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Larvicidal and pupicidal properties of *Coccinia grandis* gourd fruits against malaria transmitting vector *Anopheles stephensi* and dengue transmitting vector *Aedes aegypti*

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Abstract
Plant based natural control of vector mosquitoes is very important to prevent the environmental toxicity caused by insecticides used to control the mosquitoes. Thus, in the present research was aimed to study the larvicidal and pupicidal properties of different extracts of fruit of *Coccinia grandis* gourd against malaria vector *Anopheles stephensi* and dengue vector *Aedes aegypti*. The fourth instars larvae of *A. stephensi* and *A. aegypti* were used for larvicidal activity and kept a container containing 249 ml of distilled water and 1ml of known concentration of plant extracts and incubated for 24 hours. The mortality rate of larvae was observed at every 3 hours and recorded. Pupae of *A. stephensi* and *A. aegypti* were placed a container containing 249 ml of distilled water and 1ml of desired concentration of plant extracts and incubated for 24 hours. The mortality of pupae was observed at every 3 hours and recorded. Different extracts of fruit of *C. grandis* gourd possessed potential larvicidal and pupicidal activity against malaria vector *A. stephensi* and dengue vector *Aedes aegypti*. It was due to the presences of phytocompounds such as tannis, saponin etc. Different extracts of fruit of *C. grandis* gourd possesses larvicidal and pupicidal activity against malaria transmitting vector *A. stephensi* and dengue transmitting vector *Aedes aegypti*. Thus the fruit of *C. grandis* gourd was might be useful for control of mosquitoes after the details experiments.

Keywords: Malaria, Dengue, *A. aegypti A. stephensi, Coccinia grandis,* Phytochemicals, Larvicidal and pupicidal

Introduction
Mosquitoes are the most important bloodsucking arthropods that transmit many of the diseases to the human being. It carries the microbes that cause the diseases than any other arthropods and affects millions of people worldwide. WHO has declared that the mosquitoes are "number one public enemy" and act as a major problem for public health [1, 2]. They can transmit most life threatening diseases like malaria, dengue, chikungunya, filariasis, yellow fever, encephalitis, West Nile virus infection, etc., in almost all parts of the world. Among communicable diseases, malaria is the third most killing diseases in the world. The World Health Organization (WHO) reported that malaria, human immunodeficiency virus (HIV), and tuberculosis are the three major infectious diseases throughout the world [3]. It infecting approximately 300-500 million peoples every year [4]. In 2015, WHO reported 214 million malaria cases in the world and 438000 deaths due to this disease. Unfortunately, approximately 70% of malaria deaths occur in children less than five years of age [5].

Dengue is fastest re-emerging diseases that greatly affects the economy and health of developing and developed countries [6]. It is an acute viral disease that caused by a single-stranded RNA virus that belongs to the *Flaviviridae* family. They may lead to severe manifestations like DSS or DHF [7, 8]. There are about 390 million people were infected by dengue every year and of which 96 million cases showed significant clinical symptoms against dengue. It does not have a particular treatment, but there is a chimeric tetravalent vaccine that is made of attenuated serotypes of dengue viral strains [7].

Some of the chemical pesticides such as Anvil, Permethrin, Malathion and insecticides such as organochlorine, organophosphate, DDT, dieldrin, endosulfan and carbamates are used to control the vector mosquito. It may cause unwanted side effect to human population and environment and also target vector can develop resistance to these pesticides. Hence the control of vector mosquito is still challenging problem to the medical field.

Recent years, traditional use of natural medicines from plant origin received much attention and as they are significantly evaluated for their safety for using human beings and their efficacy[9]. Pesticides from herbal plants are less toxic to environment and human being and delayed the development of resistance to the vector mosquitoes. Herbal plants are rich source for the alternative control of mosquitoes. Bioactive compounds from herbal plants act as a specific targets to insects and they are eco-friendly [10]. Medicinal plant *Coccinia grandis* was widely used for the treatment of various diseases including cancer, infectious diseases, diabetes, inflammatory conditions, worms, infestations and malaria fever [11]. Thus the present study was focused on the biological control of malarial transmitting mosquito *A. stephensi* and dengue transmitting *A. aegypti* by fruit of *Coccinia grandis.*

2. Materials and Methods

2.1. Study Plant

The unripe fruit of the *C. grandis* was collected from local areas, dried and powdered. Then the powder was used for the preparation of extracts using ethanol, ethyl acetate, chloroform and aqueous as solvents. The plant extract was prepared by cold extraction method and the extracts were concentrated by rotary evaporator.

