A Comparative Study on Larvicidal Activity of Leave Extracts of *Acalypha Indica* L. (Kyaung- Say- Pin) and *Ageratum Conyzoides* L. (Kwe-Tae-Pan)

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Abstract

The phytochemical components and larvicidal properties of *Acalypha indica* and *Ageratum conyzoides* leaves are the subject of this study. The preliminary phytochemical tests revealed that both the leaves of *Acalypha indica* and *Ageratum conyzoides* contained alkaloids, carbohydrates, organic acids, glycosides, flavonoids, phenolic compounds, saponins, tannins, steroids, and terpenoids, but that starch, α-amino acid, and reducing sugars were not present. Larviciding is a useful approach in the control of *Aedes aegypti* species, the vector for malaria, and extensive uses of synthetic organic insecticides during the past decades have resulted in environmental pollution and the development of physiological resistance in major vector species. The larvicidal activity of the leaves of the two plant extracts of *Acalypha indica and Ageratum conyzoides* against 3rd and 4th instar larvae of *Aedes aegypti* larvae was investigated. The larvicidal activity of the leaves of two plants was determined by methanol, ethanol, and watery extracts. Methanol extracts of leaves *of Acalypha indica and Ageratum conyzoides* showed more potent larvicidal activity (LC_{50}= 0.1154 %, LC_{90} = 0.3476 %) and (LC_{50}= 0.0.1071 %, LC_{90}= 0.3538 %) respectively against the late third and four instar larvae (*Aedes aegypti* mosquito). Methanol extracts possess higher larvicidal activity than other extracts. However, the larvicidal activities of these extracts were much lower than those of synthetic larvicides (Deltamethrin). It was recommended that leaf extracts of both plants can be used as larvicides against these insect vectors of public health importance. The plant extracts may serve as larvicidal agent in insect vector control . Many sources of natural compounds suggested alternatives for conventional chemical control. Plants materials offer not only effective mosquito control but also promise to be environmentally safe, low toxicity and a high degree of biodegradation.

Key words: ***Acalypha indica*** L.**,** ***Ageratum conyzoides*** L.**,** ***Aedes aegypti and larvicidal activity***

Introduction

One of the main diseases is spread by mosquitoes in many nations. Some of the illnesses spread by mosquitoes can be fatal to humans. Aedes aegypti transmits the mosquito-borne illness dengue fever. The World Health Organization estimates that dengue is a problem in 128 nations. There are three approaches to stop the spread of diseases by mosquitoes. To do this, remove their habitats for reproducing, lessen human exposure to mosquito vectors, and administer medication to cure the illnesses. A nation can use a variety of synthetic insecticides and larvicides to reduce the mosquito population (Ranaweera *et al*., 1996).The use of larvicides is an evident practical and cost effective method of preventing the spreadof these infections to people. Larviciding is a valuable technique for malaria prevention programs, especially in areas with easily accessible, few, and small breeding sites. In urban locations, where larviciding in a central region may be paired with indoor residual spraying in a barrier zone of homes around the edge of the town or city, such parameters are frequently met. Permethrin and deltamethrin are both effective larvicides for mosquitoes. However, they are the most successful against all aquatic

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insects. Under the strict supervision of professional mosquito control organizations, they are exclusively utilized to restrict quantity (Brown,1995). Due to its effectiveness against Aedes aegypti mosquitoes, deltamethrin is the larvicide that is most frequently used commercially (Carbalho, 2003).

Acalypha indica is treated for chest pain, joint pain, heart diseases, diabetes, antipyretics, throat troubles, diseases of the eye, skin diseases, ulcers, chlorosis, and urinary disorders (Jagatheeswari, *et al.,* 2013). Besides, this plant is used for medicinal applications such as constipation, dermatology ailments, earaches, ganglions, headaches, hemorrhoids, insect bites, pimples, and wound healing. *Acalypha indica* plant extract was evaluated for phytochemical, some biological, larvicidal, and pesticidal activities (Aushi, *et al*., 2018).

Ageratum conyzoides may be alternative sources of mosquito control that contain potentially suitable bioactive compounds that are biodegradable and contain nontoxic products that are effective against mosquitoes (Mohan *et al*., 2005). *Ageratum conyzoides is a* medicinal plant that is effective against diseases and contains biologically active compounds, which are potent against diseases (Prince *et al*., 2011).

Materials and Methods

Collection of Plant Samples

The two plants selected for this study were *Acalypha indica* and *Ageratum conyzoides.* These leaves were collected from Pyay Township. After being dried at room temperature for two weeks. It was made into a powder and stored in airtight containers to prevent moisture changes and contamination.

(a) *Acalypha indica* L. (b) *Ageratum conyzoides* L.

Figure 1 Photograph of leaves of *Acalypha indica* L. and *Ageratum conyzoides* L.

