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LARVICIDAL ACTIVITY OF SOME SAPONIN CONTAINING PLANTS AGAINST THE DENGUE VECTOR *AEDES AEGYPTI.*

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ABSTRACT
The crude extracts of fruits of *Sapindus mukorossi*, leaves of *Cestrum nocturnm*, *Cestrum diurnum*, and *Asclepias curassavica* were tested under laboratory conditions against *Aedes aegypti*, a vector of dengue and chikungunya, for their larvicidal properties. Bioassay experiments carried out with crude alcoholic extracts of *Sapindus mukorossi*, *Cestrum nocturnm*, *Cestrum diurnum*, *Asclepias curassavica*, LC_{50} values of 10.05, 9.02, 8.96 and 11.74 ppm respectively. All crude extracts showed positive result for the presence of saponin. This preliminary study suggests that these plants are sources of potential mosquito larvicide, and can be effectively used to produce less expensive and safe compounds to control mosquito vectors in India.

KEY WORDS: *Aedes aegypti*, *Asclepias curassavica*, *Cestrum diurnum*, *Cestrum nocturnm*, larvicidal, *Sapindus mukorossi*, saponin.

INTRODUCTION

Natural products of plant origin are preferred over synthetic insecticides due to their eco-friendly nature. The use of natural products and their derivatives are being advocated for the control of insect vectors of human diseases. A possibly interesting class of molecules are the saponins, a group of steroidal or triterpenoid secondary plant metabolites with divergent biological activities.

Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human (Kovendan and Murugan, 2011). They do not only transmit parasites and pathogens but they also source of allergic reaction that includes local skin and systemic sensitivity (Cheng *et al.*, 2003). The most common diseases associated with mosquitoes are dengue fever, chikungunya, yellow fever and the worst, 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 that cause the disease.

Due to the pathogenic diseases and serious harms caused by mosquitoes, controlling them has been the primary subject of several new researches over the past few years (Invest and Lucas, 2008). The technique in controlling mosquitoes depends on the larval stages (egg, larvae, pupae, and adult) on target. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide (Tiwary *et al.*, 2007). Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other organisms and the environment (Hedlin *et al.*, 1997, Arnason *et al.*, 1989, Tiwary *et al.*, 2007 Instead of using synthetic larvicides, the use of these plant-derived products in controlling mosquito larvae is inexpensive and environment-friendly.

Many studies on plant extracts and their active constituent compounds against mosquito larvae have been conducted around the world [Amer and Mehlhorn, (2006), Pavela *et al.*, (2009), Rahuman *et al.*, (2009), Kamaraj *et al.*, (2010), Sreelatha *et al.*, (2010), Elango *et al.*, (2011), Kannathasan *et al.*, (2011), Khandagle, *et al.*, (2011), Pitarokili, *et al.*, (2011), Talontsi *et al.*, (2011), Maheswaran *et al.*, (2012)].

Asclepias curassavica (L.) (Tropical milkweed) is an erect, evergreen sub shrub belonging to the sub family *Asclepiadoideae*, family *Apocynaeceae* (Endress and Bruyns, 2000) (Figure 1). The sub family *Asclepiadoideae* constitutes many medicinally important plants comprising more than 250 genera and 3,000 species, of which 43 genera and 243 species are present in India. It has a woody based stem with milky sap leaves 6 -14 x 1-3.5 cm, decussate, lanceolate, puberculous along nerves, acute at both ends. Plant grows to 1 m in height, occupies

45-60 cm. In general, *Asclepiadoideae* plants are the source of cytotoxic and cardiac glycosides and contain highly valuable potential products for curing many diseases. Resinoid (galitoxin), a toxic principle in poisonous species is found in the milky latex of its stem. Several glycosides (cardiac glycosides) and an alkaloid have been isolated. Root extracts of this plant are widely used in South America an emetic and laxative. A decoction of the plant is used as an abortifacient. Roots are known as 'Pleurisy root" and used as an expectorant for pneumonia, lung problems, employed to treat warts, fever, etc. Plant is anti-ovulatory, astringent, cardiotonic and used for abdominal tumor, haemorrhages, and headache. The plant contains highly potential esterified polyhydroxy pregnane glycoside that shows antitumour and anticancer property. The use of plants as medicines has paved way for the isolation of active compounds, beginning with the extraction of morphine from opium in the early 19th century (Kinghorn, 2001). It is estimated that around 2,50,000 flowering plant species reported occur globally and approximately half of these are found in tropical forests. Medicinal plants are of great interest as pharmaceutical industries depend in part on plants for the production of secondary metabolites (Rout and Das, 2000).