2.2. Study animal

Larvae and pupae of *A. stephensi* and *A. aegypti* were collected from Vector Control Research Centre, ICMR, Madurai.

2.3. Larvicidal activity of different extracts of fruit of *Coccinia grandis* against malarial vector *A. stephensi* and dengue vector *A. aegypti*

The larvae of *A. stephensi* and *A. aegypti* were used for larvicidal activity. Six numbers of fourth instars larvae of both species were kept in 500ml beaker containing 249ml of distilled water and 1ml of desired concentration of plant extracts were added and incubated for 24 hours and 48 hours. The mortality of larvae was observed for every 3 hours and recorded [12].

$$\text{Corrected mortality} = \frac{\text{Mortality observed in extract treated} - \text{Mortality observed in control}}{100 - \text{Mortality of Control}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larva}}{\text{Number of tested larvae}} \times 100$$

2.4 Pupicidal activity of different extracts of fruit of *Coccinia grandis* against malarial vector *A. stephensi* and dengue vector *A. aegypti*

Six numbers of freshly emerged pupae of both species were kept in 500 ml glass beaker containing 249ml of distilled water and 1ml of desired concentrations of different plant extracts. Control was set up by mixing 1ml of desired solvent respectively with 249 ml of dechlorinated water. Mortalities were corrected by Abbott's formula [12].

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment-observed Mortality in control}}{\text{100-Control in mortality}} \text{x}100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead pupae}}{\text{Number of tested pupae}} \text{x}100$$

2.5 Statistical analysis

All data were expressed as mean ± SD. Hypothesis testing methods include One Way Analysis of Variance (ANOVA). LC_{50} was calculated by probit analysis. All the data were analyzed with SPSS software.

3. Results

3.1. Larvicidal activity of different extracts of *C. grandis* gourd against malarial vector *A. stephensi* and dengue transmitting vector *A. aegypti*

The larvicidal activity of *C. grandis* gourd against larvae of *A. stephensi* showed at dose dependent manner. That is higher concentration showed maximum larvicidal activity than the lower concentration. Maximum mortality 73.33±7.7% of *A. stephensi* larvae was observed in high concentration (100%) of ethanol extract of *C. grandis* gourd. Meanwhile, mortality against larvae of *A. aegypti* showed 71.4±4.4 at higher concentration (Table 1 and 2 and fig. 1 and 2). The LC_{50} value of ethanol extract was observed in 64.10% against *A. stephensi* and 62.34 for *A. aegypti* (Table 5 and 6). One way ANOVA test showed that there was a significant difference (p>0.05) among the all concentrations. Ethyl acetate extract of *C. grandis* gourd showed maximum percentage of larvicidal activity (71.1±5.8%) against *A. stephensi* and 74.4±4.0% against *A. aegypti* and LC_{50} of value of ethyl acetate was at 74.53. More or less similar larvicidal activity was observed in aqueous extract comparted to the ethyl acetate extract against the larvae of *A. stephensi* and *A. aegypti* showed 72.2±3.2 mortality percentages (Table 1 and 2 and fig. 1 and 2). Moreover LC_{50} value of aqueous extract of *C. grandis* gourd against *A. aegypti* was found at 62.37% (Table 5 and 6). One way ANOVA test proved that there were significant differences (p>0.05) among the all concentrations (Table 2). 71.1±5.9% of mortality was observed in high concentration of chloroform extract of *C. grandis* gourd against *A. stephensi* and 68.3±2.3% of mortality showed against *A. aegypti*. The LC_{50} of chloroform extract was 74.53% for *A. stephensi* and 62.37% against *A. aegypti* and (Table 5 and 6). There was a significant different (p>0.05) among the all concentrations of chloroform of *C. grandis.*

3.2. Pupicidal activity of different extracts of *C. grandis* gourd against malarial vector *A. stephensi* and dengue transmitting vector *A. aegypti*

