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Bioactivity Evaluation of *Melaleuca cajuputi* (Myrtales: Myrtaceae) Crude Extracts against *Aedes* Mosquito

Azlinda Abu Bakar[1,2]

[1]*Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan*

[2]*Department of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur*

ABSTRACT

Melaleuca cajuputi crude extract in four different solvents viz dichloromethane, ethyl acetate, hexane, and methanol were evaluated for their insecticidal properties against *Aedes aegypti* and *Aedes albopictus* mosquito. Bioassay against larva and adult mosquito was done following World Health Organization's guidelines. Late 3rd and/or early 4th instar of *Aedes* larvae were assayed for different concentrations ranging from 10 to 120 mg/L of *M. cajuputi* crude extract. Larvicidal effects were observed in dichloromethane, hexane, and methanol. Dichloromethane gave the highest of mean mortality, against *Ae. aegypti* (12.6 ± 0.98) and *Ae. albopictus* (10.2 ± 0.37) with LC_{50} of 104.8 mg/L and 106 mg/L, respectively. The adulticidal bioassay was tested against 3 - 5 days old of female mosquitoes with the range concentrations from 0.04 to 6.21 mg/cm^2. Amongst solvents used, extracts of dichloromethane and hexane showed effects against the adult mosquito. Extract in hexane gave 100% mortality against both *Aedes* with LC_{50} of 0.015 mg/cm^2 (*Ae. aegypti*) and 0.022 mg/cm^2 (*Ae. albopictus*). In conclusion, the extract of *M. cajuputi* could be exploited in the development of potential plant-based products in controlling dengue *Aedes* vectors, particularly in the adult mosquito.

Keywords: *Aedes* sp., bioactivity, crude extracts, *Melaleuca cajuputi*, solvents polarity

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E-mail address:
azlindaab@usm.my

Current affiliation

School of Biological Sciences, Universiti Sains Malaysia, 11800 Gelugor, Pulau Pinang

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INTRODUCTION

In Asia, the mosquito *Aedes aegypti* is a primary vector for dengue and chikungunya (Sam & Abu Bakar, 2006). Meanwhile, its related taxon *Aedes albopictus* has been recognized as a secondary vector for dengue and serves as a vector competence in the maintenance of dengue, and *chikungunya virus* in areas where *Ae. aegypti* is less abundant or absent (Effler et al., 2005; Grard et al., 2014; Li et al., 2012; Wong et al., 2013). The global widespread of vector mosquitoes such as *Aedes* in certain tropical and sub-tropical areas causes a major outbreak of dengue and other mosquito-borne disease-related illness (Rezza, 2014). Utilization of insecticides in the strategy for disease outbreak control is undeniably effective, due to cost-effective, immediate action, and high efficiency against a broad range of vectors. Unfortunately, the effectiveness is threatened by negative and harmful side effects on human, non-target animals, and the environment have become apparent. Nevertheless, the development of resistance among vector populations (Hamid et al., 2018) is the biggest threat to the program's efficacy. Thus, the urge and interest in searching less hazardous alternatives of vector/pest control from plant resources are therefore being renewed and continues today.

To date, many potential plant species with known insecticidal properties and phytochemicals which are rich with biodegradable active compounds are being screened and evaluated (Sharma et al., 2006). For instance, a study on the bioactivity screening of various plant extracts in Malaysia, had shown that *Melaleuca* was the most effective when tested against *Aedes* spp. larva in the laboratory (Bakar et al., 2018). Nevertheless, evaluation of the essential oils of the same plant had also shown its potential insecticidal effects as well (Abu Bakar et al., 2012; Bakar et al., 2019). According to Lowe's report (as cited in De Monte et al., 2014, p. 63), there are many different extractive techniques and approaches working together with various methodologies and solvents to improve the recovery and, the pharmacological profile of their extract products. However, as a result of the differences among the extractive processes and methods, there is a discrepancy in the qualitative and quantitative composition of the extracts obtained from the same plant.

