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Investigation the Lethal Effect of *Colotropis procera* Ait Leaves Extracts Against *Aedes aegypti* (L) Larvae

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ABSTRACT

The research objective was to examine the lethal effects of ethanol, aqueous, and hexane extracts of (*Colotropis procera Ait*) against *Aedes aegypti* (L) larvae. The leaves of *Colotropis procera* were collected in Riyadh and transported to the Biology Department, College of Science, Imam Mohammad Ibn Saud Islamic University, where they were cleaned manually and dried in the shade at room temperature. The leaves of *Colotropis procera Ait* were subjected to preliminary phytochemical screening to identify the chemical constituents. The method described by AOAC,1990. The experiments were carried out for the bioassay tests at 27± 2^{0}C and 75–85% relative humidity under a 12 L/D photoperiod. The procedure by WHO, 1996, was utilized to find out the larvicidal activities of leaves, aqueous, ethanol, and hexane extracts of *C. procera*. The regression lines at different fatal concentrations, i.e., 95% and 50% (LC_{95} and LC_{50}), were used to analyze the mean *Aedes aegypti* larval mortality after 24 hours. The result shows the phytochemical screening of the presence of tannins, flavonoids, cardiac glycosides, alkaloids, phenols, and terpenoids where saponins and steroids were absent in *C. procera* leaves. The relative efficiency of aqueous, ethanol, and hexane extracts according to the obtained $LC_{50,}$ and LC_{95} values showed that *Calotropis procera* hexane extract had relatively higher larvicidal potentiality against *Aedes aegypti* larvae (0.00250 ppm) and ethanol extract (0.00251 ppm), followed by aqueous extract (0.0028 ppm). *Calotropis procera* can be employed substantially as an eco-friendly larvicide resource with significant toxicity against *Aedes aegypti* larvae mosquito vectors.

Keywords: Lethal effect, (*Colotropis procera Ait*), Leaves extracts, *Aedes aegypti.*

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INTRODUCTION

Aedes aegypti (L) is an anthropophilic mosquito that transmits several viral pathogens worldwide, including dengue, chikungunya, yellow fever, and Zika [1]. The = Aedes aegypti mosquito was the dengue epidemic vector in Saudi Arabia and Yemen in the 21st century. The climate (especially ambient temperature and precipitation) and the population of the distributing vector (Aedes aegypti mosquito) are proportional to Dengue epidemiology and the risk of a disease outbreak. To develop dengue control strategies, we need to familiarize ourselves with the vector population ecology and phylogenetics adequately. Evolutionary history of *Aedes aegypti* from the Arabian Peninsula, the most eastern part of the Afrotropical zone bordering the Palaearctic and Oriental zones. To execute prevention programs on national and worldwide scales, we must better understand the transmission of dengue [2]. Mosquito-borne pathogens are a major cause of disease in the Kingdom of Saudi Arabia. Effective vector control and disease prevention necessitate knowledge of the mosquito fauna. The Kingdom has 51 species recorded; however, the occurrence of two of these species is unlikely. Thus, the Kingdom's mosquito fauna consists of 49 species, including 18 anophelines and 31 culicines [3]. Dengue fever (DF) transmission rates in Saudi Arabia have increased significantly since 1994. (KSA).