C. grandis gourd showed dose dependent manner of pupicidal activity against *A. stephensi* and *A. aegypti*. That is higher concentration showed maximum pupicidal activity than the lower concentration. Maximum mortality 80.0±7.69% and 68.9±5.9% was observed against pupae of *A. stephensi* and *A. aegypti* respectively in high concentration (100%) of ethanol extract of *C. grandis* gourd (Table 1 and 2 and fig. 1 and 2). The LC_{50} value of ethanol extract was observed in 58.01%

against *A. stephensi* and 51.58 for *A. aegypti* (Table 5 and 6). One way ANOVA test showed that there was a significant difference (p>0.05) among the all concentrations. Ethyl acetate extract of *C. grandis* gourd showed maximum percentage of pupicidal activity (77.7±5.9%) against *A. stephensi* and 75.5±5.1% against *A. aegypti* and LC_{50} of value of ethyl acetate was at 67.29% against *A. stephensi* and 56.85% was recorded against *A. aegypti* (Table 1 and 2 and fig. 1 and 2). Maximum level of mortality of pupae was observed in high concentration of chloroform and water extracts of *C. grandis* gourd against *A. stephensi* and *A. aegypti*. The LC_{50} of chloroform extract was 59.32% for *A. stephensi* and 53.89% against *A. aegypti* and (Table 5 and 6) and in water extract 50% of mortality of pupae against *A. stephensi* was 67.28% and 51.91% against *A. aegypti*. There was a significant different (p>0.05) among the all concentrations of chloroform of *C. grandis.*

Table 1: Larvicidal activity of different extracts of *C. grandis* gourd against malarial vector *A. stephensi*

Extracts	Hours	Concentration (%) Mortality (%)				
		20	40	60	80	100
Ethanol	24	8.9±2.2	24.4±2.2	46.7±3.8	60.0±10.2.	73.3±7.7
Ethyl acetate	24	13.3 ±3.8	15.5±5.8	31.1±5.8	55.5±5.8	71.1±5.8
Chloroform	24	11.1±2.2	26.6±3.8	37.7±5.6	46.6±3.8	62.2±2.2
Water extract	24	13.3±3.84	15.5±5.9	31.1±5.9	55.5±5.9	71.1±5.9

Table 2: Larvicidal activity of different extracts of *C. grandis* gourd against denue transmitting vector *A. aegypti*

Extracts	Hours	Concentration (%) Mortality (%)				
		20	40	60	80	100
Ethanol	24	13.3±3.7	13.9±3.1	39.8±3.2	67.8±2.2	71.4±4.4
Ethyl acetate	24	13.3±1.7	18.9±3.1	47.8±3.2	67.8±4.4	74.4±4.0
Chloroform	24	6.3 ±2.3	33.3±5.7	46.7±7.7	62.2±2.3	68.3±2.3
Water	24	13.3+1.8	18.9±3.2	47.7±3.8	65.6±3.6	72.2±3.2

Table 3: Pupicidal activity of different extracts of *C. grandis* gourd against malarial vector *A. stephensi*

Extracts	Hours	Concentration (%) Mortality (%)				
		20	40	60	80	100
Ethanol	24	13.3±3.84	31.1±5.9	51.1±5.8	57.7±8.0	80.0±7.69
Ethyl acetate	24	11.1±4.4	20.0±3.8	33.3±6.6	60.0±3.84	77.7±5.9
Chloroform	24	8.9±2.2	31.1±5.9	51.1±5.9	57.7±8.0	80.0±7.7
Water	24	11.1±4.4	20.0±3.8	33.3±6.7	60.0+3.8	77.8±5.9

Table 4: Pipcidal activity of different extracts of *C. grandis* gourd against dengue transmitting vector *A. aegypti*

Extracts	Hours	Concentration (%) Mortality (%)				
		20	40	60	80	100
Ethanol	24	11.1±2.2	28.9±2.20	35.6±5.9	62.2±5.9	68.9±5.9
Ethyl acetate	24	10.0±2.3	37.7±5.06	48.9±3.2	65.6±3.2	75.5±5.1
Chloroform	24	11.3±3.3	33.7±3.8	42.4±8.0	57.8±2.3	68.9±7.7
Water	24	11.1±2.2	43.3±4.1	54.5±3.6	65.5±3.2	81.1±2.5