It is known that various solvents of different polarities would extract different phenolic compounds from plants with a high degree of accuracy (Wong & Kitts, 2006). Furthermore, previous studies have shown that solvents with a high polarity such as methanol displayed high effectiveness as antioxidants (Altemimi et al., 2017). The objective of this present study was to evaluate the *in vitro* bioactivity of *Melaleuca cajuputi* plant extracts derived from four different solvents polarity viz. hexane (non-polar), dichloromethane (moderately polar), ethyl acetate (polar), and methanol (polar) against larvae and adult of dengue vector mosquitoes, *Ae. aegypti* and *Ae. albopictus*.

MATERIALS AND METHODS

Collection of Plant Specimens

The leaves of *M. cajuputi* were collected from Port Dickson, Negeri Sembilan area, 2° 31′ 21.1440″ N, 101° 47′ 46.6620″ E in Malaysia. Port Dickson is located 120 km towards the south from Kuala Lumpur 3° 8’ 27.0708’’ N and 101° 41’ 35.5452’’ E. The voucher specimen was sent to Forest Research Institute of Malaysia (FRIM) in Kepong, Selangor for species confirmation and specimen deposited at the herbarium.

Preparation of Mosquito

Laboratory strain mosquito was obtained from Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang in the form of eggs. The mosquito colony was cultured and reared in the laboratory under the optimized condition: relative humidity (RH) 80% ± 5% and room temperature 28.5 °C ± 2 °C. During the maintenance period, mosquitoes’ larvae were provided with prepared powdered food which contains cat’s biscuit, powder milk, grounded (dried) cow liver, yeast, and vitamin B complex. The mosquito colony was continuously maintained throughout the study period.

Preparation of the Crude Extracts

The freshly collected leaves of *M. cajuputi* were dried at room temperature (29 - 31°C) for 5 - 7 days. The dried leaves were grounded mechanically using a household blender. The grounded leaves were extracted with solvents viz: hexane, ethyl acetate, dichloromethane, and methanol with the ratio of 1g sample to 10 mL solvent in a 10L plastic container. The samples were shaken and mixed vigorously and left to sit for 72 hrs. The extracts were filtered through glass funnel with filter paper Whatman No.1. The extract was concentrated using rotary evaporator type EYELA (N-1001S-WD, Japan) at 45°C for eight hrs. The residue obtained was kept in an amber glass vial to be used for subsequent bioassay testing.

Larval Bioassay

The larval bioassay was following the standard guidelines (World Health Organization [WHO], 2005). Five (5) different concentrations of extracts were prepared at 10, 50, 80, 100, and 120 mg/L. A 10 mL stock solution was prepared at a concentration of 100,000 mg/L (100,000 ppm) and kept in a refrigerator at 4 - 5 °C. Five replicates of 20 late third instar larvae were used in each bioassay. The numbers of dead larvae were counted after 24 hrs. of exposure. Positive and negative control solutions were prepared by mixing 1mL solvent in 199mL of distilled water and 2 mL of acetone in 198 mL of distilled water respectively. During the observation, food was not supplied to the larvae. The lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis (Finney, 1971).

Adulticidal Bioassay

Bioassay of adulticide was performed as described in the WHO (2016) guideline. Five (5) different concentrations of 2.0 mL plant extracts of 0.04, 0.08, 0.12, 2.48, and

6.21 mg/cm^2 were applied homogeneously at the filter papers Whatman No 1 (12 x 15 cm). The control paper was treated with 2.0 mL acetone. The impregnated papers were dried at room temperature for 24 hrs and kept (4 - 5°C) in an aluminum foil. Four replicates of twenty-five female (3 - 5 days old, blood starved) mosquitoes were aspirated from the mosquito cage into a plastic holding tube. The mosquitoes were allowed to acclimatize in the tube for 1 hr. and later were exposed to the treated impregnated filter paper for 1 hr. At the end of the 1 hr. exposure period, the mosquitoes were transferred back to the holding tube and kept for mortality observation for 24 hrs. A pad of cotton wool soaked in 10% sugar water was placed on the mesh-screen. The number of moribund and dead mosquitoes was recorded at intervals of 1, 5, 10, 15, 20, 25, 30, 45-, and 60-minutes post-exposure. Any knocked down mosquitoes, were considered moribund and counted as dead. A mosquito was classified as dead or knocked down if it is immobile or unable to stand or take off.