The climate, geography, and demographics of KSA make it an ideal location for the spread of DF. Nonetheless, the curbing mechanisms for the Aedes species transmitting the DF virus (DENV) are inadequate. It is paramount to determine the natural locale of the Aedes species in Makkah Al-Mokarramah, KSA, to devise efficient management methods [4, 5]. Insecticide treatments are less effective on wild larvae than on laboratory-bred larvae. These findings suggest that *Aedes aegypti* populations in Makkah City, Saudi Arabia, have tolerance and a proclivity for resistance to commonly used insecticides [6]. The development of resistance is not a new phenomenon; it is becoming a global issue [7]. Dengue fever is a serious public health problem caused by *Aedes spp*. mosquitos. Current vector control methods are ineffective at reducing Aedes populations and thus reducing dengue transmission. As a result, new tools and strategies to reduce dengue transmission in various settings are urgently needed [8]. Biological control (biocontrol) techniques use an organism's natural enemies, predators, parasites, and pathogens to control it. Biological control agents for mosquitos have been discovered and used worldwide [9, 10]. Consequently, mosquito control that focuses on killing mosquito larvae or depriving them of breeding sites makes a lot of sense. It is easy to kill the mosquito larvae while still confined to the water, a medium contained in a finite, easily-managed area [11]. Control methods aimed at the larval stage are the most effective. Successful larvae control may not reduce mosquito numbers or biting activity [12]. Giant milkweed (*Calotropis procera* Ait) favors open habitats with little competition. Most of the overgrazed range land and pastures meet this stipulation. Other conventional locales include neglected urban lots, beachfront dunes, and roadsides [13]. Various illnesses can be treated with the root, including; malaria, leprosy, fever, and snake bite. In sensitive persons, the toxicity of latex can lead to blisters and rashes. It is recommended as a host plant for butterflies [14]. Their active ingredients were classified as cardio-glycosides. Cardiac glycosides are an important class of natural products whose actions include both beneficial and toxic effects on the heart. Since 1500 B.C., plant cardiac steroids have been used as heart drugs and poisons. These applications stretch from their use as arrow poisons, emetics, and diuretics, to even as heart tonics [15]. Natural products and biological agents could be used to control mosquitoes and solve the problems they cause. Plant secondary metabolic pathways generate a wide range of bioactive molecules. Most of these molecules were developed using traditional medical knowledge, and often a correspondence exists between the traditional crude extracts and the current pure substances. Plants are a rich source of new pharmacologically active compounds. Most antimicrobial and antifungal agents are still sourced from plants. Despite advances in antimicrobial therapies, many issues with most antimicrobial drugs remained unresolved [16]. In contrast to plant-borne products recently developed to kill or repel mosquito adults and other key arthropod species of medical and veterinary importance (e.g., ticks and lice), the development of commercial botanical mosquito larvicides is severely limited. More research on these topics is desperately needed [17]. Several natural compounds have been proposed as alternatives to traditional chemical control [18]. The different medicinal properties of Calotropis make it well known, spanning 175-180 genera and 2200 species in the tropics and subtropics. Most of them contain active molecules. Calotropis is a small genus of six shrubs or small trees found in Asia, North America, and tropical and subtropical Africa. In India, C. procera and C. gigantea are structurally and functionally like each other [19]. The objective of the current research was to examine the lethal effects of ethanol, aqueous, and hexane extracts of (*Colotropis procera Ait*) against *Aedes aegypti* (L) larvae.

MATERIALS AND METHODS

Plant collection

The leaf samples were collected Riyadh area and transferred to the laboratory of the Biology Department, College of Science, Imam Mohammad Ibn Saud Islamic University, and cleaned manually and dried under shadow at room temperature $27C^0$. *Colotropis procera* Ait leaves were crushed separately in a blender for five minutes and powdered, then kept until used.

Phytochemical screening of the extract

The leaves of *Colotropis procera Ait* were subjected to preliminary phytochemical screening to identify the chemical constituents. The method described by AOAC (1990) [20] was used for screening Tannins, Flavonoids, Cardiac glycosides, Alkaloids, Phenols and Terpenoids, Saponins, and steroids.

Extract preparation

Aqueous extract preparation

20 g of crushed plant materials were weighed separately, shaken with 100 ml of distilled water, and kept overnight. After 24 hours, the mixture was homogenized using a magnetic homogenizer and filtered with Whatman No. 42 filter papers. The filtrate was used to prepare a concentration of aqueous extracts and stored under 5 C◦ in a refrigerator.

Preparation of ethanol and hexane extracts

The leaves parts of *C. procera* were used. 20 g of each sample was added to 100 ml of ethanol and hexane to prepare the corresponding extracts after 24 hours. The plant extract of each solvent/part was left in the supernatant, which was filtered, evaporated, and then collected as dry powder, weighed, and made up to the original concentrations before use.