Table 5: LC_{50} value of different extracts of *C. grandis* gourd against larvae malarial transmitting vector *A. stephensi*

Extracts	Mortality Hours	LC_{50}			LC_{90}			Chi Square
		Estimate	Lower	Upper	Estimate	Lower	Upper	
Ethanol	24	64.10	55.52	75.34	178.47	134.48	291.77	8.486
Ethyl acetate	24	74.53	63.37	92.60	235.28	163.34	464.381	15.15
Chloroform	24	79.9	65.54	108.38	325.81	197.48	949.9	3.800
Water	24	74.53	63.37	92.60	235.28	163.34	464.38	15.16

Table 6: LC_{50} value of different extracts of *C. grandis* gourd against larvae of dengue transmitting vector *A. aegypti*

Extracts	Mortality Hours	LC_{50}			LC_{90}			Chi Square
		Estimate	Lower	Upper	Estimate	Lower	Upper	
Ethanol	24	62.34	52.34	72.54	168.89	130.56	241.23	17.78
Ethyl acetate	24	61.16	55.21	68.14	172.29	139.92	233.05	22.31
Chloroform	24	58.98	48.49	66.45	173.56	129.78	230.45	15.15
Water	24	62.37	56.45	70.33	184.27	147.30	256.35	18.526

Table 7: LC_{50} value of different extracts of *C. grandis* gourd against pupae of malarial transmitting vector *A. stephensi*

Extracts	Mortality Hours	LC_{50}			LC_{90}			Chi Square
		Estimate	Lower	Upper	Estimate	Lower	Upper	
Ethanol	24	58.01	49.56	68.56	178.60	132.067	304.47	11.41
Ethyl acetate	24	67.29	58.39	79.53	184.36	138.42	302.83	13.75
Chloroform	24	59.32	51.37	68.96	162.69	124.99	254.70	10.31
Water	24	67.28	58.39	79.53	184.35	138.42	302.83	13.75

Table 8: LC_{50} value of different extracts of *C. grandis* gourd against pupae of dengue transmitting vector *A. aegypti*

Extracts	Mortality Hours	LC_{50}			LC_{90}			Chi Square
		Estimate	Lower	Upper	Estimate	Lower	Upper	
Ethanol	24	51.58	38.89	65.69	163.98	140.89	233.89	13.63
Ethyl acetate	24	56.85	51.04	63.39	168.50	136.12	230.56	18.85
Chloroform	24	53.89	48.78	67.23	155.67	128.78	234.89	15.15
Water	24	51.91	46.52	57.67	151.17	124.39	201.43	14.45

