Adulticidal activity of some Malaysian plant extracts against *Aedes aegypti* Linnaeus

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Abstract. The adulticidal activity of methanol extracts from three Malaysian plants namely *Acorus calamus* Linn., *Litsea elliptica* Blume and *Piper aduncum* Linn. against adult of *Aedes aegypti* (L.) were studied. Standard WHO bioassay tests were used to evaluate the effectiveness of these plant extracts. The hexane fraction from methanol extract of *Acorus calamus* rhizome was the most effective, exhibiting LC_{50} and LC_{90} values of 0.04 $mgcm^{-2}$ and 0.09 $mgcm^{-2}$ respectively. For *L. elliptica*, the methanol fraction also displayed good adulticidal property with the LC_{50} and LC_{90} values of 0.11 $mgcm^{-2}$ and 6.08 $mgcm^{-2}$ respectively. It is found that hexane fraction of the *P. aduncum* crude extract was the least effective among the three plants showing LC_{50} and LC_{90} values of 0.20 $mgcm^{-2}$ and 5.32 $mgcm^{-2}$, respectively. However, although *A. calamus* showed lowest LC values, the LT_{50} results indicated that the methanol fraction of *L. elliptica* was most potent extract among the extracts tested.

INTRODUCTION

Interest in *Aedes* mosquito lies in the fact that it acts as a vector for dengue fever and dengue hemaorrhagic fever which is endemic in Southeast Asia, the Pacific islands area, Africa and the Americas (Maillard *et. al.*, 1993). Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society and to the increasing resistance of mosquitoes to current commercial insecticides (Ciccia *et. al.*, 2000). Years and million of money have been spent on researches on the dengue vaccine but nothing much is produced.

Plants may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals, are active against limited number of species including specific target-insects and are biodegradable (Sukumar *et. al.*, 1991). Mosquitoes develop genetic resistance to synthetic insecticides (Wattal *et. al.*, 1981) and even to biopesticides such as *Bacillus sphaericus* (Tabashnik, 1994).

Numerous workers have reported the larvicidal activity using the local Malaysian plants against mosquitoes (Lee & Chiang, 1994; Ibrahim *et. al.* 1996; Ee & Lee, 1997; Ee *et. al.*, 2000; Sulaiman *et. al.*, 2001). However, there is limited number of reports regarding the use of plant extracts as adulticidal agents. Somehow for the emergency measures for dengue outbreak, bringing down the number of adult population is the main target. Only few, namely Rohani *et. al.* (1997), has reported the efficacy of few Malaysian essential oils such as *Litsea elliptica, Polygonum minus* and *Piper aduncum* as potential mosquito adulticides while Sulaiman *et. al.* (2001)

has reported the essential oils of *Melaleuca cajuputi* and *Cymbopogon nardus* have adulticidal effects on *Aedes* mosquito at high-rise flats in Kuala Lumpur.

In our previous study (Hidayatulfathi *et. al.* 2003) has demonstrated significant mosquitoes larvicidal activity of 10 Malaysian plant extracts. The most effective plant extracts, *Acorus calamus* Linn. (Jerangau), *Litsea elliptica* Blume (Medang pepijat) and *Piper aduncum* Linn. (Sireh lada) were chosen for the present study with the aim to determine their adulticidal activity against *Aedes aegypti*.

MATERIALS AND METHODS

Plant Materials

The plants materials were collected from various locations from Peninsular Malaysia. *Litsea elliptica* was collected from Pasoh, Negeri Sembilan while *Acorus calamus* and *Piper aduncum* from Tanjung Karang and Kepong, Selangor. Identifications were made and confirmed by botanists at Forest Research Institute of Malaysia where voucher specimens are deposited.

