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ORIGINAL RESEARCH ARTICLE

Phytochemical Screening of *Calotropis procera* Flower Extracts and Their Bio-Control Potential on *Culex sp.* Mosquito Larvae and Pupae

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ABSTRACT

Mosquito larvicidal efficacy and phytochemical screening of aqueous and ethanol extracts of the flowers of *Calotropis procera*, a common weed plant were evaluated in the present study, Phytochemical screening of the flower extracts of C.procera indicated the presence of alkaloids, carbohydrates, saponins, phenols, tannins, terpenoids and flavanoids which are known to possess medicinal and pesticidal properties. Larvicidal effects of the flower extracts of *C.procera* on the fouth instar larvae and pupae of *Culex sp.*, which transmits filariasis to humans was investigated. Results revealed a dose and time dependant effect of the extracts on mosquito larvae with 5% extract concentration causing 100% mortality within 3days exposure. *Culex sp*. pupae were more resistant to the flower extract compared to the fourth instar larvae indicating differential susceptibility of mosquito life stages. The potential use of *C.procera* extract as an effective eco-friendly, bio-control agent against mosquito larvae is discussed.

Key words: *Calotropis procera* flower extract, Phytochemicals, Larvicidal activity, *Culex species* larvae and pupae.

INTRODUCTION

Plants produce diverse types of bioactive molecules, making them a rich source of different types of medicines [1]. This revival of worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in healthcare. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines. An important reason for the increasing popularity of herbal medicine is the presence in plants of a wide variety of secondary metabolites like tannins, terpenoids, alkaloids and flavonoids which are known to have anti-microbial properties [2]. The use of plant extracts and phytochemicals with known anti-microbial properties can be of great significance in therapeutic treatments [3]. According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. In the last few years, a number of studies have been conducted in different countries to prove the anti-microbial activity and efficiency of plant extracts and phytochemicals [4,5,6]. Recent studies reveal that plants possess many subtle defenses that interfere with pest growth, development and behavior and lack toxicity to higher animals. Mosquitoes constitute a major public health menace in India and serve as vectors for transmitting various diseases like malaria, filaria, dengue etc., to humans. Control of mosquito borne diseases is becoming more and more difficult because of increasing resistance to pesticides, lack of effective vaccines and drugs against mosquitoes [7]. Hence, an alternative, safe and eco-friendly approach for mosquito control is the use of extracts of plant origin [8].

The present study deals with the use of *Calotropis procera* flower extracts for their larvicidal activity against *Culex sp*. mosquito larvae. *C.procera* is considered as a common weed in some parts of the world. This plant is known as “oak” in Ayurveda and it is used

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in cases of cutaneous diseases, intestinal worms, cough, ascites, asthma, bronchitis, dyspepsia, paralysis, inflammations and tumors [9,10]. Studies showed that *C.procera* extracts had larvicidal and insecticidal activity against different insects such as *Sarcophaga haemorrhoidalis* and third stage larvae of *Musca domestica* [11]. The aim of this study was to find the larvicidal efficacy of native *C.procera* against *Culex sp*. mosquito larvae and pupae, a potential vector of filaria.

MATERIALS AND METHODS

C.procera was collected from various locations in Chennai, the flowers were separated, washed, dried, powdered and the extracts were filtered using Whatman filter paper. Chemical tests were carried out on the aqueous and ethanolic extracts using standard procedures to identify the phytochemical constituents like alkaloids, carbohydrates, saponins, phytosterols, phenols, tannins, terpenoids and flavonoids as described by [12,13,14]. *Culex sp*. mosquito larva were collected from water stagnated areas in and around Chennai, identified based on the key characteristics outlined by [15] and the fourth instar stage and pupae were selected for further studies. The larvicidal effect of the *C.procera* extracts in different concentrations (1%, 2.5% and 5%) on *Culex sp.* was monitored by counting the number of dead larvae and pupae after exposure every 24 hours. Each test was repeated twice and the percentage of larval mortality was calculated following [7].