Collection of *Aedes aegypti* Mosquito larvae

North Dagon Township strain of *Aedes aegypti* mosquito larvae and adult *Aedes* mosquitoes emerged from pupae were reared in the laboratory of Medical Entomology Research Division, Department of Medical Research. Larvae were fed on DMR larva food. The adult was provided with 10 % sucrose solution and 8 weeks old

mouse for a blood meal. The *Aedes aegypti* mosquitoes were depended on at 26 ± 2 ºC, 65-75 % relative humidity with a photoperiod of 12 h light and 12 h dark. Laboratory reared *Aedes aegypti* larvae were used for testing insecticidal properties of leaves extracts of both plants.

Mosquito species identification

Larvae and adult mosquitoes that emerged from the larva survey were identified by morphological methods (Rampa and Prachong,1994).

Procedure of Larvicidal Activity

On preliminary tests, dilutions were prepared with distilled water. Different concentrations of 95 % methanol, 95 % ethanol and watery leaves extracts of both plants. as 0.4 g, 0.2g, 0.1 g, 0.05 g, and 0.025 g were dissolved freshly 100 mL each of distilled water in 150 mL plastic cups. 3rd and 4th instars *Aedes aegypti* fifty larvae were put into different concentrations and also negative control test was done simultaneously. Detail testing was done according to the WHO standard method (WHO, 2005). Larvae were exposed 24hr for each replication in different concentrations in a laboratory at 27-29 ºC and 70 – 80 % relative humidity. Six replicates were carried out and mortality was checked and recorded after 24 h of the exposure period. Dead larvae were identified when the larvae failed to move after probing with a needle in the thorax region of the body. Lethal concentration LC_{50} and LC_{90} values for 95% confidential limits were calculated by the formula of (Finney , 1971). For comparison purpose, LC_{50} and LC_{90} values of commercially available mosquito larvicide which contain 0.0032 g, 0.0016 g, 0.0008 g, 0.0004 g and 0.0002 g deltamethrin was investigated.

Larvicidal bioassay

Larval mortality was recorded after 24 h in each of the treatments and the control mortality were corrected using Abbott's formula.

$$\text{Percentage molarity} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100\%$$

Results and Discussion

Phytochemical Examination of Both Plant Samples

Preliminary phytochemical test were carried out in order to know the different types of chemical constituents present in the plants. Phytochemical analysis of leaves of both plants indicated the presence of alkaloids, carbohydrates, organic acid, glycosides, flavonoids, phenolic compounds, saponins, tannins, steroids and terpenoids were observed to be present but starch, α-amino acid and reducing sugars were absent.

Screening of Larvicidal Activity of Some Extracts from Leaves of *Acalypha indica* L. and *Ageratum conyzoides* L.

The methanol extract of leaves of two plants found to possess larvicidal activity. Comparative studies on the larvicidal activity of plant extracts were made by commercially available larvicides, deltamethrin.

Table 1 Mortality Effect of Different Dilutions of *Acalypha indica* L. Leaves Extracts Against 3rd and 4th Instars *Aedes aegypti* Larvae within 24 Hours Exposure

Conc. (g)	Total larvae	*Acalypha indica* L. (Kyaung- Say- Pin) Extracts					
		Methanol		Ethanol		Watery	
		Mortality	Mortality (%)	Mortality	Mortality (%)	Mortality	Mortality (%)
0.40	300	283	94.33	259	86.33	235	78.33
0.20	300	216	72.00	182	60.67	121	40.33
0.10	300	114	38.00	102	34.00	64	21.33
0.05	300	42	14.00	48	16.00	30	10.00
0.025	300	16	5.33	17	5.67	14	4.67

This table shows that the highest mortality effect of *Aedes aegypti* larvae was found 94.33 % at 0.400 g dilution of *Acalypha indica* leaves methanol extract followed by 86.33 % mortality at ethanol extract and the lowest was observed 78.33 % mortality in watery extract solution. The lowest mortality effect was found 5.33 %, 5.67 %, and 4.67 % respectively in 0.025 g dilution of methanol, ethanol and watery extracts.

Table 2 LC_{50} and LC_{90} Values of *Acalypha indica* L. Leaves Extracts Against 3rd and 4th Instars *Aedes aegypti* Larvae

No.	Extracts	Duration(h)	LC_{50}	LC_{90}	P-value	X^2
1	Methanol	24	0.1154	0.3476	0.05	8.8582
2	Ethanol	24	0.1405	0.5373	0.05	3.6272
3	Watery	24	0.2113	0.9379	0.05	20.7004

Table 2 showed that dose-effect analysis of LC_{50} and LC_{90} values of *Acalypha indica* L. leaves of methanol, ethanol and watery extracts against 3rd and 4th instars *Aedes aegypti* larvae were found 0.1154 g, 0.1405 g and 0.2113 g respectively for LC_{50} and 0.3476 g, 0.5373 g and 0.9379 g, respectively for LC_{90}. The methanol extract needed the lowest amount of extract 50 % and 90 % mortality than ethanol and watery extracts. The ratio of the doses was found 1: 1.2175:1.8310 for LC_{50} and 1: 1.5457:2.6982 for LC_{90}.

