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PHYTOCHEMICAL SCREENING AND LARVICIDAL ACTIVITY OF *Murraya koenigii* LEAVES EXTRACTS AGAINST MOSQUITO LARVAE

(Saringan Fitokimia dan Aktiviti Larvisid Ekstrak Daun *Murraya koenigii* Terhadap Larva Nyamuk)

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Abstract

Exploring new use of aromatic leaves of *Murraya koenigii* lead us to test it as pesticide. Phytochemical screening of the extracts was conducted to determine the active compounds using hexane and methanol solvents. The LC_{50} and LT_{50} values for both extracts against mosquito larvae were determined. This study was comprised of several methods namely collection of leaves of *M. koenigii*, extraction *M. koenigii* leaves with hexane and methanol sequentially, phytochemical screening and larvicidal bioassay on third instar larvae using three different concentrations (250, 500 and 750 ppm). Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids and glycoside for methanol extract meanwhile negative results were obtained for hexane. Larvicidal bioassay of methanol and hexane extraction crudes gave the lowest LC_{50} and LT_{50}, which were 250 ppm and 450 ppm with 36 hours, respectively. The plant extracts showed larvicidal activity against mosquito larvae at 0.05 level of significance in terms of concentration for hexane and duration for methanol. High larvicidal activity of *M. koenigii* leaves was supported by the presence of phytochemicals that shows synergistic effects in terms of larvicidal action to mosquito larvae. This study demonstrated that *M. koenigii* could be an effective mosquito larvicide.

Keywords: *Murraya koenigii*, phytochemical screening, mosquito larvicides

Abstrak

Menerokai kegunaan baru daun aromatik *Murraya koenigii* membimbing kami untuk mengujinya sebagai racun perosak. Saringan fitokimia ekstrak-ekstrak telah dikendalikan untuk menentukan sebatian aktif menggunakan pelarut heksana dan metanol. Nilai LC_{50} dan LT_{50} untuk kedua-dua ekstrak terhadap larva-larva nyamuk telah ditentukan. Kajian ini terdiri daripada beberapa kaedah iaitu pengumpulan daun *M. koenigii*, pengekstrakan daun *M. koenigii* menggunakan heksana dan metanol secara berturutan, saringan fitokimia dan biocerakin larva ke atas larva instar III menggunakan tiga jenis kepekatan (250, 500 dan 750 ppm). Saringan fitokimia mendedahkan kehadiran alkaloid, saponin, tannin, flavonoid dan glikosid untuk ekstrak metanol manakala hasil negatif telah didapati untuk heksana. Biocerakin larva hasil ekstrak mentah metanol dan heksana memberi nilai LC_{50} dan LT_{50} terendah iaitu masing-masing 250 ppm dan 450 ppm selama 36 jam. Ekstrak tumbuhan menunjukkan aktiviti larvisid terhadap larva nyamuk pada tahap signifikan 0.05 dari segi kepekatan untuk heksana dan tempoh untuk metanol. Aktiviti larvisid daun *M. koenigii* yang tinggi telah disokong oleh kehadiran fitokimia yang menunjukkan kesan sinergi dari segi tindakan larvisid kepada larva nyamuk. Kajian ini menunjukkan *M. koenigii* boleh menjadi larvisid nyamuk yang berkesan.

Kata kunci: *Murraya koenigii*, saringan fitokimia, larvisid nyamuk

Introduction

Murraya koenigii is a native plant of India, Sri Lanka, Bangladesh and the Andaman Islands [1]. According to Arivoli et al. [2], the common names for *M. koenigii* in South Asia are 'karivepu' or 'karuveppilai' in Tamil, 'karipatta' in Hindi, 'girinimba' in Sanskrit, 'karibevu' in Kannada, 'kariveppu' in Malayalam, 'kadhilimb' in Marathi and 'karepeku' in Telugu. *M. koenigii* are widely found around the world.

