles (1902) complex are actually known (Coetzee et al., 2013). In DR Congo, 2 of them are captured i.e. *A. coluzzii* Coetzee & Wilkerson sp. n. and *An. gambiae s.s.* (Bobanga et al. 2016). This complex was recognized in the 1960s and includes the most important vectors of malaria in sub-Saharan Africa, particularly of *Plasmodium falciparum* Welch (1897) malaria (Coetzee et al. 2000).

Culex quinquefasciatus Say (1823), also known as *C. fatigans* Wiedemann (1828) the southern house mosquito, is a medium-sized mosquito found in tropical and subtropical regions of the world. It is the vector of *Wuchereria bancrofti* Cobbold (1887) causing

elephantiasis, of avian malaria *Plasmodium*, and arboviruses including St. Louis encephalitis virus, Western equine encephalitis virus, Zika virus and West Nile virus (Bhattacharya and Basu 2016).

Avoidance of mosquito bites remains the best strategy since no vaccine is yet available for most of the pathologies mentioned above. One of the methods to manage these diseases is to control the vectors in reducing their contact with humans causing an interruption in the disease transmission. The use of synthetic insecticides to control mosquitoes is very common due to their quick action, but their continuous use led to the development of resistance and to permanent residual effects on the non-target organisms, including humans (Shrivastava et al. 2011). In addition to the recent pyrethroid molecules-resistance (Kanza et al. 2013; Pennetier 2008; Sutthanont et al. 2010), all these mentioned factors create the need to develop new easily biodegradable and effective alternative insecticides.

Beside these chemical weapons, there are also biological ones represented by larvivorous fish e.g. *Gambusia affinis* Baird & Girard (1853) or *Poecilia reticulata* Peters (1859), which deserve more attention as mosquito control agent (Chandra et al. 2008) as well as microbial control agents (Padua et al. 1984) and other strategies (Benelli 2015). Plant products (e.g. extracts, secondary metabolites) are also considered to be a potential alternative approach against different species of mosquitoes and their various immature stages due to their richness in bioactive compounds, their availability, and their environmental safety (Rathy et al. 2015). Hence, products derived from plant sources can act as larvicidal agents, insect growth regulators, repellents and oviposition attractants; they can therefore play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level (Govindarajan et al. 2008a, b).

In the continuation of our previous ethnobotanical and antiprotozoal studies on Nkundo identified plants of DR Congo (Fruth 2011; Fruth and Muganza 2006; Musuyu Muganza et al. 2012), we have undertaken a general screening of plants extracts for their biocidal effects on *C. quinquefasciatus* in a first phase, and then to determine, in a second stage, the LC_{50} values of the most potent extracts on larvae of *A. aegypti*, *A. gambiae* and *C. quinquefasciatus*, the 3 most important diseases vectoring mosquitoes.

2. MATERIAL AND METHODS

2.1. Experimental site

The research was conducted between September 2012 and March 2013 in the Department of Medical Entomology and Ecology of Vectors (MEEV), at the Institut National de Recherche Biomédicale (INRB) in Kinshasa, DR Congo.

2.2. Plant collection and Processing

Plants species investigated in the current study were selected on the basis of both ethnobotanical surveys (Mato 2005; Musuyu Muganza 2006) and the screening for their antiprotozoal and cytotoxic potential (Musuyu Muganza et al. 2012). In total, thirty-five plant species belonging to 21 families and 34 genera (see Table 1), were collected between July and August 2008, at the research site LuiKotale (Hohmann and Fruth 2003) in the South-Western part of the Salonga National Park, Maï-Ndombe district, Bandundu province in the DR Congo.

The plant species were identified at the Institut National d'Etudes et de Recherches en Agronomie (INERA), Department of Biology, Faculty of Sciences, University of Kinshasa. Voucher specimens of all the selected plant species were deposited at this herbarium.

The collected plant materials were air-dried and reduced to powder (Blender IKA® A11, Germany & Sieve Retsch® ISO 3310/1 apert: 500 µm). The obtained powders were kept in brown covered glass bottles.

2.3. Preparation of extracts

2.3.1. Aqueous extracts

Hundred grams (100 g) of each powdered plant material were mixed with 1000 mL distilled water and boiled for 10 min. After cooling, the suspensions were filtered using the Whatman's N°. 1 filter paper. The filtrates were then freeze-dried (CHRIST® ALPHA 1-4 LSC, Germany). The dried extracts were kept in hermetically closed glasses, labelled accordingly and stored (20-25 ˚C).

