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Research Article

Ricinus communis (CASTOR) as Larvicide on *Anopheles arabiensis* Patton

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ABSTRACT

This research is a study to evaluate the larvicidial effect of *Ricinus communis* (Castor); the Castor plant, leaves and seeds of the Castor were used to prepare hexane, ethyl acetate and ethanol extracts; different concentrations of the prepared extracts were then used to study their potency on mortality of 3rd instar larvae of the main vector of malaria in Sudan *Anopheles arabiensis* Patton; to a varying degrees, many of the chemical extracts showed larvicidal activities to *An. arabiensis*.

Inorder to compare the different plant extracts, concentration rates and exposure periods, Analysis of variance (ANOVA) was used. The Castor leaf ethyl acetate extract obtained as the best , where mortality among the larvae is found to be 96% after 24 hours with an LC_{50} at 0.390 mg/l , 100% mortality was observed after 48 hours with LC_{50}at 0.284 mg/l. Six fractions were obtained when A column chromatography was used to fractionate Castor leaf ethyl acetate extract ; one fraction among the fractions obtained, was found to have the highest larvicidal effect with 100% mortality after 24 hours at a concentration of 0.086 mg/l; the compounds of this fraction were determined using Gas Chromatography, as Linalool and Eugenol. The results are discussed regarding the similar researches.

KEY WORDS: *Anopheles arabiensis, Ricinus communis* Castor, Larvicide , Control.

1. INTRODUCTION

Mosquitoes constitute the most important single family of insects from the standpoint of human health. Due to their high potential to exploit even adverse environmental conditions, mosquitoes can rapidly increase their population [1]. *An. arabiensis* is the principal malaria vector in Sudan. It is the most widespread member of the *An. gambiae* complex, being endemic throughout most of the Afro- tropical region, extending northwards along the River Nile to 20 °N in Sudan [2]. *An. arabiensis* is the only known vector in Northern Sudan. It is well adapted to the semi arid and arid climate in the North of Sudan [3].

Vector control is undoubtedly necessary to prevent an epidemic when the conditions leading to a sudden increase in transmission or human exposure have been detected in an epidemic-prone area. Nevertheless, in any attempt to reduce the vectorial capacity in an endemic area, it is necessary to take into account the sustainability of the resulting change in endemicity [4].

Synthetic chemical larvicides continue to be applied for controlling mosquitoes in most parts of the world and offer quick and immediate solution of vector problems [5]. Many of these chemicals are toxic to human, plant and animal life, and resistance can be problematic in maintaining control, especially with organophosphate and pyrethroid larvicides. As a result, researchers are currently investigating natural subtances to use as insecticides for controlling larval mosquitoes. Phytochemical insecticides have

received much attention, as they are considered to be more environmentally biodegradable and considered safer than synthetic insecticides [6].

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of a variety of insect pests and vectors. Insecticidal activity of plant essential oils has been well-described by [7].

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent, and ovipositor attractant and have different activities observed by many researchers [8]; [9]. However, insecticides of plant origin have been extensively used on agricultural pests and recently extended, against insect vectors of public health [10].

2. MATERIALS AND METHODS

2.1 Biological materials

The study area is the state of Khartoum. *An. arabiens*is Larvae were collected from different localities in Khartoum State; these varied from large water bodies left after the rainy season to small pools resulting from broken water pipes cisterns and tanks. Rearing and maintenance of mosquito's larvae was as described by [11]. Mature leaves and seeds of Castor plant were collected from within the study area. The Plant materials have been collected, dried under shade at room temperature; and then ground to a fine powder using an electric grinder. The leaves powder was kept in a plastic bag. The same procedure was followed to obtain and keep the seeds powder.

2.2 Chemical extracts preparation

A conventional Soxhlet apparatus was used to prepare the different chemical extracts. 500 gms of the prepared powder of each plant part was first enrolled inside a filter paper; considerable volume of Hexane was added to a 2-litres Soxhlet round bottom flask. Approximately 50-60 siphonings were excuted during the 12 hours period. The flask was placed on a rotatory evaporator and the solvent was removed under vacuum at this stage, all non-polar substances like fats, lipids, waxes were considered to be extracted. The residue was then extracted using Ethyl acetate and furthermore was re-extracted in the Soxhlet using Ethanol as a polar solvent for 12 hours so as to extract all polar substances. The crude extract was kept in dark glass bottles covered tightly with aluminum foil, labeled then stored in a refrigerator till needed for experiments.

Concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l were then obtained by adding distilled water to the initial volumes to be completed to 250 ml then transferred to suitable dishes for experimentation.

