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Larvicidal potential of *Calotropis procera* against *Aedes aegypti*

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Abstract
The present study was conducted to know the larvicidal effects of native plant, *Calotropis procera* (Giant milkweed). Leaves and stem of the plant were used to check the larvicidal activity. Microwave assisted extraction was used for material extraction. Different concentrations (20%, 40%, 60%, 80% and 100%) of the leaf and stem extract were used. Mortality of the larvae was observed after twenty four hours. Results showed the effectively of higher concentrations was greater as compared to lower concentrations. Leaf extract was found to be more significant in killing the larvae while the stem extract showed less significant results. It is concluded that all parts of this plant can be used for the prevention and control of *A. aegypti* mosquitoes due to its pesticide properties and bioactive chemicals.

Keywords: Mosquito, microwave assisted extraction, mortality, *Calotropis procera,* larvicidal activity

1. Introduction
Mosquitoes as vector have great concern to public health as they transmit different diseases dengue fever, yellow fever, malaria, chikungunya and filariasis causes great mortality in tropical and subtropical regions [1]. Dengue is a disease caused by dengue virus serotypes 1 to 4 (DENV-1 to DENV-4) and is transmitted by *Aedes aegypti* mosquitoes [2]. *Aedes aegypti* is a tropical mosquito, found in greatest profusion in Central Africa, from where it is originated [3]. Dengue infection starts with simple fever that leads to severe and sometimes fatal dengue haemorrhagic fever (DHF) the other type is dengue shock syndrome (DSS) [4]. Symptoms of usual simple dengue usually start with fever within 4 to 7 days after bitten by an infected mosquito. Through the bites of infective female *Aedes- aegypti* mosquitoes, Dengue viruses are transmitted to the vulnerable human host [5].
Ovitrap surveys could be considered a sensitive and an efficient technique for detecting and monitoring *Aedes* populations at low densities. They are economical, safe and environment-friendly [6]. There is no specific treatment for classic dengue fever. Control has mostly been approached by source reduction: the eradication of containers that are favorable sites for oviposition and development of the aquatic stages [7].
Use of chemical insecticides is one of the widely used approaches to control the mosquito population. Mosquitoes have been developed resistance against these commercial chemical used as insecticides [8]. Commercial insecticides greatly affect the living beings and environment as well. There is an imperative demand to search novel eco-friendly substitutes which are most active and cost effective than the synthetic insecticides. Plants have a great diversity of naturally occurring biologically potent chemicals that can be used as larvicidal agents, plant based biochemicals have wide range activity against target species and are considered safe for environment [9].
The plants and their products have greatly been employed from many decades for controlling the insects. Numerous plant secondary metabolites protect the plants against the insect attacks. These biologically potent phytochemicals have several activities including larvicidal activity. Plant based insecticides are non-toxic and biodegradable in nature [10]. A great number of plant and their extracts have been studied for their remarkable larvicidal potential [11]. Insecticidal activity of about 2000 plant species have been reported [12]. Recent studies have showed that efficacy of plant biocompounds have significant action against mosquitos [13, 14].
Many plants have been known to repel the mosquitoes. One such plant *Calotropis procera* has been studied for this purpose.

Bioactivity of ten plant oils was determined against the *A. aegypti.* These plant oils were tested at 125, 250, 500 and 1000 ppm concentrations against the third instar larvae of *Aedes aegypti*. Larval mortality was observed after 24 hours. Among the plant oils tested, orange oil exhibited highest larvicidal activity with LC of 85.93, followed by palmarosa 50 with 88.78, Tulasi with 92.48 and nutmeg oil with 93.62 ppm [15].

To evaluate the bio-insecticidal activity four plants were studied. In this study different solvent extracts of latex from, *Thevetia neriifolia*, *Artocarpus heterophyllus*, *Ficus glomerata*, *Calotropis procera*, on neonate larvae of *Spodoptera litura* a major pest of Indian agricultural crops. The solvent extracts (30-1000µg) of plant latex have caused very high larval mortality~90-95%, in comparison with the control. Results were found to be dose dependent. Plant latex fractions caused the pupal mortality (7- 47%) [16].