Breeding site

Mosquito larvae were collected in plastic trays containing tap water from breeding sites around Riyadh. The larvae collected were transferred to the Biology Department, College of Science, Imam Mohammad Ibn Saud Islamic University. Other mosquito species and aquatic predators accidentally collected with *Aedes aegypti* mosquito were immediately removed from the rearing dishes. Larvae were fed usually on fish food (already prepared food) or sometimes little quantity of yeast granules. The formed pupae were immediately transferred to the adult cage by using a suitable dropper. The transferred pupae were put in a similar rearing dish inside the adult cage.

Bioassay tests

The experiments were carried out at 75–85% Relative humidity and a mean temperature of 27 ± 2^{0}C under a 12 L/D photoperiod. Larvicidal activities of leaf extracts of *C. procera* were determined by following the method of WHO (1996) [21]. Twenty of the 3rd and early 4th instar larvae of *Aedes aegypti* were moved through a dropper to test cups, each containing 250 ml tap water, and applied with a known concentration (1ml-1.5ml-2ml-2.5ml-3ml and 5ml) of each of the aqueous, ethanol and hexane extracts. Each concentration was replicated thrice for each applied extract. The control batch was also designed last and kept alive. After 24 hours the larval mortality was tallied.

Statistical analysis

The Y variable (mean larval mortality after 24 hours) was plotted against the X variable (the corresponding concentrations) to the regression analysis using Microsoft excel 2016. The regression lines were created to determine the lethal concentrations of 50% and 95% (LC_{50} and LC_{95}) on *Aedes aegypti* larvae.

RESULTS AND DISCUSSION

Phytochemical constituents of C. procera leaves

Table 1 shows the phytochemical screening of the presence of tannins, flavonoids, cardiac glycosides, alkaloids, phenols, and terpenoids where saponins and steroids were absent in *C. procera* leaves.

Table 1. *C. procera* leaf phytochemical constituents

Compound	Leaves
Tannins	+
Saponins	-
Flavonoids	+
Steroids	-
Glycosides	++
Alkaloids	+
Phenols	+
Terpenoids	+

+: present, −: absent

The effect of Aqueous extract preparation of C. procera leaves on Aedes aegypti larvae

The susceptibility of *Aedes aegypti* was tested by aqueous extract preparation for a 24 hr submission period at the doses of 0.0028, 0.0042, 0.0060, 0.0069, and 0.0080 ppm. The resulting percentage mortalities were 55, 65, 75, 80, and 85, respectively **(Table 2)**. The doses reflected an LD_{50} of 0.0028 ppm and LD_{95} of 0.011 ppm. 55% mortality was realized in the lowest dose (0.0028 ppm), whereas 85% mortality was noted in the

highest dose (0.0080 ppm). The R-square was 0.79. The standard error of the log dose SE(Y) was 1.47, whereas SE(X) was 0.65.

The Aqueous extract proved to be toxic against *Aedes aegypti* larvae.

Table 2. The effect of Aqueous extract preparation on *Aedes aegypti* larvae

DOSE		MORTALITY %		PROBIT	
ppm	Log+3	Tested	Corrected	Tabulated	Calculated
0.0028	0.45	55.0	55.0	5.13	5.00
0.0042	0.63	65. 0	65.0	5.39	5.46
0.0060	0.78	75.0	75.0	5.67	5.85
0.0069	0.84	80.0	80.0	5. 84	6.00
0.0080	0.91	85.0	85.0	6.04	6.18

Control mortality was 0.0% in all cases; regression equation: Y = 11.56 + 2.75X, SE(Y) = 1.47, SE(X) = 0. 65, R-square = 0.79, LD_{50} = 0.0028 ppm, LD_{95} = 0.011 ppm

The effect of ethanol extract preparation of C. procera leaves on Aedes aegypti larvae

The susceptibility of *Aedes aegypti* larvae at the doses of 0.0011, 0.0017, 0.0022, 0.0028, 0.0032, 0.0038, 0.0048, and 0.0063 ppm was tested by ethanolic extract preparation. The results percentage mortalities were 20, 25, 40, 45, 70, 75, 80, and 85, respectively) **(Table 3)**. The doses reflected an LD_{50} of 0.00251 ml/L and an LD_{95} of 0.0096 ppm. The lowest dose (0.0011 ml/L) produced 20% mortality, whereas the highest dose (0.0063 ppm) reflected *ca*. 85% mortality. The R-square was 0.92.