2.3.2. Ethanolic extraction

An amount of 50 g of each powder was macerated 3 times with 500 mL 80% EtOH for 48 h under permanent shaking (Heidolph UNIMAX 2010, Germany). Next, each extract was

filtrated into conical flasks using Whatman's No.1 filter paper and the filtrates were evaporated under reduced pressure at 40-45 °C, yielding the corresponding dried extracts.

2.4. Larvae collections / cultures of mosquitoes

A team of trained collectors from the Department of MEEV carried out the collections. Biocidal effects were tested on late third or early fourth instar larvae. The breeding room was maintained at a temperature varying between 25 °C and 28 °C. The relative humidity varied between 75% and 80%.

Aedes aegypti larvae were collected in breeding sites found in the enclosure of the INRB and brought in the insectary of the Department of MEEV for acclimatization and selection.

Anopheles gambiae mosquito larvae used for this study were collected from a culture maintained in the insectary of the Department of MEEV.

As for *Culex quinquefasciatus* larvae, they were collected from various untreated breeding sites (wastewaters) in outlying areas of the city of Kinshasa and transported in plastic containers to the laboratory of MEEV insectary. This collection was made at sunrise. Larvae were finally placed in plastic trays containing a quantity of water from their breeding sites.

Collected larvae were pooled in prepared trays placed in cages covered with mosquito net tiles. From this farm, suitable larvae were identified using dichotomous appropriate keys (De Meillon 1947; Edwards 1941; Gillies and Coetzee 1987; Gillies and De Meillon 1968; Hopkins 1952; Nagahuedi 1994) and selected for the biocidal tests.

2.5. Effects of plant extracts on mosquito larvae

Testing of the plant extracts for larvicidal activity was carried out at different concentrations by preparing the required stock solutions following the standard procedure (WHO 2005).

In the first phase dealing with preliminary larvicidal screening, 50 aqueous extracts (1%) from 35 plants were tested for their biocidal potency on *Culex* larvae which were the easiest to collect at the time of the study. In the second phase of the study, extracts found most active (mortality rate > 80%) in the precedent phase were tested along with their ethanolic counterparts on *A. aegypti*, *A. gambiae* and *C. quinquefasciatus*. Both aqueous and ethanolic extracts were tested at 10 different concentrations ranging from 20 – 0.04 mg/ mL obtained by 2-fold dilutions.

Twenty larvae were introduced into a 100 mL glass beaker containing 50 mL of dechlorinated water or DMSO 1% used as solvents, including appropriate controls for aqueous and ethanolic extracts.

The larval mortality, in both treated and control samples, was recorded after 24 h exposure and the percentage of mortality was calculated. There was no need to correct the data with the Abbott's formula (Abbott, 1925). Larvae were counted as dead when they were not coming to the surface for respiration and were probe insensitive (Sivagnaname and Kalyanasundaram 2004).

2.6. Data analysis

The mortality rates (%) were evaluated by the Kruskal-Wallis test in order to compare rank means between extracts, with Mann-Whitney pairwise comparisons followed by Bonferroni post-hoc tests for analysis of significancy. In addition, Principal Component Analysis (PCA) was applied in order to compare the sensitivity of different mosquito larvae to the plant extracts.

Eigenvalues and eigenvectors were calculated using a correlation matrix and PCA biplot was generated using PAST software, version 2.17c, Package for Education and Data Analysis. To determine the LC_{50} values of each tested plant extract towards each selected larval species, data were treated by Logit analysis using MS Excel 2007. The data recorded as mortality % (average ± SD) represent the mean of three bioassays. Statistical significance was set at $p < 0.05$.

3. RESULTS

The results of the general larvicidal screening of 50 aqueous (1%) plant extracts on *C. quinquefasciatus* larvae after 24 h exposure are given in the Table 1. Samples in bold had the most effective biocidal effects on the tested larvae. Seven crude extracts (14%) showed high larvicidal activity, of which, 4 had a mortality rate of 100% and 3 displayed a mortality rate ranging 85%-98%. Three extracts of *Piper guineense* fruits, stem and root gave 100% mortality on the tested larvae; the leaves' extract was much less toxic towards the tested larvae.

