



Original Research Article

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## Inhibition Effects of *Elaeis oleifera* (Arecaceae) and *Launaea taraxacifolia* (Asteraceae) on Two Genotypes of *Anopheles gambiae* Larvae

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### Abstract

The management of breeding sites is one of the preventive measures against malaria. However, in view of the environmental damage caused by the various chemical insecticides used for this purpose and especially the resistances developed by *Anopheles gambiae* (the main vector) with respect to these synthetic chemical compounds, we have proposed implementing of extracts plants. Thus, organic solvents with a gradient of polarities such as *n*-hexane, dichloromethane and methanol-water were used to extract the phytochemical compounds present in the leaves of *Elaeis oleifera* and *Launaea taraxacifolia*. The three different extracts obtained for each plant were tested on 3rd stage larvae from two genotypes of *Anopheles gambiae*: the Kisumu strain of Kenyan origin and the wild strain from the breeding sites of Cotonou. Phytochemical analysis revealed that in addition to tannins, flavonoids, anthocyanins, leuco-anthocyanins and triterpenes which are present in both plants, *Elaeis oleifera* also contains mucilages and alkaloids. The hydro-methanolic extracts were found to be the most active on the two origins of larvae with lethal concentrations LC<sub>50</sub> of 448.01 ppm in 24 hrs and 51.38 ppm in 48 hrs of exposure for the Kisumu strain; 4199.63 ppm and 1456.44 ppm in 24 hrs and 48 hrs respectively for the wild population for *Elaeis oleifera*. In addition, *Launaea taraxacifolia* appeared much more interesting and displayed almost identical activities on the two larval origins with LC<sub>50</sub> of 182.68 ppm and 135.13 ppm respectively in 24 hrs and 48 hrs for Kisumu larvae and 157.36 ppm in 24 hrs then 116.88 ppm in 48 hrs for wild larvae. It is therefore concluded that *Launaea taraxacifolia* can be used for bio-larvicide production in integrated malaria vector control.

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### Introduction

Vector control is one of the main prevention measures for malaria in the absence of a vaccine. In addition to the

two widely applicable basic methods for controlling the vectors of the disease, which are long-lasting insecticidal nets and indoor spraying, the management of breeding sites appears today as a complementary

measure (Fillinger et al., 2009; OMS, 2016). This method of reduction of the number of mosquito larvae and nymphs results in larvicidal treatments, which involve the regular application of insecticides, often of synthetic chemical nature, to water bodies. However, it could induce multiple dysfunctions of aquatic and terrestrial ecosystems through bioaccumulation and appearance of resistance of the target vectors, in particular *Anopheles gambiae* (Djogbenou et al., 2011; Edi et al., 2012; Nwane et al., 2013) and then considerably reduce the effectiveness of the treatment. Although the development of resistance of mosquitoes has not yet been detected with bio-insecticides of microbial origin and for rough use such as *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* (Becker, 1998), it is important to broaden this spectrum of new biochemical insecticides in order to be ready to respond to all eventualities, as well as to have a system of effective control of these populations of vectors. The use of naturally occurring insecticides based on active plant extracts applicable to mosquito larvae is necessary for the control of malaria vectors (Chougourou et al., 2012).

Several studies have pointed to this direction with the aim of searching for phytomolecules which can serve as a basis for the formulation of bio-larvicides. *Hyptis suaveolens* has been shown to inhibit the larvae of *Culex quinquefasciatus*, a vector of lymphatic filariasis (Murugesan et al., 2015). *Ficus benghalensis* acts on the larvae of *Aedes aegypti* and *Anopheles stephensi* (Govindarajan, 2010a). In addition, recent studies in Benin have investigated the activities of polyphenol-rich plants on larvae of parasitic insects such as *Launaea taraxacifolia* on *Anopheles gambiae* larvae (Ahouansou et al., 2016) and *Elaeis oleifera* on the larvae of *Coelaenomenodera lameensis*. *Elaeis oleifera* is a species of palm tree that is resistant to the attacks of *Coelaenomenodera lameensis* larvae among the various oil palm genotypes due to its rich polyphenolic compounds (Fagbohoun et al., 2015). In addition, *Launaea taraxacifolia* is known and domesticated in all West African countries (Adebisi, 2004). Its leaves eaten fresh in salad or cooked as sauce (Koukouli et al., 2015), are nutritionally rich in vitamins, proteins, minerals, essential fatty acids, fiber and flavonoids (Adinortey et al., 2012; Dickson et al., 2012) and are widely used as an infusion for the treatment of several diseases such as blood pressure regulation, dyslipidemia, management of cholesterol blood levels, antioxidant properties and viral infections (Arawande et al., 2013; Dansi et al., 2012;