Each of the Castor chemical extracts different concentrations were presented to bioassays and larval mortality tests to determine the LC_{50}. A number of 25 third instar *An. arabiensis* larvae for each concentration were used in all experiments. The sets of 25 larvae were introduced in 500 ml beakers containing the set concentration. The treatments were replicated three times, and each replicate contained a control of the relevant solvent. Dead larvae were counted after 24 hours, 48 hours, and 72 hours.

The extract which gave the highest mortality was fractionated using column chromatography (CC); tested by thin layer chromatography (TLC) to test the purity of the fractions. The fractions were then subjected to analysis by Ultraviolet spectroscopy (UV) performed on UV Shimadzu UV-210PC, Infrared spectroscopy (IR) taken as KBr pellets and a solution in Chloroform on Jasco A-302 Infrared Spectrophotometer. Also fractions subjected to Gas Chromatography/Mass Spectroscopy (GC/MS) trying to identify active ingredients acting as larvicide in Castor.

The results were subjected to normal descriptive statistical tests, size, variance and mean. The corresponding corrected mortalities were transferred to a Probit. Log-dose and the corresponding Probit were submitted to Probit analysis to calculate the regression equation, the correlation R^2 and the slope. From the regression equation, the LC_{50} was calculated. Computerized statistical analyses were done using Excel, Microsoft Office program 2003 and Statistical Package for Social Sciences, SPSS 13.0, 2004.

3. RESULTS

3.1 Larvicidial effect of Castor extracts on 3rd instar of *An. arabiensis*

Table (1) shows the LC_{50} of both Castor leaves and seeds extracts using hexane, ethyl acetate and ethanol in part per million^{-1}Litre.

Among the leaf extracts, it can be observed that, ethyl acetate extract was gained the lowest LC_{50} over the 24 hours exposure period; while hexane extract comes second followed by ethanol extract. Among the Castor seeds extracts, the ethanol extract has the lowest LC_{50} followed by ethyl acetate extract, and the last one was hexane extract.

Figure (1) sums up LC_{50} of the different extracts on 3rd instar larvae of *An. arabiensis* after 24 hours, 48 hours and 72 hours of exposure.

3.2 Castor leaf ethyl acetate extract Spectroscopy and Chromatographic test

Castor leaf ethyl acetate extract was introduced to Ultraviolet and Infrared spectroscopies, the spectrum obtained by those tests were considered in interpretation of the extract chemical consistency. Castor leaf ethyl acetate extract has absorbance of 3.984 (λ_{max}) at a wavelength of 208.00 nm,

The extract of Castor leaf ethyl acetate was subjected to a TLC test to know components; this test was carried out using an aluminum pre-coated TLC plate. Three drops of the extract were enabled to separate their chemical compounds (Figure 2 and table 2).

3.3 Column chromatography for Castor leaf ethyl acetate extract

The Castor leaf ethyl acetate extract shows high efficiency and gave rapid mortality in 24 hours exposure against 3rd instar larvae of *An. arabiensis*. In order to gain separated pure compounds of the extract, it was subjected to column chromatography of silica mesh; fractions were monitored by TLC and the similar ones were pooled together (Figures 3a and 3b).

3.4 Different fractions of Castor leaf ethyl acetate extract against *An. arabiensis* larvae

The Castor leaf ethyl acetate extract fractions obtained were tested separately against 3rd instar larvae of *An. arabiensis* **,** and the results are shown in table 3.

Table (3) and figure (4) show the larvicidal activity of the previously mentioned fractions as $F_1 – F_7$; the mortality results have been taken after 24 hours; the concentration of the tested fractions were selected according to the literature and similar works. It can be observed that, the range between the higher and upper limits of confidence was narrow; this may indicate a homogeneous individual response to the dose of larvicide. Mortality is found to be high and significantly different among the larvae, increased by increasing concentration and the results were significantly different from the control.

3.5 Determination of active ingredients

Among the fractions tested, fraction 3 (F_3) of the Castor leaf ethyl acetate fractions has the highest larvicidal effect on 3rd instar larvae of *An. arabiensis* giving LC_{50} of 125 part per million. So this fraction was chosen for further chemical analysis using a complex test carried out with Gas Chromatography in order to know the active ingredients. It was found that fraction 3 of Castor leaf ethyl acetate extract contains: Linoleic and Ricinoleic

4. DISCUSSION

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, repeated use of synthetic insecticides for their control has disrupted natural biological control systems and lead to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents [12] [13] [14] .