Table 1: Mortality rates (mean % ± SD) of *Culex quinquefasciatus* larvae tested with aqueous (1%) extracts (Fr = fruits, Le = Leaves, RB = root bark, SB = stem bark).

Plant species	Family	Parts	Mortality (%)
Afrostyrax lepidophyllus Mildbr.	Huaceae	RB	11.67 ± 2.89
Alchornea cordifolia (Schum. & Thonn.) Muell.Arg.	Euphorbiaceae	Le	18.33 ± 2.89
Alchornea floribunda Muell.Arg.	Euphorbiaceae	Le	0.00 ± 0.00
Alstonia boonei De Wild.	Apocynaceae	SB	5.00 ± 0.00
Annickia ambigua (Robyns & Ghesq.) Set. & Maas	Annonaceae	SB	6.67 ± 2.89
Autranella congolensis (De Wild.) Chevalier	Sapotaceae	SB	38.33 ± 2.89
Cajanus cajan L.	Fabaceae-Faboideae	Le	20.00 ± 5.00
Catharanthus roseus (L.) G. Don	Apocynaceae	Le	11.67 ± 2.89
***Crossopteryx febrifuga* (Afz. ex G. Don) Benth.**	**Rubiaceae**	**RB**	**85.00 ± 0.00**
Dalhousiea africana S. Moore	Fabaceae-Faboideae	Le	6.67 ± 2.89
Drypetes gossweileri S. Moore	Euphorbiaceae	SB	10.00 ± 0.00
Greenwayodendron suaveolens (Engl. & Diels) Verdc.	Annonaceae	RB	13.33 ± 2.89
Greenwayodendron suaveolens (Engl. & Diels) Verdc.	Annonaceae	SB	25.00 ± 0.00
Greenwayodendron suaveolens (Engl. & Diels) Verdc.	Annonaceae	Le	18.33 ± 2.89
Greenwayodendron suaveolens (Engl. & Diels) Verdc.	Annonaceae	Fr	33.33 ± 2.89
Harungana madagascariensis Lam.	Hypericaceae	SB	23.33 ± 2.89
Isolona hexaloba (Pierre) Engl. & Diels	Annonaceae	Fr	8.33 ± 2.89
Isolona hexaloba (Pierre) Engl. & Diels	Annonaceae	SB	6.67 ± 2.89
Isolona hexaloba (Pierre) Engl. & Diels	Annonaceae	RB	5.00 ± 0.00
Isolona hexaloba (Pierre) Engl. & Diels	Annonaceae	Le	0.00 ± 0.00
Jatropha curcas L.	Euphorbiaceae	RB	8.33 ± 2.89
Laportea aestuans (L.) Chew	Urticaceae	Le	18.33 ± 2.89
Mammea africana Sabine	Clusiaceae	SB	36.67 ± 2.89
Manniophyton fulvum Muell.Arg.	Euphorbiaceae	SB	5.00 ± 0.00
Manniophyton fulvum Muell.Arg.	Euphorbiaceae	RB	6.67 ± 2.89
Manniophyton fulvum Muell.Arg.	Euphorbiaceae	Le	5.00 ± 0.00
Morinda morindoides (Baker) Milne-Redh.	Rubiaceae	Le	5.00 ± 0.00
Myrianthus arboreus P.Beauv.	Cecropiaceae	SB	6.67 ± 2.89
Napoleana vogelii Hook & Planch.	Lecythidaceae	SB	8.33 ± 2.89
Ocimum gratissimum L.	Lamiaceae	Le	6.67 ± 2.89
Oncoba welwitschii Oliv.	Flacourtiaceae	Le	6.67 ± 2.89
***Penianthus longifolius* Miers**	**Menispermaceae**	**RB**	**90.00 ± 0.00**
Picralima nitida (Stapf) T. Durand & H. Durand	Apocynaceae	SB	11.67 ± 2.89
***Piper guineense* Schumach.**	**Piperaceae**	**Fr**	**100.00 ± 0.00**
***Piper guineense* Schumach.**	**Piperaceae**	**RB**	**100.00 ± 0.00**
***Piper guineense* Schumach.**	**Piperaceae**	**SB**	**100.00 ± 0.00**
***Piper guineense* Schumach.**	**Piperaceae**	**Le**	**58.33 ± 2.89**
Piptadeniastrum africanum (Hook.f.) Brenan	Fabaceae-Mimosoideae	SB	6.67 ± 2.89
Quassia africana (Baill.) Baill.	Simaroubaceae	Le	35.00 ± 5.00
***Quassia africana* (Baill.) Baill.**	**Simaroubaceae**	**SB**	**100.00 ± 0.00**
***Quassia africana* (Baill.) Baill.**	**Simaroubaceae**	**RB**	**98.33 ± 2.89**
Scorodophloeus zenkeri Harms	Fabaceae-Caesalpinoideae	SB	8.33 ± 2.89
Staudtia kamerunensis L.	Myristicaceae	SB	10.00 ± 0.00
Tetrapleura tetraptera (Schum. & Thonn.) Taub.	Fabaceae-Mimosoideae	Fr	11.67 ± 2.89