Statistical Analysis

Percentage mortality that lies between 5% to 20% will be corrected using Abbott's formula (Abbot, 1925). Larvicidal and adulticidal effects were reported in median lethal concentration (LC_{50}) with a 95% confidence interval subjected to a log probit analysis test. The comparative effectiveness of crude extracts among different types of solvents and *Aedes* mean mortality were analyzed using paired t-test and one-way ANOVA. Results with the value of $p \leq 0.05$ were reported to be statistically significant. All data were analyzed and calculated using SPSS statistics software.

RESULTS

The insecticidal bioefficacy of the *M. cajuputi* crude extracts of dichloromethane, ethyl acetate, hexane, and methanol were tested at 10 mg/L, 50 mg/L, 80 mg/L, 100 mg/L, and 120 mg/L against dengue vectors, *Ae. aegypti* and *Ae. albopictus*. Table 1 summarizes the bioactivity against larvae and adults' stage of *Aedes* vectors. From the results obtained, dichloromethane, hexane, and methanol showed some larvicidal effects when tested against *Aedes* larvae. Meanwhile, adulticidal effects were only observed in dichloromethane and methanol. The bioassay test against larvae and adults *Aedes* mosquito showed a significant increase in the mortality percentage (%) with the increase of concentration.

In Table 2, the paired t-test and the one-way analysis of variance (ANOVA) were analyzed in mean mortality of *Aedes* sp. larvae, and solvents used. Statistical analysis of one-way ANOVA revealed no significant difference between and within groups among solvents and *Aedes* sp. Meanwhile paired t-test between solvents and *Aedes* sp. showed a significant difference ($p \leq 0.05$) in hexane (p = 0.04) and methanol (p=0.003) solvent respectively. The highest larvicidal activity was observed in dichloromethane against *Aedes* sp. with the LC_{50} values of 104.8 mg/L and 106.0 mg/L for *Ae. aegypti* and *Ae. albopictus* at 24 hrs. respectively

Table 1

Bioactivity of Melaleuca cajuputi *crude extracts against* Ae. aegypti *and* Ae. albopictus

Solvents	Larvicidal	Adulticidal
Dichloromethane	√	√
Ethyl acetate	-	-
Hexane	√	√
Methanol	√	-

Note. √ toxic effects

Table 2

Mean mortality (± SE) of M. cajuputi *crude extracts against larvae of* Ae. aegypti *and* Ae. albopictus

Aedes sp.	Dose	*Mean mortality ± SE		
	(mg/L)	Dichloromethane[1]	Hexane[2]	Methanol[3]
[a]*Ae. aegypti*	10	1.20 ± 0.20	0	1.40 ± 0.25
	50	3.40 ± 0.25	1.20 ± 0.20	1.60 ± 0.25
	80	6.80 ± 0.37	4.20 ± 0.37	2.40 ± 0.40
	100	9.80 ± 0.66	5.40 ± 0.51	2.80 ± 0.20
	120	12.6 ± 0.98	6.60 ± 0.51	3.00 ± 0.32
	Control**	0	0	0
[b]*Ae. albopictus*	10	0	0	0
	50	0	0	0
	80	7.40 ± 0.25	0.60 ± 0.25	0
	100	10.0 ± 0.32	1.00 ± 0.32	1.60 ± 0.25
	120	10.2 ± 0.37	2.00 ± 0.32	2.00 ± 0.00
	Control**	0	0	0

