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Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae)

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Mosquitoes are responsible for transmission of many diseases to human. They not only can carry diseases that afflict humans, but also transmit several diseases and parasites to birds, dogs, horses, etc. *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae) are urban and rural mosquito pests in Iran. Hormozgan province, southern Iran, has a favourable climate for mosquito breeding and development, with active foci of malaria transmission. Extensive application of chemical insecticides for many years in this area caused irrepairable damages to environment and resulted in resistance in *An. stephensi* to some insecticides/larvicides[1,2].

Several botanicals offer great promise as sources of phytochemicals for the control of mosquitoes. One of the earliest reports of the use of plant extracts against mosquito larvae is extraction of plant alkaloids like nicotine, anabasine, methyl anabasine and lupinine from the Russian weed in 1933. *Anabasis aphylla* killed larvae of *Culex pipiens* Linn., *Cx. territans* Walker, and *Cx. quinquefasciatus* Say[3]. Six plant families with several representative species, Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae and Rutaceae, appear to have the greatest potential for providing future mosquito control agents. Sukumar *et al*[3] has reviewed botanical derivatives in mosquito control and also discussed about botanical phytochemicals with mosquitocidal potential, using examples of different studies[4].

Some publications have described antibacterial, analgesic or schizontocidal activities for the latex of *Calotropis procera*[5–8]. Giridhar *et al*[9] found compounds with larvicidal activity in the latex of *C. procera* for the first time. Extracts of *C. procera* have insecticidal activity against different insects such as *Sarcophaga haemorrhoidalis*[10], while the latex was used against the third stage larvae of *Musca domestica*[11]. The crude latex produced by the green parts of the plant was evaluated for its toxic effects upon egg hatching and larval development[12]. They found the whole latex was shown to cause 100% mortality of III instars within 5 min. Different aqueous concentrations of this plant affected the gravid female *Aedes aegypti* mosquitoes and this behaviour continued till three gonotrophic cycles[13].

The aim of this survey was to find the larvicidal efficacy of native *C. procera* of southern Iran against *An. stephensi*, the main malaria vector of this area[1] and *Cx. quinquefasciatus*, a potential vector of some arboviral infections as well as filariasis.

Calotropis procera or milkweed naturally grows in southern parts of Iran. The fresh leaves of this plant were collected from Bandar Abbas County, an endemic area of malaria in southern Iran[14], bordered with Persian Gulf. Fresh leaves of *C. procera* became dried in the room temperature far from direct sunlight. Methanolic extracts were obtained with

maceration method in the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Latex was collected by cutting the fresh leaves using a razor blade and gathered into glass vials. Larvae were obtained from two established colonies of *An. stephensi* and *Cx. quinquefasciatus* in the insectary of Bandar Abbas School of Public Health, Hormozgan University of Medical Sciences. Larvicidal tests were perforemd based on WHO standard method[15].

Tests were conducted using concentrations of 4, 8, 16, 32, 64, 128, 256, 512 and 1024 ppm of extract, and 4, 8, 16, 32, 64, 128, 256 ppm of plant latex against the larvae of the above mentioned two species of mosquitoes. Tests were conducted using four batches of 25 larvae (a total of 100 larvae) and one batch as untreated control group for each concentration. Tested larvae were maintained under the insectary conditions (Temperature 27–29°C, relative humidity 70–75% and 12 : 12 light : dark period). Mortality rates were observed and recorded after 24 h. Tests with untreated control group mortality between 5 and 20% were corrected by Abbott's formula[16], meanwhile above 20% were repeated. The LC_{50} and LC_{90} values were obtained by Probit Analysis[17]. Toxic activity was reported as LC_{50} and LC_{90} representing the concentrations that induced 50 and 90% mortality after 24 h.

The results for the toxicity of milkweed derivatives against *An. stephensi* and *Cx. quinquefasciatus* larvae are presented in Table 1. The alcoholic extract of *C. procera* showed to be less toxic than latex in both mosquito species. The LC_{50} values were 109.71 and 387.93 mg/l for *An. stephensi* and *Cx. quinquefasciatus,* respectively. These figures were 13.06 and 86.47 mg/l respectively for latex of the plant. Overall, *An. stephensi* larvae were more susceptible than *Cx. quinquefasciatus* to both latex and extract. The 512 ppm concentration of plant extract didn't show a mortality rate >78% in *Cx. quinquefasciatus* after 24 h. But in the case of *An. stephensi* we observed >95% mortality after 24 h from 256 ppm. Tests with latex showed 99% mortality at 64 ppm for *An. stephensi*, only 44% mortality against *Cx. quinquefasciatus* and a maximum of 67% in 256 ppm.