Tetrapleura tetraptera (Schum. & Thonn.) Taub.	Fabaceae-Mimosoideae	SB	11.67 ± 2.89
Thomandersia hensii De Wild. & T. Durand	Acanthaceae	Le	11.67 ± 2.89
Thomandersia hensii De Wild. & T. Durand	Acanthaceae	SB	33.33 ± 2.89
Trema orientalis (L.) Blume	Ulmaceae	Le	0.00 ± 0.00
Triclisia dictyophylla Diels	Menispermaceae	Le	10.00 ± 0.00
Vitex ferruginea Schumach. & Thonn.	Vitaceae	SB	11.67 ± 2.89
Negative control	-	-	0.00 ± 0.00

Subsequently, the 7 most active plant species (Table 1) were submitted to the larvicidal activity testing through different concentrations of their respective aqueous (A) and ethanolic (E) extracts: [CFRA & CFRE (for *Crossopteryx febrifuga* root bark), PLRA & PLRE (*Penianthus longifolius* root bark), PGFA & PGFE, PGRA & PGRE, PGSA & PGSE (for *Piper guineense* fruits, root bark and stem bark), and QARA & QARE, QASA & QASE (for *Quassia africana* root bark and stem bark)]. The results of these tests on the different types of larvae are presented in Tables 2, 3 & 4. Both negative controls did not cause any case of mortality in any of the 3 tested larvae types and therefore, the correction of the results with the Abbott's formula (Abbott 1925) was not necessary.

Ten concentrations for each of the 14 extracts were obtained after 2-fold dilutions, and tested on the 3 larvae species. The estimates of the LC_{50} values are presented in Table 5 and the larvicidal activities were concentration-dependent (Tables 2, 3 & 4). The extracts PGRE, QASE and PGFE were the most effective against *Ae. aegypti* larvae as they displayed LC_{50} values of 0.313 mg/ mL for the 2 first cited and 0.41 mg/ mL for the last one. Larvae of *An. gambiae* seemed to be more sensitive towards the tested plant extracts; the 3 most active samples were PGFE, PGRE & PGSE with LC_{50} values < 0.039 mg/ mL; followed by PGFA (0.071 mg/ mL) with almost the half of their potency. With regard to the biocidal effects of the tested extracts towards *C. quinquefasciatus*, 5 plant extracts (PGFE, PGRE, PGSE, QARE & QASE) displayed LC_{50} values all < 0.039 mg/ mL; followed by PGFA with a LC_{50} value of 0.051 mg/ mL.

Table 2: Mortality Rate (mean % ± SD) of different plant extracts at different concentrations on *Aedes aegypti* larvae after 24 hours of exposure.