Table 3. The effect of ethanol extract preparation of *C. procera* leaves on *Aedes aegypti* larvae

DOSE		MORTALITY%		PROBIT	
Ppm	Log+3	Tested	Corrected	Tabulated	Calculated
0.0011	0.04	20.0	20.0	4.16	3.98
0.0017	0.24	25.0	25.0	4.33	4.54
0.0022	0.35	40.0	40.0	4.75	4.85
0.0028	0.45	45.0	45.0	4.87	5.13
0.0032	0.55	70.0	70.0	5.52	5.30
0.0038	0.57	75.0	75.0	5.67	5.47
0.0048	0.68	80.0	80.0	5.84	5.78
0.0063	0.8	85.0	85.0	6.04	6.12

Control mortality was 0.0% in all cases; regression equation: Y = 12.33 + 2.82X, SE(Y) = 0.81, SE(X) = 0.31, R-square = 0. 92, LD_{50} = 0.0251 ppm, LD_{95} = 0.0096 ppm.

The effect of hexane extract preparation of C. procera leaves on Aedes aegypti larvae

The susceptibility of *Aedes aegypti* larvae at the doses of 0.0011, 0.0017, 0.0022, 0.0028, 0.0032, 0.0038, 0.0048 and 0.0063 ppm. The resulting percentage mortalities were 25, 35, 40, 50, 55, 65, 75, and 85, respectively **(Table 4)**. The doses reflected an LD_{50} of 0.00250 ppm and an LD_{95} of 0.0296 ppm. 25% mortality was noted from the lowest dose (0.0011 ppm), whereas the highest dose (0.0063 ppm) reflected a mortality rate of 85%. The R-square was 0.96. The hexane preparation proved to be toxic against *Aedes aegypti* larvae

Table 4. The effect of hexane extract preparation of *C. procera* leaves on *Aedes aegypti* larvae

DOSE		MORTALITY%		PROBIT	
ppm	Log +3	Tested	Corrected	Tabulated	Calculated
0.0011	0.04	25.0	25.0	4.33	4.17
0.0017	0.24	35.0	35.0	4.61	4.62
0.0022	0.35	40.0	40.0	4.75	4.87
0.0028	0.45	50.0	50.0	5.00	5.10
0.0032	0.55	55.0	55.0	5.13	5.24
0.0038	0.57	65.0	65.0	5.39	5.38
0.0048	0.68	75.0	75.0	5.67	5.63
0.0063	0.8	85.0	85	6.04	5.90

Control mortality was 0.0% in all cases; regression equation: Y = 10.93 + 2.28X, SE(Y) = 0.47, SE(X) = 0.18, R-square = 0. 96, LD_{50} = 0.0251 ppm, LD_{95} = 0.0296 ppm.

Effective relatives of Calotropis procera preparation according to LD_{50}

The relative efficiency of aqueous, ethanol, and hexane extracts, according to the obtained LC_{50} and LC_{95} values, showed that hexane had relatively higher larvicidal potentiality against *Aedes aegypti* larvae (0.00250ppm) and ethanol extract (0.00251ppm followed by aqueous extract (0.0028 ppm) show **Table 5** and **Figure 1**.

Table 5. Effective relatives of *Calotropis procera* preparation according to LD_{50}

Preparation	*Aedes aegypti* larvae	
	LD_{50} /ppm	LD_{95} /ppm
Aqueous extract	0.0028	0.011
Ethanol extract	0.00251	0.0096
Hexane extract	0.00250	0.0296