A thorough study of literature showed that a large number of taxa in the family Asclepiadoideae are medicinally important and contain different kinds of secondary metabolites. Glycosides steroidal, pregnane, flavonoids are the major compounds reported in this family. The production of an essential oil containing 2-hydroxy 4-methoxy benzaldehyde from the in vitro derived roots of *Hemidesmus indicus* has been carried out (Sreekumar *et al.*, 2000, 1998,). The batch culture of *H. indicus* roots had been conducted for the continuous production of roots and root specific compounds (Sreekumar *et al.*, 1998,). Selected medicinal plants of Asclepiadaceae family have been screened (Averineni *et al.*, 2007). Recently, the antimicrobial activity of root extracts in A. *curassavica* (L). was reported (Waltor,1997).

Sapindus mukorossi (*Sapindaceae*), better known as the soap-nuts, generally grows in tropical and sub-tropical regions of Asia (Fig No. 2). It was reported that *S. mukorossi* possessed efficient natural surfactants and has served as commercial ingredient of shampoo and cosmetic cleansers (Tananka *et al*., 1996). In addition, some pharmacological effects including anthelmintic anti-dermatophytic, antitussive, (Nakayama *et al.*, 1986), anti-inflammatory (Takagi *et al.*, 1980), antimicrobial, (Tamura *et al.*, 2001), cytotoxic (Quetin *et al.*, 1992), haemolytic and molluscicidal (Huang *et al.*, 2003) activities were found in the plant. As for the principal constitutes of this plants, various triterpenoid saponins containing dammarane type (Hua *et al.*, 2004,), hederagenin-type (Nakayama *et al.*, 1986) tirucallane-type (Watanabe *et al.*, 1988) as well as sesquiterpene oligoglycosides, (Tanaka *et al.*, 1996, Nakayama *et al.*, 1986) have been isolated from the fruit, gall, pericarp, root, and stem.

Figure 1: *Asclepias curassavica* Plant

Figure 2: *Sapindus mukorossi* plant

Cestrum nocturnum L. and C. *diurnum* is a garden shrub from *Solanaceae* family, and its flowers exude a special sweet fragrance. *C. nocturnum* is naming Night jasmin, Lady of the Night (Figure 3) and Night Blooming Jasmine and *C. diurnum* as day jasmine (Figure 4) on the basis of their flowering time (Roig, 1988). They are widely naturalized in tropical and subtropical regions throughout the world, including Asia, Australia, southern China and the southernmost United States. In traditional medicine, leaves of *C. nocturnum* have been used for their pharmacological significance in burns and swellings. It is also used for treating epilepsy and as stupefying charm medicine in West Indian Islands (Saad and Buznego, 2008).

Figure 3: *Cestrum nocturnum* plant.

Figure 4: Cestrum diurnum plant.

The volatile oil is known to be mosquito-repellent and hence *C. nocturnum* is used to prevent malaria in several African Nations (Mimaki *et al.,* 2006). Pharmacological studies on the plant proved that the leaves have significant analgesic and bactericidal activity (Huang *et al.,* 2006). Bio-control efficacy of *C. diurnum* has been proved against anopheles, *aedes aegypti* and *culex* larvae (Ghosh and Chandra, 2006, Ghosh *et al.,* 2008, Jawale *et al.,* 2011), Local anesthetic effect, inhibitory effect on central nerve system and cardiac arrhythmic effect of plant are also documented (Zeng *et al.,* 2002). Furthermore, n-butanol and polysaccharides extracts from *C. nocturnum* had obvious in Vivo effects on tumor inhibition (Zhong *et al.,* 2008). Some phytochemical studies on leaves from *C. nocturnum* proved the presence of a calcinogenic glycoside (Mello, 2003) and other glycosides such as nocturnoside A and nocturnoside B (Ahmad *et al.,* 1995), phenol glucosides (cesternosides A and B), flavonol glycosides and steroidal saponins and glycosides (Mimaki *et al.,* 2001), (Mimaki *et al.,* 2002).