Owoeye et al., 2015). In this connection, the objective of this work has been framed to evaluate the insecticidal potential of *Launaea taraxacifolia* and *Elaeis oleifera* on larvae of *Anopheles gambiae* from different origins in a comparative study of plants with larvicidal activities.

## Materials and methods

### Collection of larvae, plants and extractions

Bio-tests were carried out on two categories of larvae of *Anopheles gambiae*: wild larvae collected in breeding sites to Cotonou according to the morphological and behavioral characteristics of the larvae using taxonomic identification keys (Gillies and Coetzee, 1987) and Kisumu larvae of Kenyan origin obtained at the Center for Entomological Research of Cotonou (CREC). The latter have been maintained in breeding in the CREC laboratory for several years and their sensitivity is regularly checked. The leaflets of *Elaeis oleifera* were harvested from the plantations of the Benin Agricultural Research Institute (INRAB) in the commune of Pobè while the aerial parts of *Launaea taraxacifolia* were harvested from corn fields at Comé in the southern part of Benin. After collection, the samples were transported to the laboratory and then dried at 16°C before being ground and reduced to powder. In a first step, the extractions were carried out with three solvents of different polarity: *n*-hexane, dichloromethane and methanol in order to obtain the crude extracts. To 100 g of powder from each plant sample, was added 500 mL of *n*-hexane. The mixture was homogenized, then kept under continuous stirring for 24 hrs, and then filtered and evaporated to dryness using a rotary evaporator (*Heidolph Laborota 4000 efficient*). The same operation is carried out for dichloromethane. As to the third extraction, we used the methanol-water mixture (70:30; v/v) with 0.5% of formic acid following the same process described above. The operations were repeated three times and the dry residue obtained at each operation was weighed out to determine the average yield of extraction.

### Phytochemical analyzes

The different chemical groups were found in the samples of *E. Oleifera* and *L. taraxacifolia* using the classical method of Houghton and Raman (Houghton and Roman, 1998), used routinely and very recently by Fagbohoun et al. (2014 and 2015). Thus the Mayer and Dragendorff tests are used for alkaloids, the Fehling test

for reducing compounds and glycosides, the Liebermann-Burchard test for triterpenoids and steroids, the Frothy test for saponins, the Shinoda tests and sodium hydroxide for flavonoids, the ferric chloride test for tannins, the Guignard test for free cyanogenic derivatives and the Borntrager test for anthraquinones.

### Biological tests on *Anopheles gambiae*

The bioassays were carried out on larvae of *Anopheles gambiae* according to the WHO standard protocol for the larval susceptibility tests against insecticides used in control campaigns with a slight modification in accordance to our conditions (WHO, 2005). Kisumu larvae were treated with solutions of extracts of concentrations ranging from 62.5 to 2000 ppm prepared from each type of extract with 2% DMSO (Dimethyl sulfoxide); while wild larvae were subjected to extract solutions with concentrations ranging from 62.5 to 15000 ppm when appropriate. The tests were carried out in transparent cups 5 cm in diameter, each containing 100 ml of solution and 20 larvae of *Anopheles gambiae* in the third stage of the same category. The same number of larvae was placed in another control cup containing only 100 mL of 2% aqueous of dimethyl sulphoxide (DMSO). For each concentration of the extracts as well as for the control, three replicates were made. Larval behavior, by counting the number of survivors, was monitored for 48 hrs and lethal concentrations (LC<sub>50</sub>) were determined every 24 hrs. Indeed, dead larvae were considered immobile even when in contact of a needle and also those that were moribund.