Larvicidal activity of *Calotropis procera* was determined against *Anopheles arabiensis* and *Culex quinquefasciatus* as a natural mosquito larvicide. The larvicidal activity was monitored against 2nd, 3rd and 4th instar larvae of each mosquito species 24 h post–treatment. Adult emergence inhibition activity was tested by exposing 3rd instar larvae of each mosquito species. The oviposition prevention activity was tested by using three different concentrations of extracts that caused high, moderate and low larval mortality rates. The results suggest that the leaf extract of *Calotropis procera* remarkable larvicidal, adult emergence inhibitor, repellent and oviposition deterrent effect against both tested mosquito, and might be used as natural biocides for mosquito control [17].

Calotropis procera belongs to the family Asclepiadaceae and it is also known as calotrope, small crown flower [18, 19]. *Calotropis procera* is native to West Africa as far south as Angola, Madagascar, North and East Africa, southern Asia, the Arabian Peninsula, Indochina to Malaysia and in Pakistan [20]. *Calotropis procera* prefers open habitat with little competition. The species grows sometimes in extremely drained soils in areas where the annual precipitation is as much as 2000 mm and also in dry habitat (150 to 1000 mm precipitation). *Calotropis procera* is a small tree or shrub 2–4 m tall (rarely up to 6 m tall), with characteristic grey-green waxy leaves [21]. The bark is light gray, furrowed and corky. Whenever stems or leaves are cut, a plentiful white juice flow. Giant milkweed has a very deep, solid taproot with few or no near-surface lateral roots [22]. All parts of the plant are reported to be toxic. The sap contains a compound called calotropin, which affects the heart, and causes irritation and blistering in people [23]. While generally considered to be inedible to cattle and other grazing stock, there are no cases of stock poisoning [24]. Preliminary studies have shown that the latex available in *C. procera* has a significant effect as a larvicide [25].

Objectives of this study was to find out the larvicidal property of plant, *Calotropis procera* by preparing extracts of stems and leaves and treating these extracts with larvae of mosquito, *A. aegypti.*

2. Materials and Methods

2.1 Collection of larvae

The larvae were obtained from the IPH (Institute of Public Health), Lahore and kept in a mosquito cage.

2.2 Plant material collection and extraction

Fresh and healthy leaves and stem branches of *Calotropis procera* were collected from their natural habitat from area of Sabzazar, Lahore, Pakistan. Leaves and stem were washed thoroughly using tap water then dried using an absorbent.

Extraction of plant material was done through microwave assisted extraction. Leaves and stem were weighted precisely (30 gm) through electric balance and ground to smaller sized pieces using pastel and mortar. Crushed leaves were added into 100 ml distilled water and heated into microwave oven for 200 seconds at 1000 watt microwave irradiation. After that the extract was filtered and further used to check the larvicidal assay. In the same manner stem extract was prepared.

2.3 Preparation of concentrations of plant extracts

Larvicidal potential of the leaves and stem of *C. procera* was checked by using five concentrations (20%, 40%, 60%, 80% and 100%) that were made by mixing of (20 mL, 40mL, 60mL, 80mL and 100mL) of leaves and stem extracts separately to (80mL, 60mL, 40mL, 20mL and 0mL) of distilled water respectively.

To check the accuracy of results of experiment, three replicates of each concentration were made. Replicates of the 20% concentration (100 mL) were prepared by pouring 30 mL solution of 20% concentration into three separate beakers. In the same manner replicates of each concentration were made. After that under the safe laboratory conditions 10 larvae of the *A. aegypti* were inoculated into each replicate carefully and the beakers were covered with polythene sheets.

2.4 Percentage mortality of larvae

Mortality of the larvae was checked after 24 hours.