Lately, the global spread of arboviruses known as Zika by mosquitoes has been alarming the public of the damage these insects can cause. Mosquitoes can be simply considered as annoying pests due to the nuisance from their bites and they can transmit diseases to humans and domestic animals. Mosquitoes are one of the oldest human enemies that can cause diseases and affect human health. To date, mosquitoes are recognized to have more than 3,000 species all over the world. According to Vongsombath [3], all mosquitoes are small, long-legged flies, and have two wings that are classified in the Order Diptera (true flies) under the Family Culicidae. Mosquito control strategies have depended primarily on the use of synthetic chemical insecticides. The non-environmental friendly effect of most of these synthetic chemical insecticides has led to alternative ways of managing this problem and one of them is by focusing on safer and effective plant products as insecticides. Bioactive botanicals are promising alternatives for mosquito control because of low toxicity to non-target organisms and their innate biodegradation ability. The present study aims to explore the phytochemical constituents and larvicidal activity of the crude leaf extracts of *M. koenigii*.

Materials and Methods

Plant collection and preparation of crude extract

Murraya koenigii leaves were collected from botanical herb garden of Universiti Teknologi MARA Pahang Jengka Campus. A total of 2.6 kg of leaves was washed using water and dried in the oven. All the dried leaves were ground and weighted. A total mass of powder obtained was 850 gram. The powdered plant materials were sequentially extracted with hexane and methanol for 72 hours before they were filtered. The filtrates were then subjected to rotary evaporator until the solvents were completely evaporated. Both methanolic and hexane crude extracts obtained were stored in the universal bottles and maintained at 4 ^{o}C in a refrigerator until it is used.

Phytochemical screening test: Saponins test

0.2 g of extract was added to a half of test tube of distilled water. The solution was then shaken vigorously and left for 30 minutes. Creamy mist with small bubbles showed the presence of saponin [4].

Tannins test

Water was added to 0.1 g of extract, heated to boil and filtered. Then, ferric chloride was added into solution. Dark green colour of solution indicated the presence of tannins [5].

Glycoside test

Few drops of glacial acetic acid and 2-3 drops of ferric chloride were added to 2 ml of extracts along with 1 ml of concentrated sulfuric acid. The appearance of brown ring at the interface confirmed the cardiac glycoside [6].

Test for alkaloids

Few drops of HCI were added into 1 ml of each plant extract in test tubes. After that, the mixture was heated for 20 minutes and left for cooling process. Two drops of Wagner's reagents were added to 1 ml of the filtrate and reddish-brown precipitate observed had shown the presence of alkaloids [5].

Test for flavonoids

10 ml of distilled water was added to 0.5 g of each plant extract in a test tube and. Then, 5 ml of diluted ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml of concentrated H_2SO_4. Yellow colour showed the presence of flavonoids [7].

Larvicidal bioassay

Bioassay for the larvicidal activity was carried out according to WHO procedures with slight modifications. In each 250 ml glass beaker, ten healthy larvae were released. For positive control, the larvae were exposed to abate molecules/crystal (Abate 1.1 g, BASF). Control negative was prepared by using the mixture of hexane to dimethyl sulfoxide (DMSO). Three replicates were set up for each concentration at three different concentrations (250, 500, 750 ppm). Mortality was observed after 24 and 48 hours post-treatments. The observed percentage mortality was corrected by Abbott's formula (equation 1):

$$\text{Percent mortality} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100 \quad (1)$$

Statistical analysis

SPSS version 19.0 package was used for determination of lethal time (LT_{50}) and lethal concentration (LC_{50}). Both LT_{50} and LC_{50} refer to times or amount of substance that, when administered to a group of experimental animals, will kill 50 per cent of the group respectively. Data from mortality and effect of concentrations were subjected using Two-way ANOVA and the differences between the treatments were determined by Tukey's test ($p< 0.05$).

Results and Discussion

The results from phytochemical tests revealed that the *M. koenigii* methanol leaves extracts showed all positive results on alkaloids, saponin, tannins, glycoside and flavonoids whereas negative results were obtained for hexane extracts (Table 1). Mathur et al. [8] reported the presence of alkaloid, saponin and flavonoid in methanol *Murraya koenigii* leaves extracts. The polarity of the solvent determines the type of active biochemical form of plant extracts. The results confirmed that polar solvent like methanol is better than hexane solution. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities [9]. Secondary metabolites in plant serve as a mean of defence mechanism of the plants to avoid from herbivore predators and other environmental factors.