### Statistical analyses

The analysis of the data is carried out using the SPSS 21.0 statistical software at the risk of 5% ( $p < 0.05$ ) in order to obtain the average mortality rate of *Anopheles* larvae according to the doses applied and to extract the

lethal concentrations (LC<sub>50</sub>).

## Results

### Phytochemical Screening

Table 1 presents the results obtained after the detection of 17 chemical groups in the two plants studied. In addition to tannins, flavonoids, anthocyanins and leuco-anthocyanins, as well as terpenes found in both plants, *Elaeis oleifera* also contains mucilages and alkaloids.

**Table 1.** Plants phytochemical Screening

Compounds	<i>Launaea taraxacifolia</i>	<i>Elaeis oleifera</i>
Tannins	+	+
Catechin tannins	+	+
Galic tannins	+	+
Flavonoids	+	+
Anthocyanins	+	+
Leuco-anthocyanins	+	+
Quinone derivatives	-	-
Saponosides	-	-
Triterpenes	+	+
Steroids	-	-
Cyanogenic compounds	-	-
Mucilages	-	+
Reducing compounds	-	-
Coumarins	-	-
Free anthracene	-	-
O-glycosides	-	-
C-glycosides	-	-
Cardiac glycosides	-	-
Alkaloids	-	+

+ = Present; - = Absent.

### Extraction yields

The yields of crude extracts of *Launaea taraxacifolia* and *Elaeis oleifera* to *n*-hexane, dichloromethane and methanol-water are reported in Table 2.

**Table 2.** Extraction yields of plants in different solvents.

Solvents	<i>n</i> -Hexane		Dichloromethane		Methanol/water	
Plants	<i>Launaea taraxacifolia</i>	<i>Elaeis oleifera</i>	<i>Launaea taraxacifolia</i>	<i>Elaeis oleifera</i>	<i>Launaea taraxacifolia</i>	<i>Elaeis oleifera</i>
Yield (%)	2.05±0.11	3.41±0.17	5.51±0.23	4.03±0.28	18.89±1.11	14.48±1.03

Table 2 shows that the hydro-methanolic mixture is the solvent which produced the greatest quantity of extracts obtained at the level of the two plants with yields of 18.89% and 14.48% respectively for *Launaea*

*Taraxacifolia* and *Elaeis oleifera*. Indeed, these results justify the polar nature of the predominant compounds in both plants. The extract yields of other solvents of the plants were found to be <6%.

### Biological screening of crude extracts on larvae of *Anopheles gambiae*

Of the three extracts used for the anti-larval tests with *Elaeis oleifera*, the hydro-methanolic extract is the one which showed notably a fairly interesting

inhibitory activity on the Kisumu larvae (Table 3). Indeed, although no larva died within 24 hrs at 62.5 ppm, half of the tested larvae population died at 62.5 ppm after 48 hrs of exposure and a 100% mortality rate was obtained at 500 ppm in extract concentration.

**Table 3.** Mortality rate (%) of Kisumu larvae with extracts of *Elaeis oleifera* and *Launaea taraxacifolia*.

Plant	Conc. in ppm	Dichloromethane		Methanol-water		n-Hexane	
		24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
<i>Elaeis oleifera</i>	2000	70.00±2.887	80.00±2.887	100±0.00	100±0.00	100±0.00	100±0.00
	1000	51.66±1.667	65.00±2.887	93.33±1.667	100±0.00	100±0.00	100±0.00
	500	10.00±0.00	26.66±1.667	65.00±2.887	100±0.00	50.00±2.887	68.33±1.667
	250	0.00	10.00±2.887	6.66±1.667	80.00±1.667	13.33±3.333	26.67±1.667
	125	0.00	0.00	0.00	71.66±1.667	0.00	0.00
	62.5	0.00	0.00	0.00	50.00±2.887	0.00	0.00
	Control	0.00	0.00	0.00	0.00	0.00	0.00
<i>Launaea taraxacifolia</i>	2000	31.66±1.667	51.66±1.667	100±0.00	100±0.00	100±0.00	100±0.00
	1000	10.00±0.00	26.66±1.667	100±0.00	100±0.00	100±0.00	100±0.00
	500	0.00	10.00±0.00	100±0.00	100±0.00	25.00±2.887	41.67±4.410
	250	0.00	0.00	75.00±2.887	81.66±1.667	5.00±0.00	13.33±1.667
	125	0.00	0.00	21.66±1.667	30.00±2.887	1.67±1.667	6.67±1.667
	62.5	0.00	0.00	0.00	0.00	1.67±1.667	5.00±0.00
	Control	0.00	0.00	0.00	0.00	0.00	0.00