In the past the use of chemical insecticides, have resulted in the development of insecticide resistance in some important vectors of malaria, filariasis and dengue fever [15]. Insecticide resistance is now a major problem facing malaria vector control programs in most African countries [16]. Repeated use of chemical insecticides has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern [17] [18]; this initiated a search for alternative control measures. In recent years, the emphasis to control mosquito populations has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials, of botanical origin. For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent activities against mosquitoes[19] [20].During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to chemical insecticides [21]. The plant-derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment [22]. The recent increased interest in developing insecticides of plant origin as an alternative to chemical insecticides, provided the basis for many studies. In search of a natural larvicide, petroleum ether, carbon tetrachloride, and methanol extracts of *R. communis* (Castor) was tested for larvicidal activity against *Cx. quinquefasciatus.* Among the extracts tested, the carbon tetrachloride extract of *R. communis* was observed the most potent with LC_{50} at 144.11 ppm after 24 hours and 92.44 ppm after 48 hours and LC_{90} at 432.42 ppm after 24 hours and 352.89 ppm after 48 hours; the extract exhibited potential results and can be exploited as a preferred natural larvicide for the control of

mosquitoes [23] . This research aims to introduce a method for controlling *Anopheles arabiensis* Patton in their larval stages, using different chemical extracts of *R. communis;* which will hopefully eventually results in reduction of the disease.

The plant-derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic ones [24]. In recent years, the emphasis to control the mosquito populations has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials. [25], reported approximately 2,500 plants in 247 families with some sort of toxic property against insects. For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent actions against mosquitoes [19] [20]. Plants are considered as a rich source of bioactive chemicals and may be an alternative source of mosquito control agents. Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Although several plants have been reported for mosquitocidal activity, only a few botanicals have moved from the laboratory to field use most of the works are restricted to preliminary screening and determination of active principals is poorly characterized. However, there are a number of botanical insecticides being marketed which are extracted from Neem, Grapefruit seeds and Garlic, among other plants [26].

The plant origin extracts tested in this research can be arranged in a descending order of potency according to their 24 hours LC_{50} calculated in part per million/liter as follows: Castor leaf ethyl acetate extract , Castor seed ethanol extract , Castor leaf ethanol extract , Castor leaf hexane extract, Castor seed hexane extract, Castor seed ethyl acetate extract . Larvicidal effect of Castor leaf extract using hexane was tested against 3rd instar larvae of *An. arabiensis* ; there was highly significant difference between the graded concentrations within the exposure time. The LC_{50} was slightly decreased by time. The Probit mortality of the LC_{50} for the three exposure periods were found to be about 50%, which support the accuracy of the obtained values of the $LC_{50.}$ The 72 hours period gave the highest mortality. The larvicidal effects of Castor seed hexane extract against 3rd instar larvae of *An. arabiensis;* behaved in the same manner as leaf extract.

The ethyl acetate extract of Castor leaves, is the strongest of them all, the second is the ethanol extract, and the least is the hexane extract. While, regarding the Castor seeds extracts ethanol extract is the strongest of them, followed by hexane extract, and the least is the ethyl acetate extract. In general, the leaf extracts are stronger compared to the seed extracts; however, the lower the concentration of an extract, the best is the insecticide, because less quantities are needed and may be less time. Castor leaf ethyl acetate extract caused high mortality of larvae (LC_{50}0.390 g/L) for the first day; and 100% mortality at a concentration 1.0 mg/L for the second day, and 100% mortality at a concentration 0.8 mg/L for the third day the (LC_{50} was 0.284 g/L) after the second day and (0.228 g/L) after 3 days. However, there is high significant difference between the graded concentrations within the exposure time ($p<0.001$). In general, the leaves extracts are stronger than the seeds extracts in larvicidial effect and this might be due to the high protein content of seeds [27]. Castor seeds have high protein content [28]. In other study the *R. communis* crude extract prepared from plants found in the southern African floral region was investigated for its larvicidal effect and found to be active on larvae of *An. arabiensis* [29]. Mucilage which is a complex carbohydrate with a great capacity to absorb water; this complex is found in great amounts considering the weight of Castor seeds ; it is well known that, the complex carbohydrate can easily reduce the effect of various functional groups of chemicals. Per 100 grams, the Castor leaves are reported to contain 57.4 g total carbohydrate, 24.8g protein, 12.4 g ash, 10.3 g fiber, 5.4 g fat, 0.5 g P and 0.003 g Ca. The seed contains 45.0–50.6% oil, 23.1–27.2% CF, 12.0–16.0% protein, 5.1–5.6% moisture, 3.1–7.0 % NFE and 2.0–2.2% ash. Seeds are high in phosphorus, about 90% in the phytic form. [30]

Running after controlling mosquitoes, many efforts have been paid to obtain active ingredients from Castor plant (*Ricinus communis)* from which the compound juvocineme II is extracted and which later on served as the model for synthesis of Piriproxifen and Fenoxicarb. Besides, there are the synthetic copies of natural active ingredients like Neonicotinoids where Imidacloprid stands out [26].