Note. *Mean value of five replicates

[1, a,b] No significant difference

[2, a, b] Significant difference $p < 0.05$ (p = 0.04)

Control** = acetone 0.1%

[1, 2, 3, a] No significant difference

[1, 2, 3, b] No significant difference

(Table 3). Figures 1 and 2 show the highest percentage mortality values of 63% in *Ae. aegypti* and 51% in *Ae. albopictus* at 120 mg/L of dichloromethane extract in *M. cajuputi*.

The results of the adulticidal activity of hexane and dichloromethane extracts of *M. cajuputi* against *Ae. aegypti* and *Ae. albopictus* are presented in Table 4. There was a significant difference between hexane and dichloromethane in mean mortality of *Aedes* spp. with the *p*-values of 0.007 for *Ae. aegypti* and 0.003 for *Ae. albopictus*. However, no significant difference was observed in mean mortality between *Aedes* spp. in each solvent used, hexane and

Table 3
LC values of M. cajuputi *crude extracts against* Aedes *sp. larvae*

Solvents	*Ae. aegypti*					*Ae. albopictus*				
	LC_{50} (mg/L)	95% Confidence Limit		df	χ^2	LC_{50} (mg/L)	95% Confidence Limit		df	χ^2
		LCL	UCL				LCL	UCL		
Dichloromethane	104.8	94.9	117.8	2	0.43	106.0	N/A	N/A	2	15.9
Hexane	164.4	132.1	260.3	2	0.71	429.0	N/A	N/A	2	1.60
Methanol	349.5	N/A	N/A	2	0.19	N/A	N/A	N/A	N/A	N/A

Note. LCL = lower confidence limit; UCL = upper confidence limit; df = degree of freedom

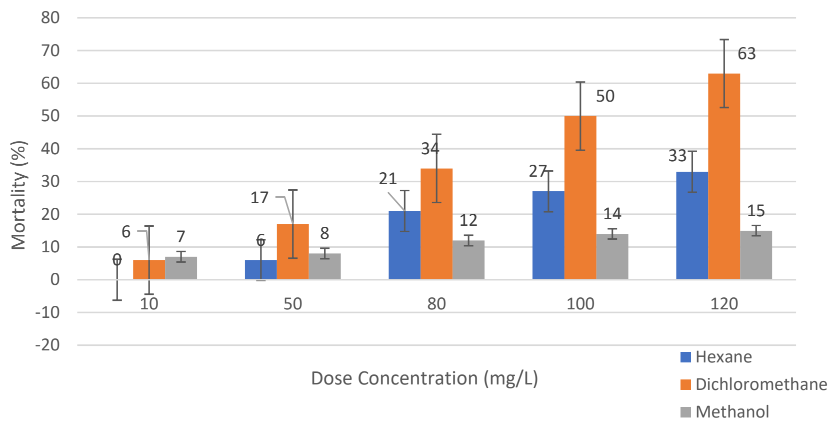

Figure 1. Percentage mortality of *M. cajuputi* crude extracts against *Ae. aegypti* larvae

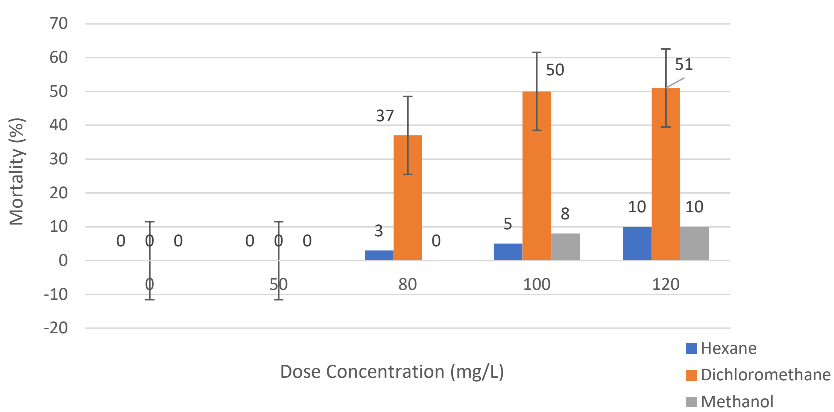

Figure 2. Percentage mortality of *M. cajuputi* crude extracts against *Ae. albopictus* larvae