	Concentrations (mg/ mL)									
Extracts	**20**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.3125**	**0.15625**	**0.078125**	**0.039**
CFRA	81.67±2.89	53.33±2.89	26.67±2.89	8.33±2.89	0±0	0±0	0±0	0±0	0±0	0±0
CFRE	100±0	85±5	75±5	56.67±2.89	46.67±2.89	16.67±2.89	5±0	0±0	0±0	0±0
PLRA	100±0	85±0	75±5	55±5	31.67±2.89	15±0	6.67±2.89	0±0	0±0	0±0
PLRE	100±0	100±0	100±0	85±5	70±5	35±5	20±0	6.67±2.89	1.67±2.89	0±0
PGFA	100±0	100±0	100±0	80±5	66.67±2.89	46.67±5.77	18.33±2.89	5±0	0±0	0±0
PGRA	100±0	100±0	90±0	75±5	55±5	35±0	13.33±2.89	5±0	0±0	0±0
PGSA	100±0	100±0	85±10	70±5	50±0	30±5	15±5	1.67±2.89	0±0	0±0
PGFE	100±0	100±0	100±0	95±0	80±5	66.67±2.89	46.67±5.77	21.67±2.89	10±0	0±0
PGRE	100±0	100±0	95±5	85±0	70±0	65±5	50±0	30±5	13.33±2.89	5±0
PGSE	100±0	100±0	91.67±2.89	81.67±7.64	65±5	51.67±2.89	40±5	20±5	10±5	5±0
QARA	91.67±2.89	78.33±2.89	38.33±2.89	10±5	0±0	0±0	0±0	0±0	0±0	0±0
QASA	100±0	88.33±2.89	71.67±2.89	38.33±2.89	15±5	0±0	0±0	0±0	0±0	0±0
QARE	100±0	100±0	100±0	91.67±2.89	75±5	56.67±5.77	28.33±2.89	10±0	0±0	0±0
QASE	100±0	100±0	100±0	100±0	90±5	70±5	50±5	21.67±2.89	8.33±2.89	0±0

Table 3: Mortality Rate (mean % ± SD) of different plant extracts at different concentrations on *Anopheles gambiae* larvae after 24 hours of exposure.

	Concentrations (mg/ mL)									
Extracts	**20**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.3125**	**0.15625**	**0.078125**	**0.039**
CFRA	100±0	90±0	75±5	50±8.66	26.67±2.89	10±5	0±0	0±0	0±0	0±0
CFRE	100±0	100±0	85±5	75±5	56.67±5.77	38.33±7.64	20±5	8.33±2.89	0±0	0±0
PLRA	100±0	98.33±2.89	83.33±2.89	66.67±2.89	45±0	10±5	5±0	0±0	0±0	0±0
PLRE	100±0	100±0	100±0	95±5	75±5	60±0	48.33±10.41	30±5	16.67±2.89	5±0
PGFA	100±0	100±0	100±0	100±**0**	100±**0**	96.67±2.89	90±0	80±5	66.67±2.89	46.67±5.77
PGRA	100±0	100±0	100±0	100±0	100±0	81.67±2.89	35±5	10±0	0±0	0±0
PGSA	100±0	100±0	100±0	100±0	100±0	73.33±2.89	53.33 ±2.89	36.67±5.77	16.67±2.89	11.67±2.89
PGFE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	88.33±2.89
PGRE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	95±0	83.33±5.77
PGSE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	73.33±5.77	66.67±2.89
QARA	100±0	100±0	98.33±2.89	93.33±2.89	81.67±2.89	73.33±5.77	56.67±5.77	31.67±5.77	15±5	6.67±2.89
QASA	100±0	100±0	86.67±5.77	70±5	55±5	33.33±5.77	18.33±2.89	5±0	0±0	0±0
QARE	100±0	100±0	100±0	100±0	100±0	100±0	90±0	73.33±2.89	40±10	16.67±7.64
QASE	100±0	100±0	100±0	100±0	100±0	100±0	70±10	43.33±5.77	11.67±2.89	0±0

Table 4: Mortality Rate (mean % ± SD) of different plant extracts at different concentrations on *Culex quinquefasciatus* larvae after 24 hours of exposure.

	Concentrations (mg/ mL)									
Extracts	**20**	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.3125**	**0.15625**	**0.078125**	**0.039**
CFRA	100±0	86.67±5.77	55±5	36.67±5.77	10±0	0±0	0±0	0±0	0±0	0±0
CFRE	100±0	100±0	90±5	75±5	48.33±2.89	16.67±2.89	5±0	0±0	0±0	0±0
PLRA	100±0	100±0	71.67±2.89	45±5	16.67±2.89	5±0	0±0	0±0	0±0	0±0
PLRE	100±0	100±0	100±0	76.67±5.77	51.67±2.89	25±0	7.5±3.54	0±0	0±0	0±0
PGFA	100±0	100±0	100±0	100±0	100±0	93.33±2.89	90±0	78.33±2.89	60±0	43.33±5.77
PGRA	100±0	100±0	100±0	100±0	100±0	81.67±2.89	50±5	40±5	18.33±2.89	10±5
PGSA	100±0	100±0	100±0	100±0	100±0	73.33±2.89	46.67±2.89	28.33±2.89	6.67±2.89	0±0
PGFE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0
PGRE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	98.33±2.89	95±0
PGSE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	81.67±2.89
QARA	100±0	100±0	100±0	96.67±5.77	83.33±5.77	70±0	46.67±5.77	26.67±5.77	10±0	0±0
QASA	100±0	100±0	100±0	100±0	100±0	95±5	80±5	66.67±5.77	36.67±5.77	0±0
QARE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	96.67±2.89	85±5	66.67±2.89
QASE	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	80±5