Extraction

Litsea elliptica leaves was collected in Pasoh, Negeri Sembilan while *Acorus calamus* rhizome was from Tanjung Karang, Selangor and *Piper aduncum* leaves and flowers were collected in Kepong, Selangor. The samples were washed and shade dried at room temperature. The dried samples were grind to produce smaller particles to enhance the extraction yield and were extracted using Soxchlet apparatus for at least 20 hours with methanol. After the filtration, the filtrates were evaporated to

dryness at 45°C and under vacuum condition. The dried extracts were then fractionated into hexane, chloroform, ethyl acetate and methanol fractions using the successive reflux procedures from Costa *et. al*. (1997) with slight modifications.

Bioassay

Bioassays for the toxicity of the extracts were carried out against adult of *Aedes aegypti* using the WHO standard bioassay procedures (World Health Organization, 1981) with slight modifications. The colonies were established at the Insectarium of Faculty of Allied Health Sciences. Preliminary bioassay tests revealed that the hexane fractions of *A. calamus* and *P. aduncum* and also methanol fraction of *L. elliptica* were more toxic than other fractions and hence further tests were conducted with these fractions. Some 5% stock solutions were prepared by dissolving a known weight in acetone. Serial dilutions from the stock were prepared in concentrations ranging from 0.01% to 5%. Malathion, at a diagnostic dosage of 5% was used as a standard for comparison purposes.

The hexane fractions of *A. calamus* and *P. aduncum* as well as methanol fraction of *L. elliptica* were prepared to concentrations of 0.01%, 0.05%, 1.0%, 2.5% and 5% respectively. Four ml of each concentration were then impregnated on filter papers (140 X 115 mm) making concentrations used were 0.02, 0.12, 2.48, 6.21 and 12.42 $mgcm^{-2}$ respectively. As for the control papers, they were impregnated with acetone only. Impregnated papers were left to dry at room temperature overnight prior to testing.

The mosquito, *Aedes aegypti* used was a Gombak strain (F-156). The 3-7 days old adult female in batches of 15 were treated using the WHO adult bioassay kit. Each test specimen was held for three hours continuously for these were natural compounds therefore the knockdown effect was assumed will be taking longer time to take place. Mortality was recorded every 10 minutes throughout the exposure period. At the end of the three hours exposure, the mosquitoes were placed in the holding tube and given 10% sugar solution enriched with vitamin B complex as the food. The test was replicated 3 times and the control was treated with acetone only. Mortality was observed after 24 hours and if there is any mortality in the control, which is not more 20%, Abbott's formula will be applied (Finney, 1971).

Data Analysis

Acute toxicity were analysed by Probit Analysis Software program to determine the LC_{50}, LC_{90} and the LT_{50} values (Raymond 1985). To determine the most effective extract, ANOVA followed by LSD tests were performed.

RESULTS AND DISCUSSION

It was observed from the results presented that the selected fractions from the plants studied produced high adult mortality against the *Ae. aegypti*. The knockdown effect demonstrated by the three plant fractions exposed for first hour are presented in Table 1. Knockdown is defined as the initial effect such as morbid or unusual behaviour due to the alteration of a specific physiological process or processes that taken placed upon contact with the toxicant. The knockdown effect occurred at 12.42 $mgcm^{-2}$ for

all extract tested as early as the tenth minute of exposure. *L. elliptica* had the best knockdown activity as the knockdown effect took place at fiftieth minute only.

Table 1. The knockdown effect for the one-hour exposure of three plant extracts on *Aedes aegypti* at different concentrations in comparison with Malathion 12.42 $mgcm^{-2}$ by bioassay test.

Extract	**Concentrations ($mgcm^{-2}$)**	**% Knockdown (min)**					
		10	**20**	**30**	**40**	**50**	**60**
Acorus calamus	0.02	0	0	0	2.04	2.04	4.08
	0.12	0	0	0	0	4.55	4.55
	2.48	0	0	2.0	12.0	20.0	24.0
	6.21	0	8.70	20.0	34.78	54.35	56.00
	12.42	4	20.00	24.00	32.00	56.00	60.87
Litsea elliptica	0.02	0	0	0	0	0	0
	0.12	0	0	0	0	0	0
	2.48	0	0	0	0	0	0
	6.21	0	0	0	0	2.22	2.22
	12.42	51.22	60.98	70.73	75.61	75.61	78.05
Piper aduncum	0.02	0	0	2.04	2.04	2.04	2.04
	0.12	0	0	0	9.10	9.10	9.10
	2.48	0	5	12.50	15.0	15.0	17.5
	6.21	2.08	30	33.33	33.33	37.50	39.58
	12.42	9.09	36.36	40.91	40.91	54.55	59.09
Malathion	12.42	0	16.13	51.61	96.77	100	100