Botanical derivatives may be the future of mosquito control programs. Phytochemicals that are tested against various life stages of mosquitoes, have potential uses as growth and reproduction inhibitors, repellents and as oviposition deterrents. In the past decades, studies on the effect of plant extracts on mosquito vectors of disease indicated that these materials are good alternatives for synthetic chemical pesticides. These studies have determined that some botanical compounds such as alkaloids, nicotine, anabasin and lupitin in the extracts produced high mortality against mosquito larvae. Mosquito species, life stage, the plant parts and solvent used for extraction, phototoxic activity and the geographical origin of a plant compound are important factors in the efficacy of a phytochemical[3,4].

Study of Ramos *et al*[12] showed that the *C. procera* latex contains the larvicidal compounds, which caused 100% mortality in III stage larvae of *Ae. aegypti* after 5 min. Study of Markouk *et al*[18] showed that *C. procera* latex caused 50% mortality against *An. labranchiae* at 28 ppm. The results of our survey

Table 1. Larvicidal activity of alcoholic extract and fresh latex of *Calotropis procera* against *An. stephensi* and *Cx. quinquefasciatus* larvae in Iran, 2008

Botanical	Species	LC_{50} (Confidence limits)	LC_{90} (Confidence limits)
Alcoholic extract	*An. stephensi*	109.71 (99.37–120.54)	234.61 (206.68–275.46)
	Cx. quinquefasciatus	387.93 (359.66–418.69)	630.66 (566.54–731.41)
Fresh latex	*An. stephensi*	13.06 (9.74–21.81)	23.53 (16.16–85.36)
	Cx. quinquefasciatus	86.47 (70.44–143.89)	973.89 (613.88–1812.05)

confirmed the efficacy of latex and extract components of *C. procera* and mortality increased with concentration. Also we found latex a strong killer against both *Cx. quinquefasciatus* and *An. stephensi* larvae compared to extract (Table 1). Considering the proof of some pesticide compounds such as Calotropin and Calotoxin in the milkweed plant extracts[19] it seems they are the most important factors in causing mortality of mosquito larvae. In the same concentrations, the fatality rate caused by latex is more than the alcoholic extract. It can be due to obtaining the plant extracts from dried parts like leaves, so they will loss some effective components that exist in fresh latex, during the drying process and therefore will induce lower mortality rates in mosquito larvae. In regard to availability of *C. procera* in southern parts of Iran, it is suggested to perform more studies to produce natural insecticide/larvicide from this native plant.

The latex of *C. procera* has shown larvicidal efficacy against all three important vector species: *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* in India[20]. They used 14 different solvents to dissolve the latex at 1000 ppm and found methanolic extract produced 100% mortality in *Ae. aegypti* larvae. Their results showed that *Cx. quinquefasciatus* is more susceptible than *An. stephensi* at same concentrations of latex. This is in contrary with our results. Study on larvicidal efficacy of *C. procera* by Markouk *et al*[18] which obtained a LC_{50} of 28 mg/l with aqueous latex of this plant against *An. labranchiae*, while the ethanolic extract of root showed LC_{50} of 315 mg/l against this *Anopheles*. In the case of *An. stephensi* Shaalan *et al*[4] reported 12 plant species tested for larvicidal effects. The values are more than those obtaind in the present study. The LC_{50} values ranged from 3.9 mg/l for seed fractions of *Calophyllum inophyllum* to 1000 mg/l for ethanolic extract of leaf of *Cannabis sativa*.

Larvicidal activity of Iranian plants *Tagetes minuta* L. and *Cymbopogon olivieri* (Boiss.) Bar against *An. stephensi* larvae are studied under the laboratory conditions[21–23]. The results of these studies showed the essential oil of *Tagetes minuta* L. is stronger larvicide against *An. stephensi*, rather than its methanolic extract; while the essential oil of *Cymbopogon olivieri* (Boiss.) Bar has interesting activity against larvae of *An. stephensi.* The only field examination of plant derived larvicides is conducted by Vatandoost and Vaziri[24], who reported LC_{50} and LC_{90} values of 0.35 and 1.81 mg/l for *An. stephensi*, and 0.69 and 3.18 mg/l for *Cx. quinquefasciatus* respectively using Neemarin, a plant derived larvicide which obtains from *Azadirachta indica*.

From the results, it can be concluded that *C. procera* extracts possess good larvicidal activity against mosquitoes and more studies are indicated to extract the active compounds for future studies and use in mosquito control.

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