The aim of this study is to carry out bioassay experiments with crude alcoholic extracts of *Sapindus mukorossi*, *Cestrum nocturnm*, *Cestrum diurnum*, *Asclepias curassavica*, to determine the larvicidal activity against the dengue-carrying mosquito, *Aedes aegypti*.

MATERIALS 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: Two hundred fifty grams 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.

Rotary Evaporation and Phytochemical Analysis: The methanolic plant extracts were sent to the Botany Laboratory of same institute for the rotary evaporation. Plant samples have undergone rotary evaporation to make it highly concentrated until it is semi-solid in form. The extracts were phytochemically screened using different chemical assays to identify the presence or absence of the phytochemical components in the plant extracts. The method described by Connolly (Connolly *et al.,* 1970) was used for detection of sterols and/or triterpenes; that described by Wolf (Wolf *et al.,* 1962) for detection of carbohydrates and saponins; Harbone (1973) for detection of flavonoids and alkaloids; that reported by Farnsworth (1966) for detection of coumarins; and that described by Geissman (1962) for detection of tannins.

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). Batches of 20 third-instars larvae of *Aedes aegypti* were placed in a small plastic container with 50ml dechlorinated water

and lay in the netted area in the Laboratory room at 30-32^{0}C. For the control group, the mosquito larvae were exposed to 60ppm methanol since it is the solvent used in the extraction of different plant samples. The experimental group is the methanolic extracts of leaf of *Sapindus mukorossi*, *Cestrum nocturnm*, *Cestrum diurnum*, *Asclepias curassavica*, with 20 ppm, 40 ppm, 60 ppm concentrations.

These concentrations were chosen after the pre-test//pre-treatment conducted. Identification of the mosquito larvae were done by tapping it with a needle in the siphon or cervical area. Each treatment was conducted in three replicates. The effects of the plant extracts were monitored through carefully counting the number of dead larvae after 24 and 48 hours of treatment, and the percentage mortality was computed.

$$\text{Percentage mortality} = \frac{Number\ of\ dead\ larvae\ X\ 100}{\text{Number of larvae introduced}}$$

Statistical Analysis: The statistical tools that were used in this study are the following: the Arithmetic Mean to get the average number of dead mosquito larvae, and Probit Analysis to calculate LC_{50} and LC_{90} values to determine Lethal concentrations of the plant extracts on *Aedes aegypti* mosquito larvae after 24 and 48 hours of treatment.

Table 1: Phytochemicals present in the methanolic extracts of the different plants.

Plant extract	Alkaloids	Saponin	Flavonoids	Steroids	Tannins
Sapindus mukorossi	-	+++	+	++	+
Cestrum nocturnum	+	+++	+	+++	++
Cestrum diurnum	+	+++	+	+++	++
Asclepias curassavica	+	++	+	++	+

Legend: (+) present, (-) absent

Table 2: Mean and percentage mortality of *Aedes aegypti* mosquito larvae in the control and experimental group after 24 hrs.

Plant species	Concentration (ppm)	Mean mortality	Mean percentage
Sapindus mukorosii	20	12.42	62.1
	40	16.4	82.0
	60	20	100
Cestrum nocturnum	20	9.66	48.33
	40	17.42	87.14
	60	20	100
Cestrum diurnum	20	6.52	32.64
	40	14.12	70.61
	60	20	100
Asclepias curassavica	20	6.44	32.24
	40	10.90	54.53
	60	16.13	80.67
Control	--	0.34	1.72

Table 3: Mean and percentage mortality of *Aedes aegypti* mosquito larvae in the control and experimental group after 48 hrs.

Plant species	Concentration (ppm)	Mean mortality	Mean percentage
Sapindus mukorossi	20	10.22	51.13
	40	17.84	89.21
	60	20	100
Cestrum nocturnm	20	8.30	41.52
	40	14.86	74.31
	60	20	100
Cestrum diurnum	20	7.884	39.42
	40	12.53	62.67
	60	20	100
Asclepias curassavica	20	5.506	27.53
	40	8.324	41.62
	60	12.86	64.33
Control	--	0.71	3.43%

Table 4: Lethal concentration (LC_{50} and LC_{90}) values of the plant extract on *Aedes aegypti* mosquito larvae after 24 hrs. of treatment.