Control (Acetone)		0	0	0	0	0	0

Table 2 showed the initial mortality occurred after three hours of continuous exposure. All extracts showed mortality between 8.75 – 97.83%. The highest mortality ranged of 8.16 – 97.83 was shown by *A. calamus*. For the first one-hour exposure period, all plant with their selected fraction produced high knockdown at 12.42 $mgcm^{-2}$. Based on these results, the concentration of 12.42 $mgcm^{-2}$ was chosen as the concentration of extracts used as comparisons among the extracts to obtain the median lethal time (LT_{50}) values.

Table 2 Preliminary effect of three plant extracts on *Ae. aegypti* adults exposed continuously for 3 hours at different concentrations in comparison with 12.42 $mgcm^{-2}$ Malathion by bioassay test

Extract	**Concentration ($mgcm^{-2}$)**	**Knockdown**	**Mortality**	
		1 hour	**2 hour**	**3 hour**
Acorus calamus	0.02	4.08	6.12	8.16
	0.12	4.55	13.64	13.64
	2.48	32.0	56.0	74.0
	6.21	56.00	76.00	96.00
	12.42	60.87	95.65	97.83
Litsea elliptica	0.02	0	0	8.7
	0.12	0	8.70	9.30
	2.48	0	9.76	17.07
	6.21	2.22	11.11	26.67

	12.42	78.05	85.37	96.00
Piper aduncum	0.02	2.04	4.08	6.12
	0.12	9.10	9.10	9.10
	2.48	17.5	27.50	35.0
	6.21	39.58	47.92	54.17
	12.42	59.09	63.64	63.64
Malathion	12.42	100	100	100

Table 3 showed the median lethal concentration (LC_{50}) and 90% of the lethal concentration (LC_{90}) values after 24 hours of exposure. The results showed that the hexane fraction *A. calamus* demonstrated the lowest LC_{50} and LC_{90} values i.e. 0.04 $mgcm^{-2}$ and 0.90 $mgcm^{-2}$, respectively while the methanol fraction of *L. elliptica* exhibited LC_{50} and LC_{90} of 0.11 $mgcm^{-2}$ and 6.08 $mgcm^{-2}$. This was followed by the hexane fraction of *P. aduncum*, which exhibited LC_{50} and LC_{90} values of 0.20 $mgcm^{-2}$ and 5.32 $mgcm^{-2}$, respectively.

Table 3 The LC_{50} and LC_{90} of the three extracts (in $mgcm^{-2}$) against *Aedes aegypti* adult female after 24 hours

Extracts	**[a]LC_{50} ($mgcm^{-2}$) (95% CL)**	**[b]LC_{90} ($mgcm^{-2}$) (95% CL)**	**Slope ± SE[c]**
Acorus calamus	0.04 (0.018 – 0.085)	0.90 (0.317-2.311)	0.98 ± 0.16
Litsea elliptica	0.11 (0.049 – 0.210)	6.08 (2.818 – 18.787)	0.74 ± 0.01
Piper aduncum	0.20	5.32	0.89 ± 0.12

	(0.089 – 0.362)	(2.62 – 14.742)	
Malathion	0.01	0.50	
			0.77 ± 0.14
	(0.003 – 0.026)	(0.22 – 2.00)	

[a]LC: Lethal concentration, [b]95% CL: Confidence Interval at 95% Confidence Level, [c] SE: Standard Error

Therefore, the order or potency of these extracts based on the LC_{50} values are as below:

Acorus calamus > *Piper aduncum* > *Litsea elliptica*

Table 4 showed the LT_{50} value obtained at 12.42 $mgcm^{-2}$ of the plant extracts after three hours of exposure to *Ae. aegypti*. For the 1st hour of exposure, *L. elliptica* demonstrated lowest LT_{50} values of 9.41 mins against *Ae. aegypti.* The LT_{50} value obtained by malathion at 12.42 $mgcm^{-2}$ concentration was 35.06 mins and is not significantly difference when statistically compared to *L. elliptica* at $p<0.05$. While the data for I hour of exposure showed the LT_{50} value for *P. aduncum* was 43.75 mins while for *A. calamus* was 51.82mins.

Data for 2 and 3 hours exposure at 12.42 $mgcm^{-2}$ revealed similar pattern to that 1 hour exposure and *L. elliptica* appeared to be the most potent plant extract . The results showed that the toxicity index for *L. elliptica* was 0.56, 0.51 and 0.65 times more than malathion respectively. After 3 hours of exposure, there was a slight change in toxicity index but *L. elliptica* remained the most potent extract. In general, longer exposure increased the effectiveness of all the the plant extracts tested.

Table 4. Toxicity index of *Acorus calamus*, *Litsea elliptica* and *Piper aduncum* in comparison to 12.42 $mgcm^{-2}$ at 1, 2 and 3 hours of exposure against *Aedes aegypti* adult mosquito.

Extracts	Exposure					
	1 hour		2 hours		3 hours	
	LT_{50}	Toxicity index	LT_{50}	Toxicity index	LT_{50}	Toxicity index*
Acorus calamus	51.82	3.10	44.77	2.68	44.77	2.68
Litsea elliptica	9.41	0.56	10.80	0.65	9.62	0.57
Piper aduncum	43.75	2.62	56.27	3.36	62.44	3.73
Malathion	16.73		16.73		16.73	

$$\text{*Toxicity index} = \frac{LT_{50}\ \text{plant extract}}{LT_{50}\ \text{Malathion}}$$

Results from this study showed that these plant extracts have potential as adulticidal against *Ae. aegypti* which is one of the main vector for dengue and DHF fever. The hexane fraction of *A. calamus* and *P. aduncum* and also methanol fraction *of L. elliptica* possessed marked adulticidal activity which suggests that the active constituents are extracted in greater measures with respective solvents. All extracts had LC_{50} values that less than 0.20 $mgcm^{-2}$ indicating promising adulticidal properties. This value is better than those reported for the well-known insecticidal plant *Azadirachta indica* (Zebitz, 1986) and those reported for *Asimina triloba*

(Mikolajcczak *et. al.*, 1988). Jeyabalan *et. al.* (2003) also have reported the adulticidal effect of *Pelargonium citrosa* on *An. stephensi*, with LC_{50} and LC_{90} value of 1.56% and 5.22% respectively. However, it is worth to note that their LC_{50} and LC_{90} values were much higher than the extracts, which were tested in this study.

Many plant extracts tested to date have shown potential larvicidal activity against insect pests including mosquitoes. However, many have failed to demonstrate adulticidal effects. A study by Lee and Chiang (1994) showed a good larvicidal property of *Stemona tuberosa* , however no adulticide was detected. Choochote *et. al.* (1999) also tried to demonstrate the adulticidal property of *Kaempferia galanga,* however it only caused a knockdown effect at the initial stage of exposure but after transferring to the holding tube, the mosquito recovered from the knockdown effect. Therefore they concluded perhaps *K. galanga* might be useful as a repellent instead.

These results also confirmed with parts of Rohani *et. al.* (1997) study which showed that *L. elliptica* and *P. aduncum* has potentials as adulticides but its a new finding to record that *A. calamus* also has such property. Therefore these results should encourage further efforts to investigate the compounds that might possess higher adulticidal properties when isolated in pure form.

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Table 1. The knockdown effect for the one-hour exposure of three plant extracts on *Aedes aegypti* at different concentrations in comparison with Malathion 5% by bioassay test.