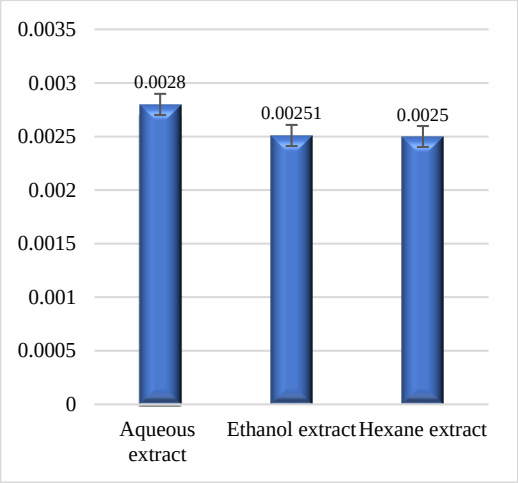

Figure 1. Histograms of the obtained LC_{50} values of all extracts on *Aedes aegypti* larvae

Pesticides, insect growth regulators, and microbial agents are commonly used to target mosquito larvae. In addition, bed nets treated with insecticide and indoor residual spraying methods are utilized. However, these chemicals harm human health and the environment and cause resistance in various vectors [22]. Finding "green" insecticide alternatives is therefore critical. The Saudi Arabian plant Calotropis procera (Aiton) Dry and (Apocynaceae) was selected for the study. LC-MS/MS was used to examine the metabolic contents of various *C. procera* extracts. Cardenolides such as calactin, uscharidin, 15-hydroxy-calactin, 16-hydroxy-calactin, and 12-hydroxy-calactin were abundant in *C. procera* leaves [23]. Secondary metabolites produced by plants such as C. procera have a wide range of physiological and biological activities, including deterrent and antifeedant activity [24]. Phytochemical screening and biological activity of *Calotropis procera* were investigated by Doshi *et al*. (2011) [25], and the obtained results of the phytochemical screening were presented. The result obtained by the current study was consistent with previous findings. The aqueous leaf extract of *C. procera* showed a high level of toxicity against the larvae of mosquitoes *An. arabiensis* and *Cx. quinquefasciatus*. The 50% mortality (LC_{50} values) was shown at 273.53, 366.44, and 454.99 ppm for the 2nd, 3rd, and 4th instar larvae, respectively, of *An. arabiensis* and 187.93, 218.27 and 264.85 ppm for 2nd, 3rd and 4th instar larvae, respectively of *Cx. quinquefasciatus* [26]. This result supported the result obtained in the current study, which found that larvicidal potentiality against *Aedes aegypti* larvae of aqueous extract was (0.0028 ppm) considering the difference in mosquito species. The present study divulges the possibility of phasing out harmful chemical pesticides with the aqueous extract of this leaf, owing to its natural larvicidal product [27]. Milkweed (*Calotropis procera*) fresh leaf extract was discovered to have larvicidal characteristics against Fresh leaf extract was discovered to have larvicidal characteristics against Diptera order, especially mosquito larvae. However, methanolic extracts of the same plant were more efficient as a larvicide. However, methanolic extracts of the same plant were more efficient as a larvicide [28]. This result supports our finding that the toxicity of *Calotropis procera* had larvicidal potentiality against mosquito larvae. *Calotropis procera* extract at 0.6 mg/mL had the highest mortality rate of 100% for L1, L2, and L3 cells of *Culex quinquefasciatus. Calotropis procera* extract had LC_{50} values of 0.194, 0.251, 0.258, and 0.284 mg/mL for L1, L2, L3, and L4, respectively. The results of the larvicidal bioassays revealed that these plant extracts have significant larvicidal properties [29]. Plant extract may guide the development of new entomological surveillance and control methods for *Aedes aegypti* [30]. Hexane extracts were less effective than the chloroform extracts of *A. indica* and D. metal against the late-third instar larvae a day after treatment. Respectively, they resulted in larval mortality rates of 62% and 87, at 1000 ppm concentration. The larvicidal effect of *A. indica* and *D. metel* against *C. quinquefasciatus* poises these plant products to be alternatives to synthetic insecticides in the plans to control mosquitoes [31]. *C. procera* leaves were extracted in hexane and tested for larvicidal activity against dengue vectors. Larvicidal bioassays with *C. procera* hexane leaf extract revealed efficacy with LC_{50} and LC_{90} values of 78.39 and 100.60 ppm, respectively. After prolonged exposure of the larvae to the extracts, the toxicity potential of the extract increased, with the LC_{50} values decreasing by 2.3%. *C. procera* extract caused increased wriggling speed and violent vertical movements in larvae [32].