Mortality rate = no of total tested larvae – no of dead larvae / no of total tested larvae x 100

2.5 Statistical analysis

Results were analyzed by applying one way analysis of variance (ANOVA) and replicates means were compared for significance by Duncan's New Multiple range test at 5% level of significance using SPSS computer software

3. Results

The larvicidal activity of plant extracts is often well known due to the presence of biologically active phytoconstituents. In this assay, larvicidal activity of *Calotropis procera* was tested against the larvae of *A. aegypti*

The results of the larvicidal tests performed against larvae of *A. aegypti* with different extracts prepared from leaf and stem extract concentrations of *Calotropis procera*. The results clearly showed that all the applied concentrations of extract were found most effective against larvae of *A. aegypti* with various mortality rates. Larvicidal affectivity of the different concentrations of leaf and stem extracts of plant *Calotropis procera* was tested by placing 10 *Aedes Aegypti* 3rd and 4th instar larvae in a 50 ml glass beaker containing different concentrations. Percentage mortality of larvae was noted after 24 hours. Larvicidal activity of *C. procera* leaf extract against *A. aegypti* showed the mortality rate 13%, 20%, 33%, 66% and 100% against 20 mL, 40 mL, 60 mL, 80 mL and 100 mL respectively as shown in (Figure 1).

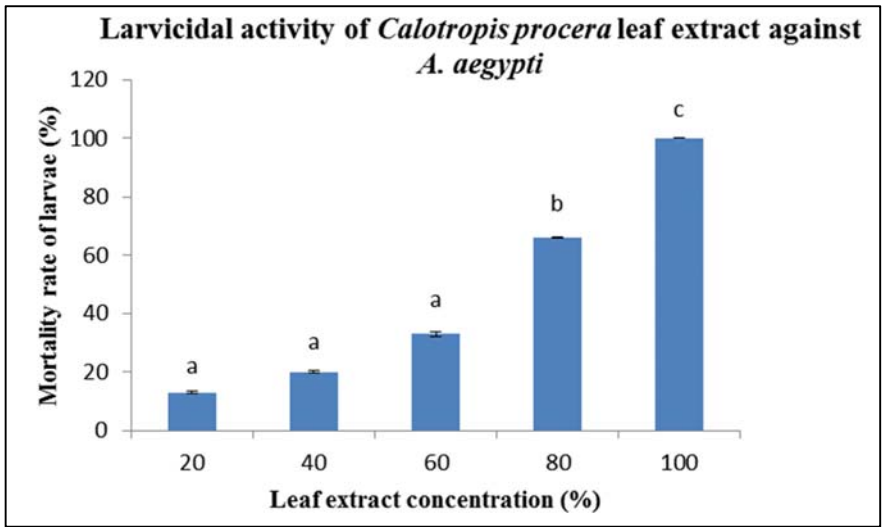

Fig 1: Effect of different concentrations of *Calotropis procera* leaf extract on the mortality rate of *Aedes Aegypti* larvae**.** Each value is a mean of three replicates with standard deviation. One way ANOVA with Duncan's multiple test range is applied to compare means by using SPSS software.

Larvicidal activity of *C. procera* stem extract against *A. aegypti* showed the mortality rate 6.6%, 26%, 56%, 56% and 73% against 20 mL, 40 mL, 60 mL, 80 mL and 100 mL respectively as shown in (Figure 2).