Table 3 Mortality Effect of Different Dilutions of *Ageratum conyzoides* L. Leaves Extracts Against 3rd and 4th Instars *Aedes aegypti* Larvae within 24 Hours Exposure

Conc. (g)	Total larvae	*Ageratum conyzoides* (Kwe-Tae-Pan) Extracts					
		Methanol		Ethanol		Watery	
		Mortality	Mortality (%)	Mortality	Mortality (%)	Mortality	Mortality (%)
0.40	300	280	93.33	256	85.33	245	81.67
0.20	300	220	73.33	173	57.67	169	56.33
0.10	300	128	42.67	92	30.67	98	32.67
0.05	300	63	21.00	46	15.33	51	17.00
0.025	300	20	6.67	20	6.67	17	5.67

Table 3 shows that highest mortality effect of *Aedes aegypti* larvae was found 93.33 % at 0.4g dilution of *Ageratum conyzoides* leaves methanol extract followed by 85.33 % mortality at ethanol extract and lowest was observed 81.67 % mortality in watery extract solution. Lowest mortality effect was found 6.67 %, 6.67 %, and 5.67 % respectively in 0.025 g dilution of methanol, ethanol and watery extracts.

Table 4 LC_{50} and LC_{90} values of *Ageratum conyzoides* Leaves extract of Methanol, Ethanol and Watery against 3rd and 4th instars *Aedes aegypti* larvae

	Extracts	Duration	LC_{50}	LC_{90}	P value	X^2
1	Methanol	24 hrs	0.1071	0.3538	0.05	3.6096
2	Ethanol	24 hrs	0.1479	0.6032	0.05	8.9380
3	Watery	24 hrs	0.1550	0.6673	0.05	2.4412

Table 4 Showed that dose effect analysis of LC_{50} and LC_{90} values of *Ageratum conyzoides* leaves extract of methanol, ethanol and watery against 3rd and 4th instars *Aedes aegypti* larvae were found 0.1070 g, 0.1479 g and 0.1550 g respectively for LC_{50} values of methanol, ethanol and watery extracts and 0.3538 g, 0.6032 g and 0.6673 g respectively for LC_{90} values of methanol, ethanol and watery extracts. Methanol extract needed the lowest amount of extract 50 % and 90 % mortality than ethanol and watery extracts. The ratios of the doses were found 1: 1.3822 : 1.4486 for LC_{50} and 1: 1.7049 : 1.8861 for LC_{90}.

Table 5 Mortality Effect of Deltamethrin Against 3rd and 4th Instars *Aedes aegypti* Larvae within 24 Hours Exposure

Concentration (g)	Total larvae	Mortality	Mortality %
0.0032	300	283	94.33
0.0016	300	201	84.67
0.0008	300	156	66.33
0.0004	300	96	48.67
0.0002	300	34	29.67

Table 6 LC_{50} and LC_{90} Values of Synthetic Larvicide Deltamethrin Against 3rd and 4th Instars *Aedes aegypti* Larvae

No.	sample	Duration	LC_{50}	LC_{90}	P-value	X^2
1.	Deltamethrin	24 hr	0.0005	0.0031	0.05	0.048

Table 7 Comparison of LC_{50} and LC_{90} for Tested Samples and Std

Tested samples (Extracts)	LC_{50}	LC_{90}
A.indica (Watery)	0.2113	0.9379
(EtOH)	0.1405	0.5373
(MeOH)	0.1154	0.3476
A. conyzoides (Watery)	0.1550	0.6673
(EtOH)	0.1479	0.6032
*(*MeOH)	0.1071	0.3538
deltamethrin (Std)	0.0005	0.0031

Conclusion

The present study investigates the bioactivity of two plants extract against the third and fourth instar larvae of *Aedes aegypti* mosquitoes.CH_3OH extracts of two plants leaves showed potent larvicidal activity (LC_{50}= 0.1154 %, LC_{90}= 0.3476 %) and (LC_{50}= 0.1071 %, LC_{90}= 0.3538 %) respectively against the late third and fourth instar larvae (*Aedes aegypti* mosquito). However, the larvicidal activities of these extracts were much lower than those of synthetic larvicides (Deltamethrin). According to these observations, it can be determined that crude extracts of both plants leaves showed larvicidal activity. So many sources of natural compounds suggest alternatives to conventional chemical control. Plant materials offer not only effective mosquito control but also promise to be environmentally safe, low in toxicity, and have a high degree of biodegradation.

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