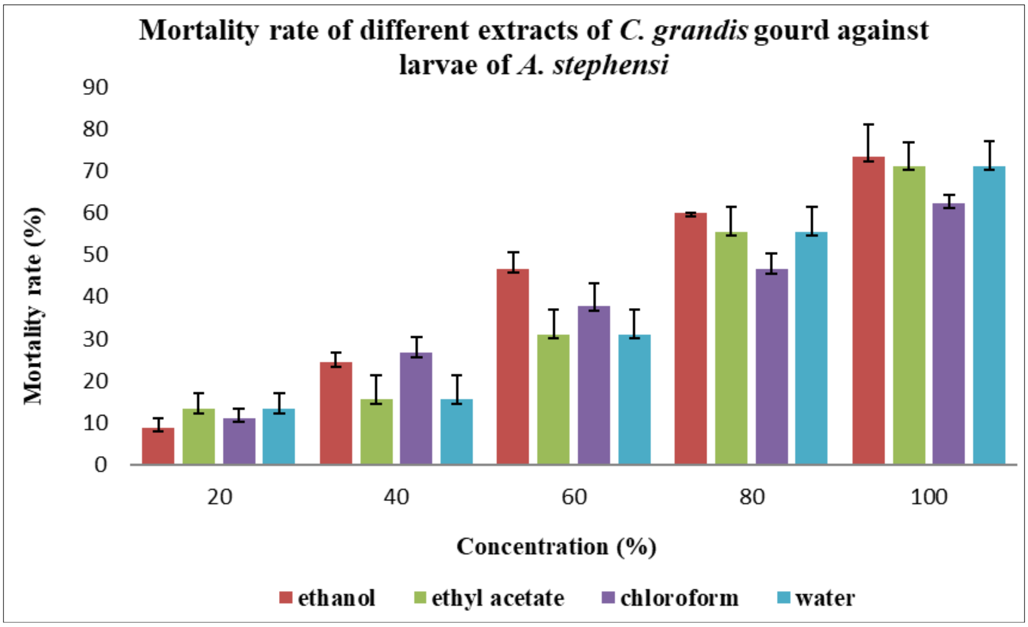

Fig 1: Mortality rate of different extracts of *C. grandis* gourd against larvae of *A. stephensi*

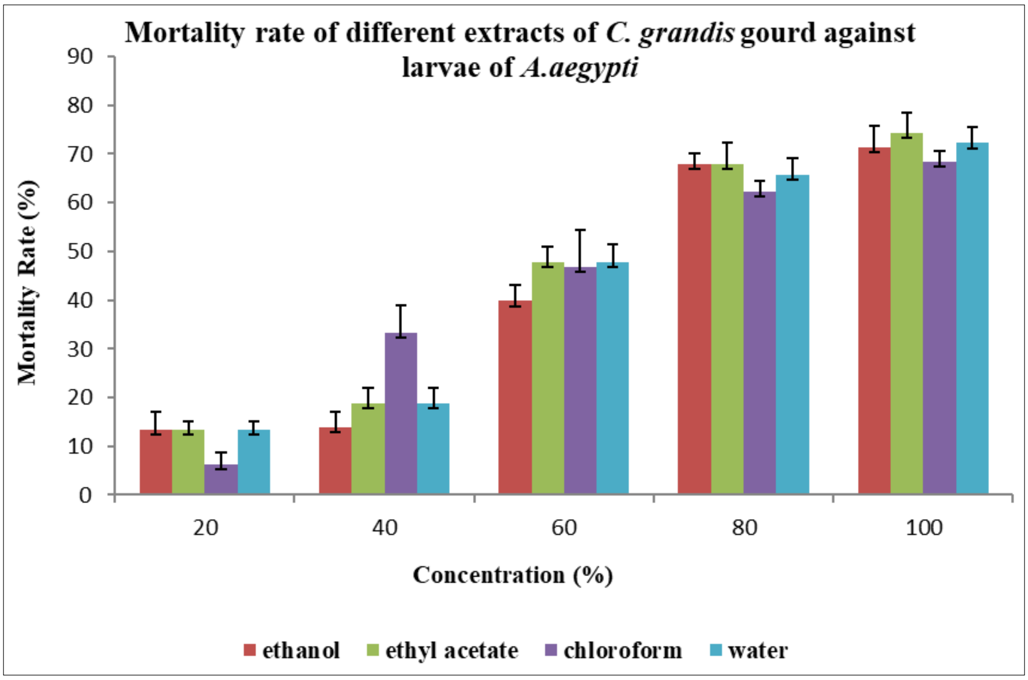

Fig 2: Mortality rate of different extracts of *C. grandis* gourd against larvae of *A. aegypti*