Plant species	LC_{50} values	LC_{90} values
Sapindus mukorossi	10.05 mg/lit SD 0.117 SEM 0.06	18.24 mg/lit SD 0.273 SEM 0.15
Cestrum nocturnum	9.025 mg/lit SD 0.199 SEM 0.115	15.271 mg/lit SD 0.397 SEM 0.229
Cestrum diurnum	8.960 mg/lit SD 0.437 SEM 0.252	17.11 mg/lit SD 0.745 SEM 0.4305
Asclepias curassavica	11.740 mg/lit SD 0.109 SEM 0.063	21.393 mg/lit SD 0.243 SEM 0.140

Table 5: Lethal concentration (LC_{50} and LC_{90}) values of the plant extract on *Aedes aegypti* mosquito larvae after 48 hrs. of treatment.

Plant species	LC_{50} values	LC_{90} values
Sapindus mukorossi	12.246 mg/lit SD 0.288 SEM 0.166	20.823 mg/lit SD 0.407 SEM 0.235
Cestrum nocturnum	16.455 mg /lit SD 0.150 SEM 0.086	27.147 mg /lit SD 0.265 SEM 0.153
Cestrum diurnum	10.531 mg /lit SD 0.195 SEM 0.112	18.390 mg /lit SD 0.307 SEM 0.177
Asclepias curassavica	19.079 mg/ml SD 0.228 SEM 0.132	30.599 mg/lit SD 0.436 SEM 0.252

RESULT AND DISCUSSION

Phytochemical screening:

Table 1 shows the phytochemicals present in the methanolic extracts of leaf of *S. mukorossi*, C. *nocturnum*, *C. diurnum*. and *A. curassavica*. However, the leaves of *S. mukorossi* and *A. curassavica* contain less flavonoid than those of the *Cestrum* species. The extracts of *Cestrum nocturnum* and *diurnum* contain good amount of alkaloids, saponins,

flavonoids, steroids and tannins as compare to other two plants. The phytochemicals of the plants serve as huge storage of compounds that have biological action (Howard *et al.,* 2007). Alkaloids, saponins, and tannins are known to possess medicinal and pesticidal properties (Azmathullah *et al.,* 2011).

In addition, compounds such as flavonoids, alkaloids, tannins and saponins in the plants are responsible for the insecticidal and toxicity to other animals (Nweze *et al.,* 2004, Akinyemi *et al.,* 2005). Saponins are known by their toxicity to harmful insects (Chaieb, 2010). Saponins isolated from *Achyranthes aspera* through bioassay guided fractionation possessed a larvicidal efficacy against *A. aegypti* and *C. Quinquefasciatus* (Bagavan *et al.,* 2008). Moreover, Flavonoids isolated from water extracts of *Annona squamosa* is effective as insecticides against mosquito killing 80% of *C. Chinensis* (Kotkar *et al.,* 2002).

Larvicidal Efficacy of Plant Extracts: The larvicidal activity of the methanolic extracts of the plants against the larvae of the dengue-vector, *Aedes aegypti* mosquito was determined through mosquito larval bioassay. The mortality of the mosquito larvae were noted in the 20 ppm, 40 ppm and 60 ppm concentrations of the plant extracts after 24 and 48 hours of exposure. Table 2 shows the average and percentage mortality of *Aedes aegypti* mosquito larvae treated in three various concentrations of plant extracts and the control group after 24 hours of treatment. Variations of the death percentage of mosquito larvae among the plant extracts were observed. On the other hand, the least percentage mortality was observed in the control group (1.72%) which is extremely low than those in the experimental groups. The 60 ppm concentration of *S. mukorossi*, *C. nocturnum* and *C. diurnum* leaf extract shoes the highest percentage of mortality equal to 100 % of the mosquito larvae. On the other hand, *A. curassavica* leaf extract shows the least percentage of mortality compared to other plant samples.

Besides, the plant extracts exhibited a concentration dependent activities against mosquito larvae since the percentage mortality were observed to increase with increasing concentrations of the plant extracts. The increase of percentage mortality of the treated mosquito larvae is supported by the presence of phytochemicals in the plant extracts which have pesticidal activities. Table 3 shows the average and percentage mortality of *Aedes aegypti* mosquito larvae treated with various concentrations of the plant extracts and the control group after 48 hours. The least percentage mortality was noted in the control group (3.43%) which is extremely lower compared to those in the experimental groups. It reveals that all tested concentrations of the three plant extracts caused mortality of mosquito larvae in comparison to those in the control group.