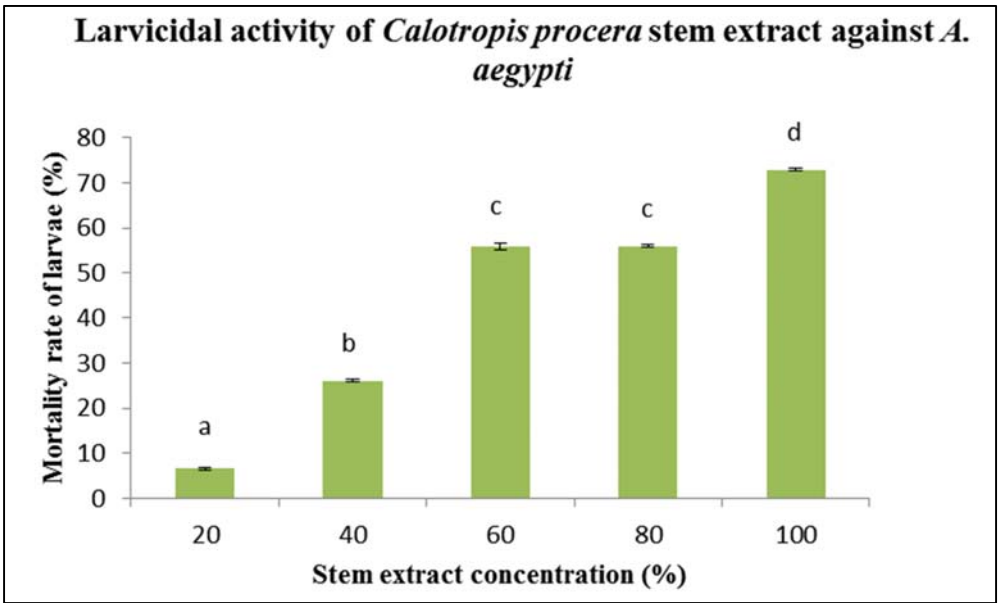

Fig 2: Effect of different concentrations of *Calotropis procera* stem extract on the mortality rate of *Aedes Aegypti* larvae**.** Each value is a mean of three replicates with standard deviation. One way ANOVA with Duncan's multiple test range is applied to compare means by using SPSS software.

Results showed that 100% and 73% mortality of larvae was observed in beakers containing 100% concentration of leaf and stem extract respectively. It was seen that lower concentrations proved to be least effective against larvae while higher concentrations showed remarkable results. Larvicidal effect of 20% concentration of stem extract was found to be least significant against larvae.

4. Discussion

It has been established worldwide that use of synthetic chemicals for eradication and control of insects are associated with many problems as insects especially mosquitoes develop resistance against chemicals. Therefore, the development of new strategies is important. Bio-pesticides are more useful than chemical pesticides because they are less harmful, toxic and cause less environmental pollution. During the last decades, a lot of studies on natural plants as larvicides against mosquitoes has indicated positive results and can be used as alternatives against pesticides. Plants have medicinal and pesticidal properties as well as they are rich in bioactive chemicals. The mixtures prepared from different parts of plants are used against mosquitoes are effective [26]. Insecticides have developed resistance against synthetic chemicals because of their excessive use. Additionally,

synthetic chemicals disturb the natural environment. These chemicals act as immunosuppressant which reduces the immunity of living beings. All parts of the *Calotropis procera* plant are reported to be toxic [23].

In this study it was observed that the 100% concentrations of leaf and stem extracts were found to be the most effective against larvae and showed 100% and 73% death of larvae after 24 hours. The stem extract was found to be less efficient against *Aedes aegypti* larvae. Finding of this study showed that due to its larvicidal effect, all parts of the plant can be used for the prevention and control of *Aegypti* mosquitoes.

Study conducted revealed that *Calotropis* spp has been suggested as a medicinally important plant and since long it has been in use for the prevention and treatment of many diseases including cancer [27]. Different species of Calotropis had received great interest with respect to its many effects, including cytotoxicity, and anti- inflammatory function [28]. The larvicidal action of aqueous latex extracts of two medicinal plants *Calotropis procera* and *Ficus benghalensis* was assessed against the fourth instar larvae of the lymphatic filariasis vector *Culex quinquefasciatus* using the conventional methods recommended by the WHO. The dose/response mortality relationship was statistically determined using double transformation regression analysis [29].

To evaluate the comparative antioxidant activity and larvicidal activity the methanolic extract of two common species of *Calotropis*, viz. *Calotropis gigantea* and *Calotropis procera* were used. The leaves of *C. procera* were found to have higher antioxidant potential than flower and root extract. *C. procera* possess comparatively higher antioxidant activity in reducing ferric ions than *C. gigantea*. The leaf methanolic extract showed a concentration dependent larvicidal activity with a low LD50 value of 387 mg/l compared to other extracts [30].

Calotropis procera plant is considered an effective and natural larvicide against *Anopheles arabiensis* and *Culex quinquefasciatus* [17]. This study also favors the finding of our study as the larvicidal activity of targeted plant. The results suggest that the leaf extract of *Calotropis procera* remarkable larvicidal, adult emergence inhibitor, repellent and oviposition deterrent effect against *Aedes aegypti*, *An. Arabiensis* and *Cx. quinquefasciatus*, and might be used as natural biocides for mosquito control.

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