Extract	**Concentrations ($mgcm^{-2}$)**	**% Knockdown (min)**					
		10	**20**	**30**	**40**	**50**	**60**
Acorus calamus	0.02	0	0	0	2.04	2.04	4.08
	0.12	0	0	0	0	4.55	4.55
	2.48	0	0	2.0	12.0	20.0	24.0
	6.21	0	8.70	20.0	34.78	54.35	56.00
	12.42	4	20.00	24.00	32.00	56.00	60.87
Litsea elliptica	0.02	0	0	0	0	0	0
	0.12	0	0	0	0	0	0
	2.48	0	0	0	0	0	0
	6.21	0	0	0	0	2.22	2.22
	12.42	51.22	60.98	70.73	75.61	75.61	78.05
Piper aduncum	0.02	0	0	2.04	2.04	2.04	2.04
	0.12	0	0	0	9.10	9.10	9.10
	2.48	0	5	12.50	15.0	15.0	17.5
	6.21	2.08	30	33.33	33.33	37.50	39.58
	12.42	9.09	36.36	40.91	40.91	54.55	59.09
Malathion	12.42	0	16.13	51.61	96.77	100	100
Control (Acetone)		0	0	0	0	0	0

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Table 2 Preliminary effect of three plant extracts on *Ae. aegypti* adults exposed continuously for 3 hours at different concentrations in comparison with 5% Malathion by bioassay test

Extract	**Concentration ($mgcm^{-2}$)**	**Knockdown**	**Mortality**	
		1 hour	**2 hour**	**3 hour**
Acorus calamus	0.02	4.08	6.12	8.16
	0.12	4.55	13.64	13.64
	2.48	32.0	56.0	74.0
	6.21	56.00	76.00	96.00
	12.42	60.87	95.65	97.83
Litsea elliptica	0.02	0	0	8.7
	0.12	0	8.70	9.30
	2.48	0	9.76	17.07
	6.21	2.22	11.11	26.67
	12.42	78.05	85.37	96.00
Piper aduncum	0.02	2.04	4.08	6.12
	0.12	9.10	9.10	9.10
	2.48	17.5	27.50	35.0
	6.21	39.58	47.92	54.17
	12.42	59.09	63.64	63.64
Malathion	12.42	100	100	100

Table 3 The LC_{50} and LC_{90} of the three extracts (in percentage) against *Aedes aegypti* adult female after 24 hours

Extracts	[a]**LC_{50} (mgcm^{-2}) (95% CL)**	[b]**LC_{90} (mgcm^{-2}) (95% CL)**	**Slope ± SE**[c]
Acorus calamus	0.04 (0.018 – 0.085)	0.90 (0.317-2.311)	0.98 ± 0.16
Litsea elliptica	0.11 (0.049 – 0.210)	6.08 (2.818 – 18.787)	0.74 ± 0.01
Piper aduncum	0.20 (0.089 – 0.362)	5.32 (2.62 – 14.742)	0.89 ± 0.12
Malathion	0.01 (0.003 – 0.026)	0.50 (0.22 –2.00)	0.77 ± 0.14

[a]LC: Lethal concentration, [b]95% CL: Confidence Interval at 95% Confidence Level, [c] SE: Standard Error

TABLE 4. Toxicity index of *Acorus calamus*, *Litsea elliptica* and *Piper aduncum* in comparison to 5% at 1, 2 and 3 hours of exposure against *Aedes aegypti* adult mosquito.

Extracts	Exposure					
	1 hour		2 hours		3 hours	
	LT_{50}	Toxicity index	LT_{50}	Toxicity index	LT_{50}	Toxicity index*
Acorus calamus	51.82	3.10	44.77	2.68	44.77	2.68
Litsea elliptica	9.41	0.56	0.51	10.80	9.62	0.65
Piper aduncum	43.75	2.62	56.27	3.36	62.44	3.73
Malathion	35.06		35.06		35.06	

$$\text{*Toxicity index} = \frac{LT_{50}\ \text{plant extract}}{LT_{50}\ \text{Malathion}}$$