dichloromethane. The probit analysis of 95% confidence limits LC_{50} (UCL-LCL) was also calculated and presented in Table 5. The chi-square values were not significantly different at $p \leq 0.05$. Among the solvents used, hexane gives lower LC_{50} of 0.015 mg/cm^2 (0.005-0.025) and 0.022 mg/cm^2 (0.009-0.003) in *Ae. aegypti* and *Ae. albopictus*, respectively.

In this study, the results showed that bioactivity of *M. cajuputi* crude extracts against *Aedes* spp. was varied according to the species, stage of life, and solvents used (Tables 1, 2, and 4). The extract of *M. cajuputi* in dichloromethane possessed moderate effects against *Ae. aegypti* larvae. On the other hand, the sensitivity of dichloromethane, hexane, and methanol extract against *Ae. albopictus* showed minimal larvicidal effects after 24 hr. of exposure at various concentrations. Meanwhile, observation in adulticidal assays using dichloromethane, ethyl acetate, hexane, and methanol showed nonconformity with the results of the larvicidal assays. Of these, dichloromethane and hexane extracts of *M. cajuputi* showed adulticidal effects against *Ae. aegypti* and *Ae. albopictus*. However, hexane extract was the most effective against *Ae. aegypti* and *Ae. albopictus* adult's mosquito. From Table 4, more than 50% mortality was observed in *Ae. aegypti* (74%) and *Ae. albopictus* (69%) at lowest concentration of 0.04 mg/cm^2 and 100% mortality when exposed at higher concentrations of 2.48 and 6.21 mg/cm^2.

Table 4

Adulticidal effects of M. cajuputi *crude extracts against* Aedes aegypti *and* Aedes albopictus

Solvents Used	Dose (mg/cm^2)	[1] *Ae. aegypti*		[2] *Ae. albopictus*	
		Mortality (%)	*Mean mortality ± SD	Mortality (%)	*Mean mortality ± SD
[a]Hexane	0.04	74	18.50 ± 1.29	69	17.25 ± 1.26
N=100	0.08	77	19.25 ± 0.96	71	17.75 ± 1.89
	0.12	79	19.75 ± 0.96	89	22.25 ± 3.30
	2.48	100	25.00 ± 0.00	100	25.00 ± 0.00
	6.21	100	25.00 ± 0.00	100	25.00 ± 0.00
	**Control	0	0	0	0
[b]Dichloromethane	0.04	31	7.75 ± 1.15	54	13.50 ± 3.00
N=100	0.08	50	12.50 ± 3.00	56	14.00 ± 2.16
	0.12	55	13.75 ± 0.96	57	16.75 ± 2.50
	2.48	80	20.00 ± 1.83	80	20.00 ± 0.00
	6.21	87	19.25 ± 6.29	81	20.20 ± 0.5
	**Control	0	0	0	0

Note. *Mean value of four replicates
**Control=acetone 0.1%
[1, 2, a] No significant difference $p > 0.05$
[1, a, b] Significant difference $p \leq 0.05$ ($p = 0.007$)
[1, 2, b] No significant difference $p > 0.05$
[2, a, b] Significant difference $p \leq 0.05$ ($p = 0.003$)