Generally, the toxicity of Castor on 3rd instar larvae of *An. arabiensis* has been observed to determine the LC_{50} of both leaves and seeds of Castor extracts using hexane, ethyl acetate and ethanol in ppm/L.

From the foregoing results, it is justifiable to take Castor leaf ethyl acetate extract to further analysis. Castor leaf ethyl acetate was introduced to Infrared (IR) spectroscopy; IR spectra were taken as KB_r pellets and a solution in Chloroform on Jasco A-302 IR spectrophotometer; the spectra obtained by this

test were considered in interpretation of the extract chemical consistency. Castor leaf ethyl acetate extract has absorbance of 3.780 (λ_{max}) at wavelength of 281.00 nm. Also Castor leaf ethyl acetate was introduced to Ultra Violet (UV) spectroscopy performed on UV Shimadzu UV-210PC. The spectrum obtained by this test was considered in interpretation of the extract chemical consistency. The spectra and the peaks height values obtained from IR and UV were compared to the stated data of the similar works and were found comparable.

Seven fractions were obtained, one of them showed the highest effect against 3rd instar larvae of *An. arabiensis.* This fraction found to contain: Linalool, Eugenol in addition to small quantities of Cinoele estragol, Limonine and Methyl chavicol.

Several works revealed isolated compounds from plants and studied their larvicidal effect such as: [31][32][33]. [34], studied larvicidal activity of the *terpenoid naphthylisoquino* in *An. stephensi*; [35], studied mosquitocidal compounds and a triglyceride,1,3-dilinoleneoyl-2-palmitin,from *Ocimum sanctum*; [36], studied larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species; [37], studied larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana* seed; [38], studied the effects of *Pelargonium citrosa* leaf extracts on malarial vector *An. stephensi*; [39], studied synthesis and absolute configuration of cordiaquinone, which is meroterpenoid larvicide isolated from *Cordia curassavica* and [40], has studied mosquitocidal activities of leaf oil and Its constituents from *Calocedrus macrolepis* .

Resistance to synthetic Insecticides and polluting the environment are challenges facing vector control; therefore botanical insecticides may serve as efficient, low cost powerful larvaicides and a suitable alternative biocontrol techniques in the future.

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Table (1): LC_{50} of the Castor different extracts on the 3rd instar larvae of *An. arabiensis* after 24, 48 and 72 hours of exposure

Part used	Extraction medium	LC_{50} part per million^{-1}Litre		
		24 Hrs	48 Hrs	72 Hrs
Leaf	Hexane	0760	0516	0394
	Ethyl acetate	0439	0307	0237
	Ethanol	1108	0981	0747
Seed	Hexane	0851	0486	0348
	Ethyl acetate	1216	0876	0680
	Ethanol	0763	0573	0404

Table (2): Chromatographic separations of Castor leaf ethyl acetate extract (Based on Figure 2).

Band No.	Colour in visible light	Colour under UV	Colour after spray with (KOH alcohol)
1	Yellowish	Colourless	Yellow
2	Yellowish	Colourless	Yellow
3	Light Brown	Light blue	Colourless
4	Light Green	Light Red	Brown
5	Green	Red	Light Green
6	Dark Green	Red	Green
7	Brown	Yellow	Brown

Table (3): Mortality rate of different concentrations of Castor Leaf Ethyl acetate extract fractions against *Anopheles arabiensis* larvae

fraction Number	fraction concentration (µg/mL)	Mortality %	LC_{50}	95% Confidence limits (µg/ml)		LC_{90}	$\chi 2$	*p*
				Lower	Upper			
F_1	Control	**0**	216	190	285	353	2.960	0.085
	100	**34**						
	200	**41**						
	400	**69**						
F_2	Control	**0**	612	501	806	1353	0.180	0.671
	100	**14**						
	200	**25**						
	400	**34**						
F_3	Control	**0**	125	107	142	315	0.274	0.601
	100	**37**						
	200	**76**						
	400	**94**						
F_4	Control	**1**	385	281	498	532	0.164	0.686
	100	**23**						
	200	**38**						
	400	**52**						
F_5	Control	**0**	4130	1007	7389	9088	0.237	0.627
	100	**10**						
	200	**17**						
	400	**21**						
F_6	Control	**0**	300	217	524	624	0.003	0.955
	100	**31**						
	200	**43**						
	400	**55**						
F_7	Control	**0**	1712	1053	2859	3474	0.003	0.958
	100	**20**						
	200	**24**						
	400	**29**						