Conc : Concentration ; Larvicidal activities of *Elaeis oleifera* and *Launaea taraxacifolia* extracts on Kisumu larvae.

**Table 4.** Mortality rate (%) of wild larvae with extracts of *Elaeis oleifera* and *Launaea taraxacifolia*.

Plant	Conc. in ppm	Dichloromethane		Methanol-water		n-Hexane	
		24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
<i>Elaeis oleifera</i>	15000	76.66±3.33	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	10 000	43.33±1.667	55.00±2.887	75.00±2.887	91.66±1.667	51.67±1.667	73.33±3.333
	5000	11.66±1.667	25.00±2.887	60.00±2.887	76.66±1.667	28.33±1.667	50.00±1.667
	2000	0.00	21.66±1.667	28.33±1.667	58.33±1.667	0.00	21.67±1.667
	1000	0.00	10.00±0.00	25.00±0.00	50.00±2.887	0.00	10.00±1.667
	500	0.00	0.00	0.00	26.66±1.667	0.00	0.00
	Control	0.00	0.00	0.00	0.00	0.00	0.00
<i>Launaea taraxacifolia</i>	1000	30.00±0.00	56.66±1.667	100±0.00	100±0.00	100±0.00	100±0.00
	500	11.66±1.667	21.66±1.667	100±0.00	100±0.00	1.67±1.667	26.67±1.667
	250	0.00	6.66±1.667	100±0.00	100±0.00	0.00	10.00±0.00
	125	0.00	0.00	71.66±1.667	83.33±4.41	0.00	0.00
	62.5	0.00	0.00	16.66±1.667	33.33±1.667	0.00	0.00
	Control	0.00	0.00	0.00	0.00	0.00	0.00

Conc. : Concentration; Larvicidal activities of *Elaeis oleifera* and *Launaea taraxacifolia* extracts on wild larvae

In addition to the wild larvae, the hydro-methanolic extract remains the most active but at very high concentrations; it is necessary to reach 15 000 ppm before obtaining 100% mortality at 24 hrs (Table 4). These same observations were made at the level of *Launaea taraxacifolia* with a few differences. The hydro-methanolic extract is also the most active with a mortality rate of 100% to 500 ppm after 24 hrs of contact with Kisumu larvae (Table 3) and 250 ppm for

wild larvae at the same exposure time (Table 4). Note that the control leads to 2% DMSO has no inhibitory effect on the larvae of strain Kisumu as wild, which continue to live during the 48 hrs of experience. Lethal concentrations responsible for 50% mortality of Kisumu and wild larvae in 24 hrs and 48 hrs are summarized in Table 5. At 24 hrs, the LC<sub>50</sub> (448.01 ppm) of the hydro-methanolic extract on the Kisumu larvae is followed by close to that of the *n*-hexane extract (529.36 ppm)

followed by that of the dichloromethane extract (1161.18 ppm) with *Elaeis oleifera*. This tendency is maintained in 48h of exposure, this time with a LC<sub>50</sub> (51.38 ppm) which is quite remarkable for the hydro-methanolic extract.

Conversely, *Elaeis oleifera* has a very moderate inhibitory effect on wild larvae, as evidenced by the doses inducing 50% mortality of the larvae: 4199.63 and

1456.44 ppm obtained in 24 hrs and 48 hrs respectively with the hydro-methanolic extract. It should be noted that the order of inhibition of the three extracts on the Kisumu and wild larvae for *Elaeis oleifera* is also maintained at *Launaea taraxacifolia*. On the other hand, the LC<sub>50</sub> obtained with the hydro-methanolic extract is almost the same: 157.36 and 116.88 ppm respectively in 24 hrs and 48 hrs on the wild larvae compared to those obtained in the Kisumu larvae (182.68 and 135.13 ppm).