The LC_{50} (median lethal concentration) estimates for the extracts of the most promising plant species are given in Table 5 together with the corresponding mortality rate for each extract on the different selected larvae types.

In the same column (of mortality rates) values marked with the same superscript indicate pairwise comparisons significantly different by the Kruskal-Wallis test followed by Bonferroni post-hoc tests at $P < 0.05$.

In order to compare the sensitivity of mosquito larvae towards the different extracts, a PCA was performed with the mean mortality rates. Through this multivariate analysis, it was possible to deduce some variation patterns for the larvicidal efficacy of plant extracts. Figure 1 shows distribution of component types of plant extracts on the biplot representation defined by the two first principal components (PC1 and PC2), allowing to grouping extracts by their efficacy. PCA showed that this correlation was statistically strong with these two principal axes. The first principal component (PC1) accounted for 83.06% of total variation and displayed strong correlation values (ranging from 0.47 to 0.99) for the sensitivity of *C. quinquefasciatus* and *An. gambiae* larvae towards all plant extracts tested after 24 h exposure. On the second axis (PC2), the projection represented 16.94% of variation demonstrating lower correlation values (ranging from 0.05 to 0.88) for the sensitivity of *C. quinquefasciatus* and *A. aegypti* larvae to plant extracts such as CFRE, PLRE, PGSA, QARA, QASA, QARE, and QASE.

Table 5: LC_{50} values and percentage mortality (mean ± SD) of different mosquito larvae tested with different plant extracts after 24 hours of exposure.

	Aedes aegypti		***Anopheles gambiae***		***Culex quinquefasciatus***	
EXTRACTS	**Mortality Rate (%)**	**LC_{50} (mg / mL)**	**Mortality Rate (%)**	**LC_{50} (mg/ mL)**	**Mortality Rate (%)**	**LC_{50} (mg/ mL)**
CFRA	42.50±31.99 a,b,c,d,e,f	9.632	58.61±35.85 a,b,c,d,e,f,g	2.500	57.67±36.58 a,b,c,d,e,f,g,h,i	4.081
CFRE	55.00±35.04	1.967	60.42±35.45 h,i,j,k,l	1.048	62.14±39.44 j,k,l,m,n,o	1.449
PLRA	52.62±35.98	2.252	58.33±39.56 m,n,o,p,q,r	1.724	55.56±41.96 p,q,r,s,t,u,v,w	3.224
PLRE	57.96±41.20 a,g	0.847	63.00±36.66 s,t,u	0.468	65.83±38.49 x,y,z,α,β	1.179
PGFA	64.58±3.830	0.799	88.00±18.37 a,h,m,v	0.071	86.50±20.01 a,j,p,γ	0.051
PGRA	59.17±38.12	1.072	78.33±35.67	0.369	70.00±36.83	0.313

			w		b,q,δ,ε	
PGSA	56.88±37.60	1.250	69.17±36.73 b,x	0.284	72.78±36.63 c,ζ,η,θ	0.338
PGFE	68.89±35.19 b,h	0.410	98.83±3.69 c,i,n,s,w,x,y,z	< 0,039	100.00±0.00 d,k,r,x,γ,δ,ζ,ι,κ	< 0,039
PGRE	61.33±35.53 c,i	0.313	97.83±5.33 d,j,o,t,α,β	< 0,039	99.33±1.61 e,l,s,y,λ	< 0,039
PGSE	56.50±36.77 d,j	0.583	93.00±15.27 e,k,p,u,γ,δ	< 0,039	98.17±5.80 f,m,t,z,ε,η,μ	< 0,039
QARA	54.58±37.38 g,h,i,j,k	6.386	65.67±36.19 y,α,γ	0.273	70.37±34.66 ι,λ,μ,ν,ξ	0.374
QASA	62.67±35.35	3.571	58.54±36.82 v,z,β,δ,ε	1.1167	77.83±34.37 g,u,κ	0.108
QARE	70.21±35.27	0.534	82.00±30.03 f,l,q,ε	0.108	94.83±10.96 h,n,v,α,ν	< 0,039
QASE	71.11±36.28	0.313	73.00±38.94 g,r	0.207	98.00±6.32 i,o,w,β,θ,ξ	< 0,039