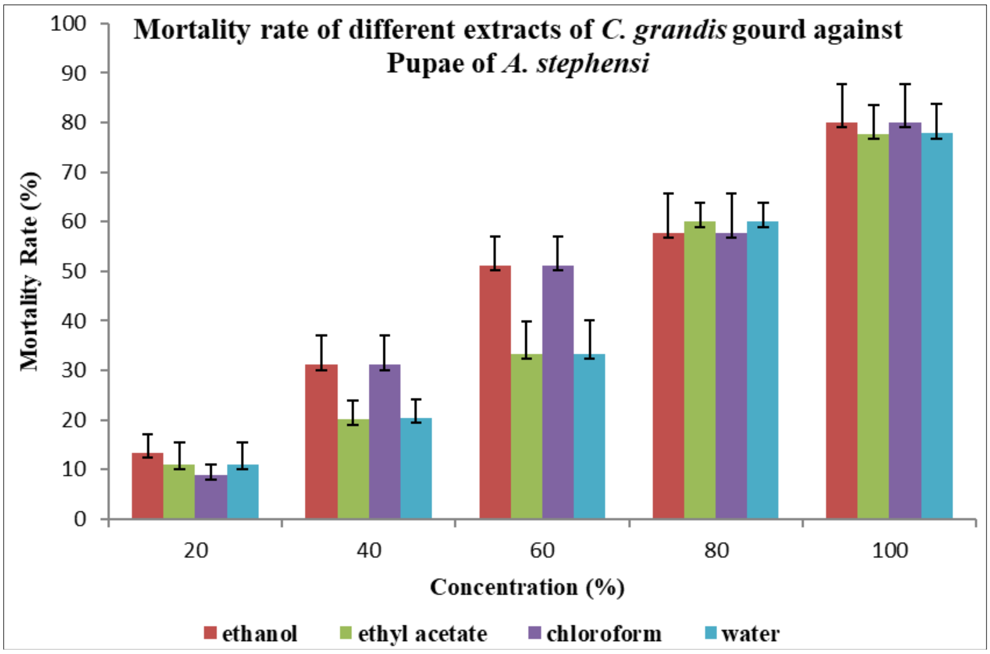

Fig 3: Mortality rate of different extracts of *C. grandis* gourd against pupae of *A. stephensi*

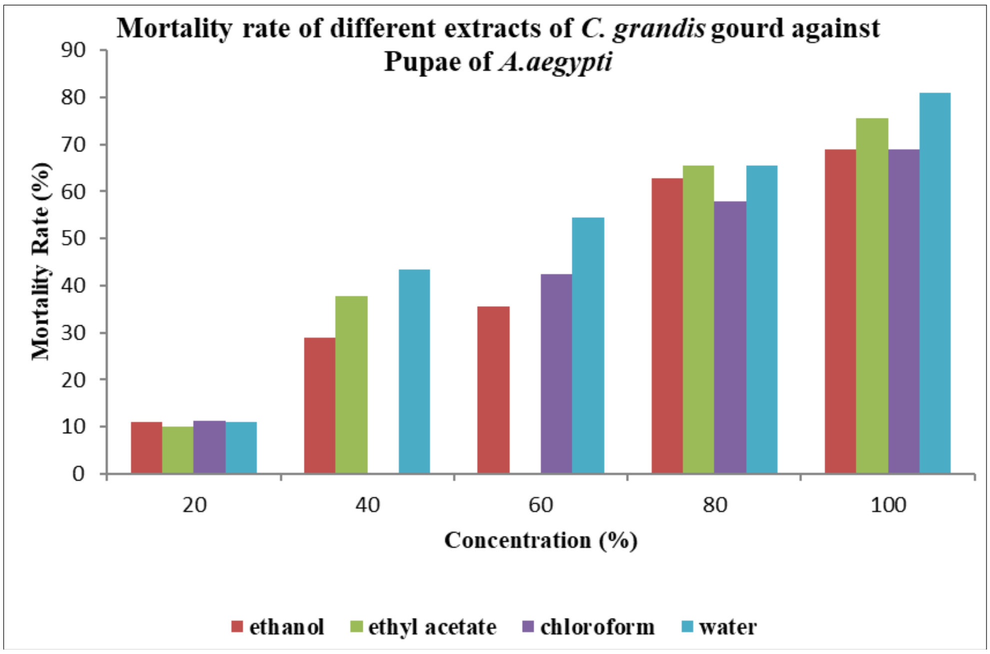

Fig 4: Mortality rate of different extracts of *C. grandis* gourd against pupae of *A. aegypti*