Result also indicates that 40 ppm and 60 ppm concentrations of *S. mukorossi* and *C. nocturnum* leaf extract have the highest percentage of mortality. Among the various concentrations of the plant extract. The plant's high larvicidal activity is supported by the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and tannins which are known to have insecticidal and pesticidal properties. These phytochemicals present in the *S. mukorossi* and *C. nicturnum* leaf extract could show synergistic effects in terms of larvicidal action of mosquito larvae. This is supported by the previous study on the larvicidal efficacy of the these plant extracts, Results showed the presence of phytochemicals such as steroids, alkaloids, terpenes, saponins, etc. that are accountable for the positive effects. Other studies have also reported that plants that contain larvicidal activity could act in combination or independently (Ndung'u *et al.,* 2004). On the other hand, 40 ppm concentration of Asclepias curassavica extract shows the least percentage mortality in the experimental group. The plant's low mortality effect on mosquito larvae is due to the less concentration of saponins and tannins in the leaf extracts of the said plant.

It is further noted that the percentage mortality increased with increasing concentrations of the three plant extracts. Moreover, the mortality of mosquito larvae was also increased in relation to the time of exposure. The larvicidal activity of the highest concentration (60 ppm) of the plant extracts on A. aegypti mosquito larvae within 48 hours of exposure showed the following order i.e. *S. mukorossi* > *C. nocturnum* > *C. diunrum* > *A. curassavica* leaves.

Moreover, the results also exhibited that there is a significant difference on the mortality of mosquito larvae between the control group and the 60 ppm concentration of the various plant extracts. This result denotes that higher concentration of the plant extracts would lead to greater number of mortality in the mosquito larvae. Furthermore, it implies that the efficacy of the plant extracts as larvicide is not due to the use of methanol as the extracting solvent. Hence, it is attributed to the different phytochemicals present in these plant extracts.

Determination of LC_{50} and LC_{90} values:

The lethal concentrations (LC_{50} and LC_{90}) values of these four plant extracts on *Aedes aegypti* mosquito larvae after 24 hours of exposure are summarized in Table 4. *C. nocturnum* and C. diurnum leaf extract reveals the lowest LC_{50} and LC_{90} values of 9.02 ppm, 15.27 ppm and 8.96 ppm, 10.05 ppm respectively. It shows that *C. diurnum* leaf extract is the most effective in terms of pesticidal activity compared to the other three plant samples. On the other hand, *Asclepias curassavica* leaf extract found to be the least effective among the plant samples since it has the highest LC_{50} and LC_{90} values of 11.74 ppm and 21.39 ppm, respectively. Results show that *C. diurnum* is highly lethal to *A. aegypti* larvae which were followed by *C. nocturnum* and *S. mukorossi* respectively.

It is evident from the result that the various concentrations of the three plant extracts were the main cause of mortality in *A. aegypti* larvae. 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 LC_{50} values of 92.9 ppm and found extract positive for the presence of saponin. Various authors have evaluated larvicidal activity of cestrum species on mosquitoes; where they found a steroidal bioactive compounds responsible for mosquitocidal activity (Ghosh and Chandra, 2006; Ghosh *et al.*, 2008). Jawale *et al.*, (2010) reported Cestrum nocturnum as larvicide against *Aedes* aegypti mosquito where methanol extract outstand as highly active larvicide, achieving 100 % larval mortality in 24 hours when tested in the concentration of 45 µg/mL (soxhlet) and 25 µg/mL (percolation). Wiesman and Chapagain (2006) reported a strong correlation between saponin content and mortality for Aedes aegypti (yellow fever mosquito) larvae exposed to extracts of Balanites aegypticiaca. 0.0014% (v/w) of the most active fraction proved to be sufficient to kill 50% of the larvae before formation of adults. Here they also showed that extraction of this active fraction is relatively easy (as unpure mixtures of saponins work well in most cases). Therefore suggested saponins, as a cheap way to reduce the mosquito population and could play an important role in mosquito control.