The best repellent effect was obtained from leaves of *C. procera* (weight loss = 0.034%) and left a good anti-termite [33]. However, in our finding, when using hexane, the result showed a

higher larvicidal potentiality against *Aedes aegypti* larvae (0.00250ppm) between tested solvents; this finding is similar to earlier studies. In addition, the hexane extract of the leaf produced LC_{30}, LC_{50}, and LC_{90} values of 67, 83, and 140 ppm, respectively, while exposure to hexane extract of the stem had values of 55, 68, and 115 ppm. Extract-exposed larvae showed significant damage, shrinkage, distortion, and vacuolization of gut tissues and peritrophic membranes at various lethal levels [34]. At 500 microg/ml, hexanoic and ethanolic extracts from 27 plant species from Brazil's Cerrado biome were tested for larvicidal activity against 3rd-stage *Aedes aegypti* larvae. Fourteen extracts from seven species were active against the larvae (>65% mortality). At 56.6, 162.31, 232.4, 285.76, and 384.37 microg/ml, respectively, *Dugeutia furfuracea, Piptocarpha rotundifolia*, *Casearia sylvestris* var. lingua, *Serjania lethalis*, and *Xylopia aromatica* were active. *Annona crassiflora* and *Cybistax antisyphilitica* were active at 23.06 and 27.61 microg/ml, respectively. The isolation of active chemical compounds can be done based on the species' larvicidal properties described [35]. All plant extracts used, showed larvicidal activity, however there was a significant difference between the ethanolic extract and the aqueous. The characteristic relative safety, degradability, and abundance in many areas of the world set the use of plant-derived insecticides as a suitable alternative to chemical insecticides in the near future [36]. Plant extracts with proven insecticidal properties can be used instead of these pesticides [37]. The current research showed that toxicity in ethanol extract (0.00251ppm had activity against Aedes aegypti larvae. These results were similar to the developments in previous studies with differences in LC_{50} due to different environments. Since ancient times, Plant-derived active toxic agents have been used as an alternative mosquito control strategy. Various vector mosquitoes have been controlled by the target-specificity of the non-toxic, inexpensive, biodegradable, and have a broad spectrum. Much research focused on phytoconstituents sources and quinoidal activity, their action mechanism on the target populations, instar specificity, variation in larvicidal activity by mosquito species, the extraction-solvent polarity, nature of the active ingredient, and promising advances made in biological control of mosquitoes by plant-derived secondary metabolites [38]. The emergence of resistance to synthetic insecticides poses a huge threat to vector control techniques. Plants are rich sources of bioactive compounds and synthesize several secondary metabolites to severe as defensive chemicals for controlling insect pests. Plants are advantageous over synthetic pesticides, therefore increasing their preference for use over chemical insecticides [39]. Components and metabolites have low bioavailability and poor host solubility [40]. Create and direct the most effective material for the behaviour faced [41]. Populations of the insects of the same species in different environments provide chances to keep a large adaptive genetic base, which may or may not progress to full speciation [42]. For the previous reason, mosquitoes became resistant to many pesticides, therefore discovering new materials as pesticides from plants is necessary. Synthetic pesticide applications are an important tool for managing pests, but they have negative effects on the environment and are incompatible with organic agriculture [43]. The biological data collected through breeding and fieldwork are compared and discussed in relation to known life-cycle data [44, 45]. The previous method is reasonable for studying the sensitivity of plant extracts as insecticides.

CONCLUSION

According to the obtained LC_{50} and LC_{95} values, the current study concluded that *Calotropis procera* hexane extract had relatively higher larvicidal potentiality against *Aedes aegypti* larvae (0.00250ppm) and ethanol extract (0.00251ppm followed by aqueous extract (0.0028 ppm). *Calotropis procera* is a highly toxic larvicide against *Aedes aegypti* larvae mosquito vectors, and due to its eco-friendly sources, it can be expanded to a wider scale of use.

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