In short, Figure 1 shows that *C. quinquefasciatus* and *An. gambiae* larvae are sensitive to all plant extracts tested after 24 h exposure, whereas *Ae. aegypti* larvae appear to be more tolerant.

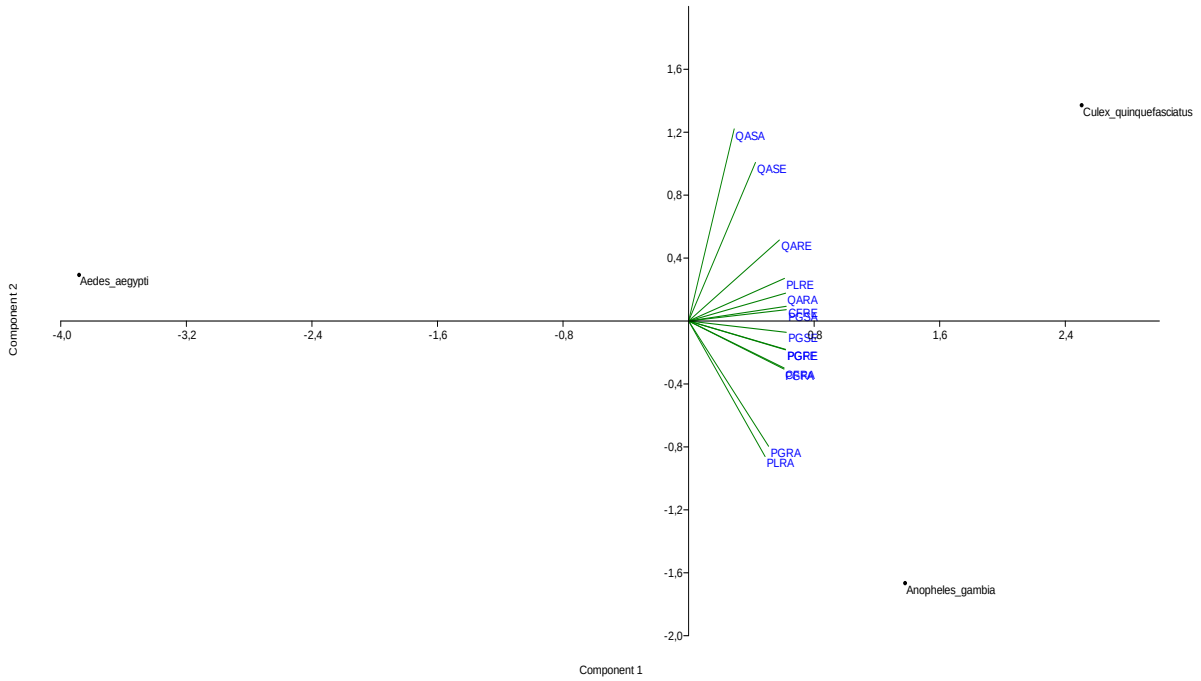

Fig. 1: Principal Component Analysis (PCA) showing the sensitivity of mosquito larvae to plant extracts tested after 24 h of exposure (with PC1 and PC2 noted at 83.06% and 16.94% of variances respectively); Codes of the different extracts are explained underneath Table 1.

4. DISCUSSION

Identification of various plant extracts that have biocidal potential against mosquito larvae can be of advantage as one of the solutions in reducing the problem of resistance and concerns for environmental safety. Control of vectors, especially parasitic vectors, is a common way of disease control. Control of mosquito larvae can reduce the population of the insects which could result in reducing the burden of the diseases (Adewole et al. 2013).