RESULTS AND DISCUSSION

The aqueous extract of *C.procera* showed the presence of many phytochemicals like carbohydrates, alkaloids and saponins, while the ethanolic extracts showed the occurrence of carbohydrates, alkaloids, saponins, phenols, tannins, terpenoids and flavonoids. Phytosterols were absent in both aqueous and ethanolic extracts (Table-1).Many of these phytochemicals screened are known to show medicinal as well as physiological activity[13]. Flavonoids are widely distributed in plants and have many functions including producing pigmentation in flowers and protection from microbial and insect attack [16]. Terpenoids have different functions like growth regulation, colour, odour and antimicrobial activity [14] and the presence of terpenoids has also been reported from many other plants like clove, chillies, turmeric etc.,[17]. Tannins and other polyphenols have been identified in cinnamon, eucalyptus, lemon balm and neem and may be responsible for its broad spectrum anti-microbial activity against viruses, bacteria and fungi [18]. The saponins are naturally occurring surface-active glycosides mainly produced by the plants. Saponins are known to be anti-microbial and to inhibit moulds and protect plants from insect attacks [19]. The larvicidal activity of flower extract of *C.procera* against *Culex sp,* larva and pupae are given in Table-2&3. It is evident that the mortality of *Culex sp.* fourth instar and pupae show a dose dependent and time dependent result. In the case of *Culex sp.* fourth instar at 1% concentration of *C.procera* extract, the mortality rate was 0%, 60% and 100% at the end of 1, 3 and 4 days of exposure; 20%, 80% and 100% at 2.5% concentration for the same study period and at 5% concentration, the mosquito larvae showed 100% mortality within 3 days post exposure. This is in agreement with the findings of Rajmohan [20] who also reported that there were differences in the mosquito larval mortality of various species. There was a prolongation in the duration of the mortality rate of *Culex sp*. pupae exposed to various concentrations of *C.procera* extracts. At 1% concentration, 100% mortality was recorded only after 6 days of exposure and similarly at 2.5% concentration, 100% mortality of pupae was seen at the end of the fifth day and at 5%, 100% mortality was seen after the fourth day. This shows that the pupae are more resistant to the effects of the extracts of *C.procera* compared to the instar stages. The results obtained show a similar trend to the observations reported by [21] for *Aedes aegypti* and *Culex sp*. Thus, this study shows that the leaf extracts of *C.procera* exhibit promising larvicidal properties against the late fourth instar larvae and pupae of *Culex sp*. Since *C.procera* is a commonly occurring weed plant, vast quantities of the leaf samples can be collected easily to obtain large quantities of the leaf extract for the use of mosquito larvae control in public health operations. The

extract can also be used for spraying the stagnant water bodies which are known to be the breeding grounds for mosquitoes. Some of the phytochemicals identified in the flower extracts of *C.procera* like saponins, phenols, tannins, etc., have been reported to possess potent mosquito larvicidal activity[7].A commercial mixture extracted from *Q. saponaria* showed increasing toxicity (100% larval mortality) in *A.aegypti* and *Culex pipiens* when both saponins concentration and the duration of the experiment were increased [22]. Isoflavonoids from tubers of *Neorautanenia mitis* had larvicidal effect against the malaria and filariasis transmitting mosquitoes, *Anopheles gambiae* and *C.quinquefaciatus* respectively [23].

This study showed that a wide variety of phytochemicals like alkaloids, flavonoids, phenols, tannins, saponins and terpenoids are present in the flower extracts of *C. procera*. Considering their antimicrobial activity and potential source of useful drugs, further studies are desirable in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. Human filariasis is a major public health hazard and remains a challenging socio-economic problem in many of the tropical countries. Lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by the mosquito *C.quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *C.quinquefasciatus* infects more than 100 million individuals worldwide annually [24]. Repeated use of insecticides for mosquito control has disrupted natural biological control systems and led to resurgence in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concerns. This study provides an alternative, eco-friendly approach for mosquito control in the use of extracts of plant. Search for natural insecticides, which do not have any ill effects on the non-target population and are easily degradable, remains to be one of the top priority issues for the tropical countries [25].

Table 1: Qualitative Analysis of Phytochemicals in the Flower Extracts of *Calotropis procera*.

Phytochemicals	Extracts of *Calotropis procera*	
	Aqueous	Ethanol
Alkaloids	+	+
Carbohydrates	+	+
Saponins	+	+
Phytosterols	-	-
Phenols	-	+
Tannins	+	+
Terpenoids	-	+
Flavonoids	-	+

Table 2: Mortality of *Culex sp.* Mosquito larvae in *Calotropis procera* Extracts.

Extract concentration (%)	Mortality Rate (%)		
	1^{ST} Day	3^{RD} Day	4^{TH} Day
Control	0	0	0
1%	0	60	100
2.5%	20	80	100
5%	60	100	_

Values are the mean of duplicate trials

Table 3: Mortality of *Culex* sp. Mosquito Pupae in *Calotropis procera* extracts.

Extract concentration (%)	Mortality Rate (%)			
	1^{st} Day	3^{rd} Day	5^{th} Day	6^{th} Day
Control	0	0	0	0
1%	0	40	80	100
2.5%	10	50	100	_
5%	30	80	100	_

Values are the mean of duplicate trials

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