4. Discussion

Mosquitoes are one of the most promising blood sucking insects that cause serious life threatening disease to public health, transmitting several diseases. Insecticides are the residues in the environment due to the insecticide synthesized using chemical have turned the researcher's to attend towards the natural products [10]. In the past years, the phyto kingdoms have been substantial alternative for insecticidal products because of the presence of bioactive principles in the plant materials. Many species in the plant kingdom showed lot of secondary metabolites which are play crucial role against insects/mosquitoes. Plant secondary metabolites are an alternative source for mosquito control and repellent agents [11]. Bioactive principles from plants can be used, either insecticide for killing larvae or adult mosquitoes or repellents for the protection of mosquito bites, depending on the type of active principles [7]. Allelochemicals are considered as a potential source for natural products for insecticides and can be used for management of insect/mosquito control[12]. Phytocompounds are usually less harmful or no harm full than the chemicals drugs and are renewed the interest in the research on phytocompounds, considering as an ecologically safe alternative for synthetic insecticides [13]. Shaalan *et al*. [14] demonstrates that identification of novel effective mosquitocidal compounds from botanicals containing active phytochemicals. Phytochemicals obtained from plants were proved potential of mosquito control and can be used for the alternative source for synthetic insecticides or along with other insecticides under the integrated vector control. Herbal plants are the source of secondary metabolites that useful for the treatment of various diseases [15, 16]. In this study the different extracts of *C. grandis* gourd possessed larvicidal and pupicidal activities against malarial vector *A. stephensi*. The LC_{50} value of different extracts of *C. grandis* gourd against larvae of *A. stephensi* were 78.20% for ethanol extract, 68.46% for ethyl acetate, 66.35% for chloroform extract and 61.97% for water extract respectively. The results of present study were in accordance with the observation of Mwangi and Rembold [17]. Murugan and Jayabalan [18] study suggested that 90% mortality was observed at 4% concentration of *L. aspera* leaf extract against fourth instar larvae of *An. stephensi*. Petroleum ether extract of *L. aspera* showed LC_{50} value between 100 and 200 ppm against the larvae of *Cx. quinquefasciatus*, *A. aegypti*, and *An. Stephensi* [19]. Phytochemicals act as larvicides, insect growth regulators, repellent, ovipositor attractant and have different activities which have been observed by many researchers [20].

Triterpenoids are the effective bioactive compounds for mosquito larvicidal activities [21]. Larvicidal activity of *L. aspera* could be attributed by the presences of phytochemicals such as terpenoids, triterpenoids and alkaloids. It is concluded that natural product from plant source mighty be act as an insecticidal and medicinal values has higher efficiency in reducing mosquito menace due to their larvicidal toxicity. In the present study, the larvicidal and pupicidal activity of *C. grandis* gourd showed that the presences of phytocompounds such as, terpenoids, triterpenoids and alkaloids [22].

Many of the communicable diseases are associated with mosquito-human interaction. Mosquitoes carry parasites that cause well-known illnesses such as malaria, dengue fever, arboviral, chikungunya fever, West Nile virus, encephalitis and yellow fever. These communicable diseases cause significant morbidity and mortality in humans and livestock around the world. In the present study plant is known to be ecofriendly, not toxic to vertebrates. Moreover, it is clearly indicated that crude or partially purified plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts [23-26]. In the present study, high bioactivity of the different extracts from *C. grandis* against *A. aegypti*. Toxicity of the tested plant extracts against the 3rd instar larvae differed according to the solvent and concentration. The larval mortality percent increased as extract concentration increased for all plant extracts. These results agree, to some extent, with the previously mentioned suggestions of [25, 26].

The pupicidal activity of different extracts of *C. grandis* gourd showed pupicidal activity against the *A. stephensi* and *A. aegypti*. The LC_{50} of the *C. grandis* gourd was 71.81%, 88.67%, 75.37% and 71.35% for ethanol, ethyl acetate, chloroform and water extracts respectively. Likewise, Maheshkumar *et al*. [27] recorded that the efficacy of *Solanum xanthocarpum* leaf extracts in the larval and puppal of *A. stephensi* with LC_{50} value of initial instars to fourth instars respectively 155.29, 198.32, 271.12, 377.44 and 448.41 ppm. Likewise, the LC_{90} value of first to fourth instars larvae and pupae were 687.14, 913.10, 1011.89, 1058.85 and 1141.65 ppm, respectively. This result was effectively coincided with our results.

5. Conclusion

From the result of present study, it is concluded that the different extracts of *C. grandis* gourd possesses effective larvicidal and pupicidal activity against malaria transmitting vector *A. stephensi* and dengue transmitting vector *A. aegypti*. The phytochemical such as terpenoids, saponins might be useful for the control of vector mosquitoes. Thus the *C. grandis* gourd was might be useful for control of mosquitoes.

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