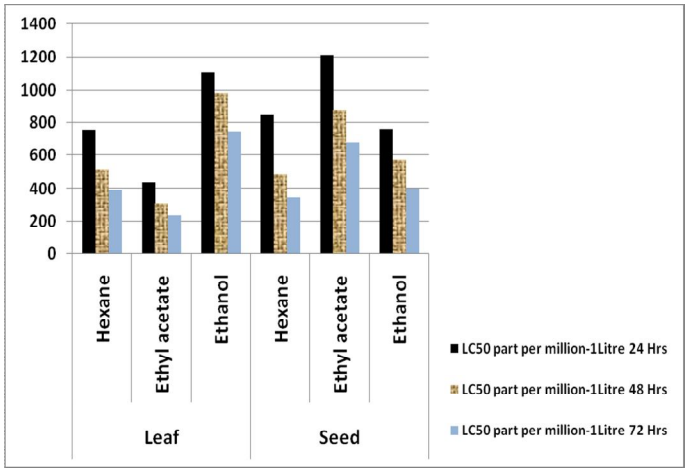

Figure (1): LC_{50} of the Castor different extracts on the 3rd instar larvae of *An. arabiensis* after 24, 48 and 72 hours of exposure

Figure (2): the TLC plate showing separation of Castor leaf ethyl acetate extract under UV

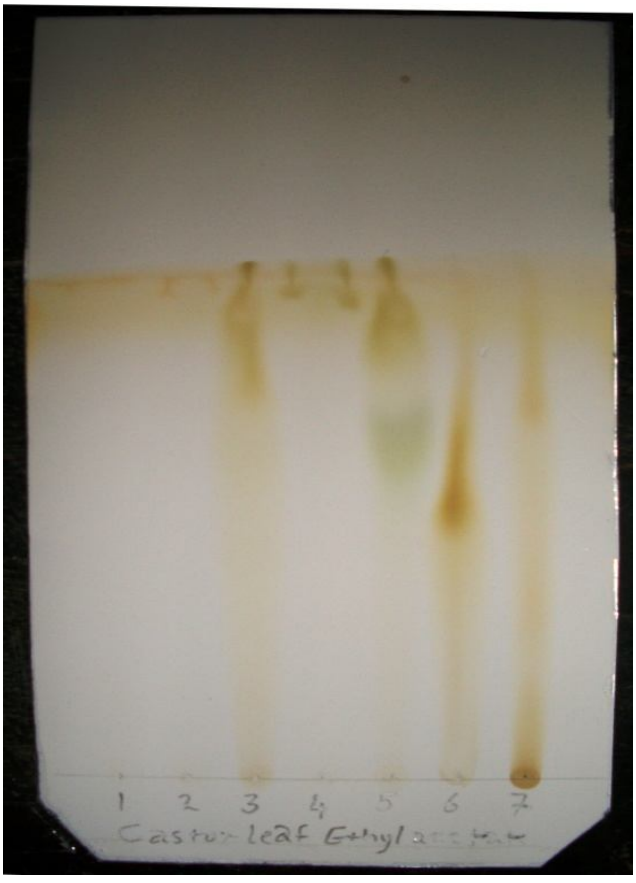

Figure (3a): TLC plate showing separation of fractions of Castor leaf ethyl acetate extract under visible light.

Figure (3b): Fractions separated from Castor leaf ethyl acetate extract

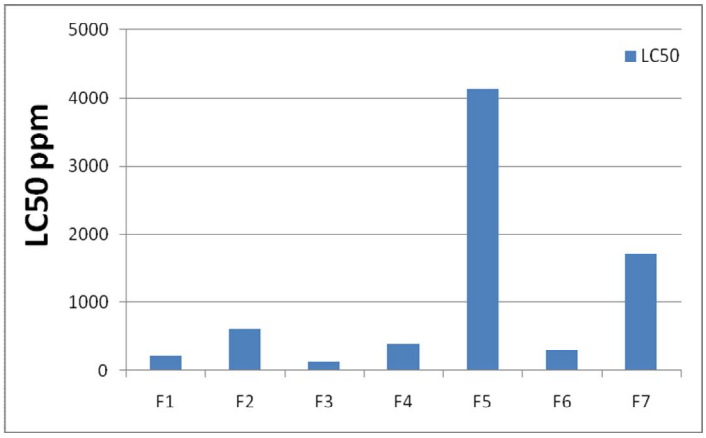

Figure (4): Mortality rate of different Castor Leaf Ethyl acetate extract fractions against *Anopheles arabiensis* larvae

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