Pelah *et al.* (2002) also found high mortality of *A. aegypti* and *Culex pipiens* (northern house mosquito, vector of the western Nile virus) mosquito larvae when exposed to serial concentrations of *Quillaja* bark saponins. Table 5 shows the lethal concentrations (LC_{50} and LC_{90}) values of the plant extracts on *Aedes aegypti* mosquito larvae after 48 hours of exposure. *C. diurnum* extract reveals the most effective as mosquito larvicides since it has the lowest LC_{50} and LC_{90} values of 10.531ppm and 18.390 ppm respectively. However, *A. curassavica* extract shows the least larvicidal activity among the four plant samples with the highest LC_{50} and LC_{90} values of 19.07ppm and 30.59ppm respectively. Results show that the leaf extract of *C. diurnum* is highly lethal followed *C. nocturnum* and *A. curassavica* respectively. In addition, The LC_{50} values of extracts of medicinal plants *Plumbago zeylanica* and *Cestrum nocturnum* against *Aedes aegypti* were reported by Patil *et al.*, (2011) as less than 50 ppm. The results of the LC_{50} and LC_{90} significantly decreased in relation to the time of exposure with the least value after 48 hours of exposure. It means that increasing the period of exposure increases the mosquito larvae mortality. Results of the present study with the extracts from different plant species exhibited variable larvicidal efficacy. The presence of several bioactive chemicals like alkaloids, saponins, tannins, flavonoids and steroids can be attributed to the susceptibility of the plant extracts as killing agent against mosquito larvae. 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. that 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 defence mechanisms against microorganisms, insects and herbivores.

Saponins are known to have various biological properties. They have membrane-permeabilising, haemolytic, antioxidant, anti-inflammatory, immunostimulant and anticarcinogenic activities, they affect feed intake, growth and reproduction in animals, and they can be used as fungicides, molluscicides and pesticides, as well as against some bacteria and viruses (Francis *et al.*, 2002; Sparg *et al.*, 2004; Avato *et al.*, 2006; Tava and Avato 2006). Saponins give rise to increased mortality levels, lowered food intake, weight reduction, retardation in development, disturbances in development and decreased reproduction in pest insects. The mechanism underlying these actions is, however, still largely unknown, but it is likely that saponins have multiple activities. The main hypotheses are that saponins could either make the food less attractive to eat (repellent / deterrent activity), bear digestive problems, because of moulting defects or have toxic effects on cells. (Ellen De Geyter *et al.*, 2007). Moreover, saponins are freely soluble and can be extracted in both aqueous and organic solvents and perform their action by attacking with the cuticle

membrane of the larvae, eventually disturbing the membrane, which is the main cause for larval death (Hostettmann and Marston 2005). Another study reported that saponin extracted from fruit of *Balanites aegyptica* showed 100% mortality against larvae of *Stegomyia aegypti* (Hostettmann and Marston 2005). On the other hand, flavonoids revealed an extensive scope of biocontrol potential such as antimicrobial and insecticidal activities. Important phenolics in terms of insecticidal, repellent and feeding deterrent functions are flavonoids which are characteristics of higher plants (Dakora 1995, Ndung'u *et al.*, 2004). Further research can be made to investigate the detailed insecticide mode of action of saponin, for a better understanding of their structure-activity relationship and specificity.

CONCLUSION

The study on the larvicidal activity of the methanolic leaf extract of *Sapindus mukorosii*, *Cestrum nocturnum*, *Cestrum diurnm*, 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. These compounds are known to possess insecticidal and larvicidal activities of insects and other animals. These four plant samples showed larvicidal activity to *Aedes aegypti* mosquito larvae which is manifested by a high percentage of mortality in comparison to those in the control group. Furthermore, *C. diurnum* extract shows the most effective larvicide among the various plant extracts with the percentage mortality of 100% in 24 and 48 hours of exposure respectively.

The plant's high larvicidal activity is supported by the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and tannins which showed combination effects in terms of larvicidal action to mosquito larvae. Moreover, *A. curucovas* extract displayed least effective in larvicidal activity compared to the other extract which is manifested by the highest LC_{50} and LC_{90} values after 48 hours of exposure. Its phytochemical test also suggested the presence of fewer amounts of phytochemicals. On the other hand, *C. diurnum* extract is the most effective in larvicidal activity compared to the other extract which is manifested by the lowest LC_{50} and LC_{90} values after 48 hours of exposure. Both cestrum species also exhibited presence of more amount of phytochemicals specially alkaloids and saponin.

All these four plants show effective larvicidal activity. It differs according to the plant species. The presence of variability in bioactive chemicals like alkaloids, saponin, tannins, flavonoids and steroids can be attributed to the susceptibility of the plant extracts as killing agent against mosquito larvae.

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