

POTENTIAL MOSQUITO LARVICIDE OF *CESTRUM NOCTURNUM* AND *SAPINDUS MUKOROSI*

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ABSTRACT

Aedes aegypti is a mosquito species responsible for transmission of dengue fever. WHO stated the best way to control the transmission of dengue virus is to fight the vector mosquito that cause disease. In present work we evaluated the potential larvicide from *Cestrum nocturnum* and *Sapindus mukorossi* leaves. Methanol extract showing highest activity of larvicide were separated and fractionated using chromatographic techniques and bioassay guided fractionation. Bioactive fractions were subjected for LC₅₀ evaluation on 3rd instars larvae of *Aedes aegypti* according to WHO protocol. Using hexane ethyl acetate solvent system, CN-3 and SM-5 fractions are separated containing larvicide with LC₅₀ 7.2 µg/ml and 18.3 µg/ml respectively for 24h. These fractions will be further characterized to identify the compound and biologically evaluated for mode of action on targeted organism. This study further lead to produce less expensive and safe compound to control mosquitoes in India.

KEYWORDS: *Aedes aegypti*, *Cestrum nocturnum*, larvicide, *Sapindus mukorossi*, Chromatography.

INTRODUCTION

There is no assured vaccine to prevent this infection or drugs to fight the disease in infected persons, so vector control is the most prevalent and economical solution available so far for reducing incidence. Most of the widely used vector destruction methods are synthetic insecticide. These synthetic insecticides not only affect the non-target population, but also constantly increase resistance to the vector (Wattal *et al.*, 1981). The search for natural insecticides which do not have any adverse effects on the non-target population and are easily degradable remains the top priority (Rodwane *et al.*, 2005).

Mosquitoes serve as vector for various tropical and subtropical diseases which cause negative effects to human health (Kovendan and Murugan, 2011). They not only transmit parasites and pathogens but also serve as source of various allergic reaction (Cheng *et al* 2003). The most common diseases associated with mosquitoes are chikungunya, Malaria, dengue fever, yellow fever and deadly, dengue hemorrhagic fever where *Aedes aegypti* is one of the mosquito species responsible for the transmission of these vector borne diseases (Kovendan and Murugan, 2011). World Health Organization (WHO) stated that about 2/5 of the global human population are currently threaten of dengue and the best way to control the transmission of dengue virus is fight the mosquitoes as vectors that cause the disease. Due to environmental concern on use of existing synthetic insecticides for vector control and further risk of development of widespread insecticides resistance in disease vector; interest on possible use of environment friendly natural products such as extracts of plants or plant parts increased for vector control. Sukumar *et al.* (1991) listed 346 species for 276 genera and 99 families which have been tested against mosquitoes for various effects such as toxicity, growth inhibition, ovipositional determinacy and repellent. This list includes many species from Sapindaceae and Solanaceae family. recently many scientist reported some of those plants as mosquito larvicides on various species of mosquito (Arruda *et al.* 2003a,b; Ferreira Barreto *et al.* 2006; Koodalingam, *et al.*, 2009; Rawani *et al.*, 2014; Jawale *et al* 2010, Jawale, 2014). taking into consideration of these facts, present study is aimed to isolate larvicides from *Cestrum nocturnum* and *Sapindus mukorossi* using bioassay guided fractionation.

MATERIAL AND METHODS

Preparation of the Plant Samples: The collected plant samples were separated for their leaves and then washed with tap water and rinsed with distilled water. All plant samples were air dried for 48 hours at room temperature. Dried leaves of the plant samples were cut and pulverized using an electric blender.

Extraction of Plants: 250gm of pulverized plant samples were placed in a glass container. The samples were soaked with methanol in the 1:1 ratio and were left to stand for 48 hours and then filtered. the resulting filtrates were then concentrated in a rotary evaporator until syrup consistency was obtained.

Collection of Mosquito Larvae: Larvae were reared (Pelah *et al.*, 2002) and third instars larvae were selected for bioassay.

Mosquito Larvicidal Bioassay: The efficacy of the plant extracts as larvicide against the dengue-vector *Aedes aegypti* mosquito was evaluated in accordance with the guidelines of World Health Organization (WHO). Larvae were transferred into the test solution with pasture pipette (20 larvae/solution). As a solvent, DMSO is used to soluble the extract in test water. Mortality of each test extract and fractions were determined after 24 hours exposure at 28°C following the protocol of WHO (1981). Mortality was corrected using Abbot formula (Finney, 1971) and the concentration at which 50% of the test population were dies (LC₅₀) was determined by probit program (Finney, 1971).