In overall, the obtained results were somewhat in accordance with previous similar reports. Ihemanma et al. (2014) showed that the leaf and seed ethanolic extract of *P. guineense* had larvicidal effects at high concentrations on mosquito larvae. *P. guineense* seeds and leaves had larvicidal effects on mosquitoes with 90% and 80% mortality, respectively, after an exposure of 12 h at 100 mg/mL. In another study, the obtained LC_{50} values indicated that ethanolic extract of *P. guineense* (0.028 mg/ mL) was the most active, followed in descending order by its aqueous extract with 0.09 mg/ mL (Aina et al. 2009); these results seem to be in accordance with ours.

The tropical plant family Piperaceae, especially the genus *Piper*, is known to contain piperamides (isobutylamides), compounds that act as neurotoxins in insects [Scott et al. 2008]. In addition, materials from *Piper* species are generally considered safe for humans because they are providing since centuries a source of diverse spices, condiments or vegetables and above all diverse medicines (Amusan and Okorie 2002; Mato 2005; Musuyu Muganza 2006). Extracts from *Quassia africana* stem and root barks of the present study have showed some biocidal action towards the selected larvae as given in Tables 1-5. The most prominent effect was exhibited for both ethanolic extracts in particular against *C. quinquefasciatus* larvae (Table 5). Ajaiyeoba et al. (2009) have previously found that methanolic extracts of the leaves, stem and root barks of *Q. africana* showed some larval toxicity on *A. gambiae* after 24 h of exposure, with the root and stem extracts displaying 100% mortality at 50 mg/mL.

The findings in this study further confirm the root and stem barks of *Q. africana* as sources of phytochemicals with larvicidal effects. Species of the Simaroubaceae family are known to contain bitter substances called quassinoids which display a wide range of biological activities *in vivo* and *in vitro* including anti-feedant and insecticidal (Guo et al. 2005). Many previous studies have already demonstrated the larvicidal effects of extracts from *Q. africana* and a substance, simalikalactone D, has even been isolated from MeOH extract of roots and

found to exhibit a LC_{50} value of 1.25 µg/ mL towards *A. gambiae* larvae (Sama et al. 2014). The present study has the advantage of testing simultaneously different types of extracts from different parts of *Quassia africana* against different types of larvae, offering thereby the possibility to isolate more other larvicidal substances.

The other active extracts of the present study were from *C. febrifuga* root bark and *P. longifolius* root bark with respective mortality rates of 85 and 90% on *C. quinquefasciatus* larvae in the preliminary screening. Ethanolic and aqueous samples from *P. longifolius* were more potent than those from *C. febrifuga* towards the 3 larvae species tested as reported in Table 5. No previous data could be found concerning the testing of any larvicidal activity of *P. longifolius* and *C. febrifuga*, so this study is most probably the first report on the larvicidal activity of these two plant species.

Overall, it appears that 80% ethanol extracts are all more active than their aqueous counterparts. Hence, 80% ethanol is probably more suitable for qualitative and quantitative extraction of phytochemicals that act more efficiently against the tested larvae.

5. CONCLUSION

The most active plant species can be utilized in the production of plant-based larvicides which could be used to a certain extent as a substitute for the synthetic ones as they are readily available in many areas of the world, affordable, and above all eco-friendly and biodegradable. In descending order of their effectiveness towards the different larvae species, the most interesting plant species of the present study are *P. guineense*, *Q. africana*, *P. longifolius* and *C. febrifuga*. The studied extracts offer thus a useful source of biopesticide materials for controlling small-scale insect out-breaks and reducing the presumed resistance development. They can be used alone or in potentially synergistic combinations including with other known plants-based insecticides.

Further studies on the larvicidal mode of action, their possible effects on non-target organisms and the suitable formulations for improving their larvicidal potency are to be carried out for their standardization. In addition, further studies aiming at identifying more other active molecules from the most active extracts have to be carried out.

ACKNOWLEDGEMENTS

We acknowledge the excellent field and technical support provided by researchers (G. Binene & S. Fasine) and trainees (M-C. Babeki & W. Kasongo) of the Department of Medical Entomology and Ecology of Vectors (Institut National de Recherches Biomédicales, Kinshasa). We are very grateful to the Nkundo people of all the visited villages of Bolongo (Bandundu Province, DR Congo) for their participation to the ethnobotanical surveys and the collection of plant material. This work was partially supported by the Project Cuvette Centrale/ Max Planck Institute for Evolutionary Anthropology (Leipzig/ Germany).

CONFLICT OF INTEREST

Authors have declared that there is no conflict of interests.

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