**Table 5.** LC<sub>50</sub> values in ppm of extracts tested on larvae.

Plant	Extracts	LC <sub>50</sub> (Kisumu)		LC <sub>50</sub> (wild)	
		24 hrs	48 hrs	24 hrs	48 hrs
<i>Elaeis oleifera</i>	Dichloromethane	1161.18	790.63	10980.60	6817.71
	Methanol-water	448.01	51.38	4199.63	1456.44
	<i>n</i> -Hexane	529.36	411.42	8776.49	6367.31
<i>Launaea taraxacifolia</i>	Dichloromethane	2675.62	1951.75	2412.99	1833.65
	Methanol-water	182.68	135.13	157.36	116.88
	<i>n</i> -Hexane	729.08	614.94	834.23	747.94

*p*<0.05 significant level; Concentrations causing mortality of half of larvae.

## Discussion

The phenomenon of resistance to insecticides developed by *Anopheles gambiae* has prompted the search for phyto-larvicides which are beneficial to the environment and will target mosquitoes that are resistant to chemical insecticides. Indeed, although the integrated control program for *Anopheles gambiae* proposed by (Coosemans et al., 1992), which includes the use of deltameter-treated mosquito nets and repellent and/or intra-residential treatments, may have reduced malaria prevalence, these authors had shown that this method did not in any way reduce the density or the burden of the target parasite populations.

In this study we conducted bioassays of different extracts of *Launaea taraxacifolia* and *Elaeis oleifera* against wild-type and Kisumu-type 3<sup>rd</sup> instar mosquito larvae at different concentrations. Our results showed that the mortality rate of the larvae differs according to the concentrations and duration of exposure, whatever the species and the extract used. The lowest LC<sub>50</sub> are derived from hydro-methanolic extracts. These results, although somewhat preliminary, reveal the value of the hydro-methanolic extracts of *Launaea taraxacifolia* and *Elaeis oleifera* compared to the hexanic and dichloromethanic extracts. Moreover, similar studies carried out by Komalamisra et al. (2005) and Govindarajan (2010a) showed a larvicidal efficacy of the methanolic extract of *Rhinacanthus nasutus*, *Derris*

*elliptica* and *Ficus benghalensis* respectively on larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* compared to the solvents acetone and benzene. In addition, the highest dry extract yield obtained at the level of the two plants studied comes from the hydro-methanolic solvent. This result is the outgrowth of the presence of a majority of polar chemical groups responsible for the larvicidal activity shown by this extract in both plants compared to the extraction with hexane and dichloromethane. Indeed, the characterization of the major chemical groups in the two species *Launaea taraxacifolia* and *Elaeis oleifera*, revealed the presence of tannins, flavonoids, anthocyanins and leuco-anthocyanins which are exclusively polyphenols and triterpenes accessorially within both plants. *Elaeis oleifera* also contains mucilages and alkaloids that are not found in *Launaea taraxacifolia*. These results are in line with those obtained in the previous work carried out by Fagbohoun et al. (2015) and Koukouï et al. (2015).

From the set of the presented results, a first classification of the toxic efficacy of the extracts tested can be demonstrated; thus, the hydro-methanolic extract of *Launaea taraxacifolia* has an inhibitory effect on the two genotypes of larvae of *Anopheles gambiae* studied, whereas its *Elaeis oleifera* counterpart is only active on Kisumu larvae and has a very moderate inhibitory effect on the wild larvae. This tolerance of wild larvae to *E. oleifera* can be related to

the compounds of the alkaloid group characterized in this plant. Similar studies have been carried out on two species of *Senecio*: *S. jacobaeae* and *S. vulgaris*, the leaves of which contain a series of pyrrolizidinic alkaloids (Harry et al., 1994), have shown that larvae of certain lepidoptera such as *Arctia caja* and *Tyria jacobaeae* have developed a certain accommodation in the face of alkaloid toxins and managed to accomplish their complete development. It is possible that, despite the presence of polar compounds in this latter, tolerance to alkaloids, would contribute to the resistance observed by *Anopheles gambiae* in the presence of certain chemical insecticides from the group of organophosphates, pyrethroids and carbamates (Djogbenou et al., 2011; Edi et al., 2012; Nwane et al., 2013). Moreover, it could also be explained by a differential presence in structure and quantity of the larvicidal substances incriminated in the two species studied above.