Table 5
LC values of M. cajuputi *crude extracts against* Aedes *sp.* a*dults*

Solvents	*Ae. aegypti*					*Ae. albopictus*				
	LC_{50} (mg/L)	95% Confidence Limit		df	χ^2	LC_{50} (mg/L)	95% Confidence Limit		df	χ^2
		LCL	UCL				LCL	UCL		
Dichloromethane	0.116	0.071	0.176	3	3.73	0.030	0.007	0.069	3	0.83
Hexane	0.015	0.005	0.025	3	4.57	0.022	0.009	0.033	3	5.86

Note. LCL = lower confidence limit; UCL = upper confidence limit; df = degree of freedom

DISCUSSION

Many researchers have reported the potential of plant extracts for controlling mosquito-borne diseases (Ghosh et al., 2012; Kamaraj et al., 2010; Rehimi et al., 2011). Up to date, there are now more than 2,000 potential plant species that have been evaluated for their insecticidal properties worldwide (Maiza et al., 2013; Roark, 1947; Shaalan et al., 2005; Sukumar et al., 1991). In some developing countries, pesticidal plants offer unique and challenging opportunities for the exploration and development of their botanical sources. Furthermore, one of the most important factors affecting the benefits and efficiency of bioactivity from plant materials and their consequent health is the extraction solvents used (Ngo et al., 2017). Thus, the selection of solvents used depends on the purpose either as of choice for yielding high content or for specific extraction of phytochemical compounds that would be useful for its medicinal and/or insecticidal properties.

The polarity effect depends on the reactivity and selectivity of radical chemistry that has been identified over 50 years ago (Walling, 1957). Most chemical reactions that are carried out in laboratories or the industry are in the form of solutions. Hence the proper and appropriate solvent selection as one of the reaction parameters is important for a good and successful reaction (Reichardt, 2005). According to Rawani et al. (2010), phytochemicals found in plants may play an important role (bioactivities) in vector control if applied appropriately. The phytochemicals in plants can be obtained from the whole plant or specific parts of the plant with different solvents such as petroleum ether, benzene, chlorophyll, methanol, and acetone.

Ghosh et al. (2012) showed that the extraction of active biochemical from plants depended upon the polarity of the solvents. Polar solvents will extract polar molecules and non-polar solvents extract non-polar molecules. In this study, hexane (polarity index of 0.1), dichloromethane (polarity index of 3.1), ethyl acetate (polarity index of 4.4), and methanol (polarity index of 5.1) (Corradini et al., 1998; Harris, 2015) had been used to investigate the insecticidal properties of *M. cajuputi* extract against *Ae. aegypti* and *Ae. albopictus*. From the results obtained, hexane and dichloromethane solvents that had lower to moderate polarity index were observed to give moderate effects against *Aedes* mosquito. These findings agree with the previous study by Ghosh et al. (2012), which described the efficacy of solvents polarity in the bioassays. Biochemical extracted using moderate polarity index solvents showed good results in a few bioassays. This study revealed the bioactivity variance of *M. cajuputi* crude extracts when tested against larvae and adults of *Aedes* sp. Even though larvicidal effects were observed in dichloromethane, hexane, and methanol extracts of *M. cajuputi*, the effectiveness was slightly weak. However, the adulticidal activity showed good effects against *Ae. aegypti* and *Ae. albopictus*.

CONCLUSION

The bioactivity of crude plant extracts is characterized by a mixture of complex active compounds. Thus, plants containing beneficial phytochemicals may supplement and would be useful plant-based insecticides for future development. Variations of an insecticidal potential of *M. cajuputi* crude extract varied with the different solvents used in the extraction process. Due to the variation's efficacy in the larvicidal and adulticidal effects against *Ae. aegypti* and *Ae. albopictus* mosquitoes. More work is still needed to confirm its effectiveness, especially in the field. A study on the phytochemicals of an active compound of the *M. cajuputi* crude extract in different solvents used can be carried out to characterize the insecticidal properties in different research settings. It can be used as a solution of variants efficacy in a situation of chemical instability of whole or unprocessed plant products.

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