Bioassay guided fractionation:

In our earlier finding (Jawale, 2014) methanolic extracts of leaf of *Sapindus mukorossi*, *Cestrum nocturnum*, *Cestrum diurnum*, *Asclepias curassavica* were tested for larvicidal activity on *Aedes aegypti* larva. where LC₅₀ for 24 hrs when compared among these four plant. it was observed that *C. nocturnum* and *S. mukarossi* extract showed highest mortality which is also supported by presence of various phytochemicals like Alkaloids, saponin, flavanoids, steroids, and tannins. In present work these methanolic extracts of *C. nocturnum* and *S. mukarossi* were further subjected to bioactivity guided fractionation by TLC and preparative TLC. The most potent fractions were further fractionated in column chromatography to evaluate LC₅₀ for *Aedes aegypti* third instars larvae.

Test solution : methanolic extract of leaves of *Sapindus mukorossi*, *Cestrum nocturnum*.

The stationary phase: silica gel GF 254 (Merck), standardized plates of 10x10 cm and 0,25 mm thick.

The mobile phase: Hexane: ethyl acetate (varying ratios)

Table 1: Lethal concentration (LC₅₀) values of fractions isolated from methanol extract of *C. nocturnum* and *S. mukorossi* on *A. aegypti* larvae for 24h. exposure.

Extracts	Hexane: Ethyl acetate	LC ₅₀ (µg/ml)	Regression equation
CN2	1:10	28.1	Y=2.1195X+2.1285
CN3	1:1	07.2	Y=1.0035X+2.0148
CN4	10:1	33.4	Y=2.1241X+1.2773
SM2	1:10	39.1	Y=1.7262X+2.0126
SM5	6:4	18.3	Y=2.0371X+1.0047

CN = *Cestrum nocturnum*, SM= *Sapindus mukorossi*

RESULT AND DISCUSSION

The study on the larvicidal activity of the methanolic leaf extract of *Sapindus mukorossi*, *Cestrum nocturnum*, *Cestrum diurnum*, *Asclepias curassavica* against the dengue-vector, *Aedes aegypti* mosquito showed that the leaf methanolic extracts of *S. mukorossi* contains no alkaloids, very few flavonoids and tannins while the methanolic extracts of *C. nocturnum* and *C. diurnum* are rich in alkaloids, saponins, tannins, flavonoids and steroids (Jawale, 2014). Methanolic extract of *C. nocturnum*, *C. diurnum*, *A. curassavica*, *S. mukorossi* were prepared by percolation method and tested for mosquito larvicidal activity on 3rd instars larvae of *Aedes aegypti*. In earlier finding *C. nocturnum* and *S. mukorossi* presented potential larvicidal activity among these four plants (Jawale, 2014). These methanolic extract of *C. nocturnum* and *S. mukorossi* leaves were separated on TLC with Hexane and ethyl acetate solvent system with increasing polarity. It showed total 12 bands appearing both with *C. nocturnum* and *S. mukorossi*. All these bands were then tested for mosquito larvicidal activity using preparative TLC. Where CN-2 (*C. nocturnum* fraction-2), 3, 4, and SM-2 (*S. mukorossi* fraction-2), 5 presented significant larvicidal activity. Whereas CN-1, 5, 6 and SM-1, 3, 4, 6 didn't showed remarkable activity at concentration lower than 350 µg/ml (results not shown). Various authors have evaluated larvicidal activity of saponin containing plants (Ghosh and Chandra, 2006 and Ghosh *et al.* (2008) isolated a phytosteroid compound from *C. diurnum* which exhibited remarkable biocontrol potentiality against larval mosquitoes. The volatile oil of the species of *C. nocturnum* and *C. diurnum* are used as mosquito repellent in several African nations (Ntonifor *et al.*, 2006). Eight steroidal glycosides have been isolated from the leaves of *C. nocturnum* (Mimaki *et al.*, 2002). Fractions of *C. nocturnum* methanol extract with LC₁₀₀ 12-75 ppm against *A. aegypti* were reported by Jawale *et al.* (2010). Sukumar *et al.* (1991) reported 3 species of family Sapindaceae, namely *Koelreuteria paniculata* (extracts of

seeds and leaves), *Poullinia fuscescens* (extracts of seeds and fruits) and *Sapindus saponaria* (extracts of seeds and fruits) were found to be effective against mosquito larvae. Surendran *et al.* (2009) when tested fruit extract of *Sapindus emarginatus* against *A. aegypti* revealed the LC₅₀ values of 92.9 ppm and found extract positive for the presence of saponin.