The alkaloids found in *Elaeis oleifera* could thus influence the toxicity of active molecules in the wild larvae of *An. gambiae*. Indeed, at 500 ppm of hydroalcoholic extract, all Kisumu larvae die after 48 h of exposure, whereas to obtain the same result on the wild larvae, it will be necessary to apply thirty times the previous dose (15000 ppm). This selection of larvicidal activity on two genotypes of the same mosquito species shows that the use of *Elaeis oleifera* in the formulation of an insecticide against *An. gambiae* would be somewhat hypothetical. However, this species showed the lowest  $LC_{50}$  (51.38 ppm) of all the bioassays carried out during the 48 hrs of contact with the Kisumu larvae. This potential for inhibition, in line with the resistance of this plant attacks by the larvae of *Coelaenomenodera lameensis*, an oil palm leafminer, has been demonstrated by Fagbohoun et al. (2015). It is all the more interesting in terms of toxicity to larvae of anopheles Kisumu, as shown by Aouinty et al. (2006), with the lowest  $LC_{50}$ , 530 ppm and 600 ppm, those of extracts of *Tetraclinis articulata* and *Ricinus communis* on mosquitoes of *Culex pipiens*, *Anopheles caspius*, *Culex longiareolata* and *Anopheles maculipennis*.

Moreover, the hydro-methanolic extract of *Launaea taraxacifolia*, was more active on both larval categories regardless of the time of exposure, with the only difference that after 48 hrs of exposure of the larvae Kisumu, its  $LC_{50}$  (135.13 ppm) is significantly higher than that obtained with *Elaeis oleifera* (51.38

ppm). On the whole, we observe that the larvae of the wild population of *Anopheles gambiae* of Cotonou present approximately the same sensitivity to the larvicidal substances retained by the hydro-methanolic mixture of *Launaea taraxacifolia*, compared to the Kisumu larvae called "sensitive", with a lethal dose of 116.88 ppm. This dose reflects significantly lower toxicity than the methane extract of *Sida acuta* against mosquito larvae: *Anopheles stephensi* ( $LC_{50}$  = 38.64 ppm), *Aedes aegypti* ( $LC_{50}$  = 42.08 ppm) and *Culex quinquefasciatus* ( $LC_{50}$  = 47.91 ppm), with reference to the work of (Govindarajan, 2010b). However, it shows a very interesting activity compared to that obtained on *Anopheles stephensi* larvae through the studies carried out on the leaves of *Ajuga remota*: 330 ppm in 24 hrs and 290 ppm in 48 hrs (Preeti et al., 2004), as well as that carried out by Muema et al. (2016) in Kenya on larvae of *Anopheles gambiae* and *Anopheles arabiensis* with the methanolic extract of *Agerantum conyzoides* and gave  $LC_{50}$  = 232.70 ppm and 406.35 ppm in 24 hrs of treatment. Moreover, it should be mentioned that, apart from their traditional use as food, numerous studies (Adimonyemma et al., 2016; Gbadamosi et al., 2012) have proved a proven activity of *Launaea taraxacifolia* against microorganisms such as: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans* and *Proteus* spp.

In all the results presented, it can be deduced that, apart from its well-known nutritive properties, *Launaea taraxacifolia* possesses a very interesting larvicidal power with respect to *Anopheles gambiae*. The results of this study are encouraging in the direction of malaria eradication through anti-larval control using green chemistry. It is therefore necessary to continue this work in the interest of specifically identifying the compounds responsible for the larvicidal activity in this edible legume, in order to produce a bio-larvicide that is accessible to vulnerable populations.

### Conflict of interest statement

Authors declare that they have no conflict of interest.

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