The LC₅₀ values of extracts of medicinal plants *Plumbago zeylanica* and *C. nocturnum* against *A. aegypti* were reported by Patil *et al.*, (2011) as less than 50 ppm. Wiesman and Chapagain, (2006) reported a strong correlation between saponin content and mortality for *A. aegypti* (yellow fever mosquito) larvae exposed to extracts of *Balanites aegyptiaca*. 0.0014% (v/w) of the most active fraction proved to be sufficient to kill 50% of the larvae before formation of adults. Therefore suggested saponins, as a cheap way to reduce the mosquito population and could play an important role in mosquito control. A study conducted by Rawani *et al.*, (2009) showed the larvicidal activity of *Carica papaya*, *Murraya paniculata* and *Cleistanthus collinus* extracts against *Culex quinquefasciatus* and revealed the presence phytochemicals such as saponins, steroids, alkaloids, terpenes, etc. are accountable for their larvicidal efficacy potential. Phytochemicals of plants possess a broad scope of bio-control potential. Roopa and Wadje, (2012) stated that some of these substances serve as plant defense mechanisms against microorganisms, insects and herbivores.

In present investigation, percolation method is applied for the extraction of complex mixture of natural products over other accelerated solvent extraction, as it gives more reliable and help extraction of biologically active secondary metabolites (Jawale *et al.*, 2010). Bioassay guided fractionation of these potent plants through thin layer, preparative thin layer and column chromatography is proved economical and efficient method of separation of phytochemicals instantaneously. Silica gel of separated band when mixed with DMSO can be used for biological testing. Further these potent fractions of CN-2, 3, 4 and SM-2, 5 were separated on column chromatography with hexane and ethyl acetate solvent system from methanol extract and used for LC₅₀ evaluation on larvae of *A. aegypti*. Where LC₅₀ (24h) of fraction CN-3 and SM-5 is found 7.2 µg/ml and 18.3 µg/ml respectively (Table 1).

Hexane ethyl acetate system is used for separation and fractionation, which assure removal of natural sugars and fatty acids like waxes etc. (Harborn, 1998). Thus increasing polarity of these solvent system assure isolation of larvicidal principal. (Rajendra and Estari, 2013). Similarly hexane and ethyl acetate solvent combinations are reported to extract antimicrobial principle from flavonoids, saponin and tannins group (Egharevba *et al.*, 2010). Various author have used different solvent for extraction of active larvicide from different plants. Elango *et al.* (2009) showed that the ethyl acetate extract of *Aegle marmelos* and *Eclipta prostrata*, hexane, and methanol extracts of *Andrographis paniculata* and *Cocculus hirsutus* were effective against the larvae of *A. subpictus* (LC₅₀= 167.00, 78.28, 67.24, 142.83 ppm) and against the larvae of *Culex tritaeniorhynchus* (LC₅₀= 99.03, 119.89, 88.50, 105.19 ppm), respectively. Mathew *et al.* (2009) reported that leaf chloroform extracts of *Nyctanthes arbortristis* showed lethal values (LC₅₀= 526.3. 780.6ppm 24h and LC₅₀=303.2, 518.2ppm 48h) against *A. aegypti* and *A. stephensi*, respectively. Flower methanol extracts of the above plants showed lethal values (LC₅₀=679.4, 244.4 ppm; LC₅₀=1071.3, 433.7ppm) against *A. stephensi* after 24 and 48 h, respectively. *Clitoria ternatea* leaf methanol extract showed dose-dependent larvicidal activity against *A. stephensi* with LC₅₀ values of 555.6 (24h) and 867.3 (48h)ppm, also the root extracts with LC₅₀ value of 340 ppm (48h). Seed extract showed larvicidal activity (LC₅₀= 116.8, 195 ppm) after 24h and (LC₅₀=65.2, 154.5 ppm) after 48h treatment against *A. stephensi* and *A. aegypti*, respectively.

Larvicidal activity of flower methanol extract showed LC₅₀ values 233 and 302.5 ppm against *A. stephensi* and *A. aegypti*, respectively, after 48h treatment. Amer and Mehlhom, (2006) reported that *Lippia citriodora* essential oil having LC₅₀ value of 101.4 ppm was effective against third instar larvae of *A. stephensi*. In future research can be extended to evaluation of mode of toxicity and chemical characterization of complex larvicidal compound from such saponin containing plants.

CONCLUSION

In present investigation four saponin containing plants were evaluated for the presence of larvicidal principle. *Cestrum nocturnum* and *Sapindus mukorosi* showed promising larvicidal activity. When separated using chromatographic techniques, it showed 2 potent fractions containing larvicidal principle among 12 fractions from both the plants. Further characterization of these fraction will lead to the discovery of complex larvicidal compound from *Cestrum nocturnum* and *Sapindus mukorosi*.

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