Saponin is responsible in increasing mortality of insects, lowering food intake, causing instability and retardation in development and reproduction [10]. The presence of saponin in *M. koenigii* can be the reason of larvicidal activity. Besides being popular as anthelmintic, mosquito repellent, and antiseptic, tannin also functions as antidiarrheal and haemostatic [11, 12]. Glycosides presence in the plants is to increase the force of cardiac contraction. Through the history, alkaloids are the part of constitutive defence. Flavonoids play important roles in plants as insect feeding attractants or excitants, repellents or deterrents and as oviposition inhibitors. Flavonoids are believed to contribute to larvicidal activity through its toxicity.

Table 1. Phytochemical test of leaves extracts of *M. koenigii*

Phytochemicals	Compounds	Solvent	
		Hexane	Methanol
Alkaloids		-	+
Tannins		-	+
Glycoside		-	+
Saponins		-	+
Flavonoids		-	+

+ = present, - = absent

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides. Mosquitoes in the larval stages are attractive targets to pesticides because mosquitoes breed in water, thus, it is easy to deal with them in this habitat. However, the use of

conventional pesticides in the water sources introduces many risks to people and the environment. Natural pesticides, especially those derived from plants, are more promising in this aspect. A considerable number of plant derivatives have shown to be effective against mosquitoes in a safe manner. The results of the larvicidal bioassay are presented in Table 2 for hexane and Table 3 for methanol extracts. The larvae were considered dead when there was no movement detected if they were disturbed with a needle. Moribund larvae are those incapable to rise up to the water surface (within reasonable time) or show characteristic of diving reaction when water was disturbed. Table 2 indicates the number of larval death proportional to the level of concentration in hexane extract. The percentage of plant extracts used influenced the mortality rate of the larvae [13]. At 250 ppm and 500 ppm, the number of death larval was not significantly different. After 48 hours exposure with 750 ppm of crude hexane leaf extract, the mortality achieved was 100%. Abate 1.1 g; a standard insecticide achieved 100% mortality at much lower concentration, which was 110 ppm.

Table 2. Number of mosquito larval death by crude hexane leaf extract of *M. koenigii* after 24 and 48 hours of exposure

Concentration (ppm)	**No. of Larvae (24 hours)**	**No. of Larvae (48 hours)**
250	4.67 ± 3.79^{b}	5.33 ± 2.52^{b}
500	5.67 ± 1.53^{b}	7.68 ± 1.53^{b}
750	9.33 ± 0.58^{a}	10.00 ± 0.00^{a}
Control	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
Abate 1.1g (110)	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}

Values are mean number of larval death of three replicates ± standard deviation. Different superscript alphabets indicate statistical significant difference at $p< 0.05$ levels by Two-way ANOVA followed by Tukey's test

Table 3 shows the significance between concentrations of *M. koenigii* methanolic leaves extract against mosquito larvae in both durations. Impressively, as low as 250 ppm, it has given high mortality rate that is 83.0% after 48 hours of exposure.

Table 3. Number of mosquito larval death by crude methanol leaf extract of *M. koenigii* after 24 and 48 hours of exposure

Concentation (ppm)	**No. of Larvae (24 hours)**	**No. of Larvae (48 hours)**
250	3.33 ± 0.578^{a}	8.33 ± 1.53^{a}
500	4.67 ± 1.16^{a}	7.00 ± 1.00^{a}
750	6.00 ± 3.464^{a}	8.67 ± 1.53^{a}
Control	0.00 ± 0.0^{b}	0.00 ± 0.0^{b}

Values are mean number of larvae death of three replicates ± standard deviation. Different superscript alphabets indicate statistical significant difference at $P<0.05$ levels by Two-way ANOVA followed by Tukey's test

Another experiment was conducted using 250, 300, 350, 400 and 450 ppm extractions to determine the exact value for LT_{50} and LC_{50}. Through the experiment, the results obtained were 450 ppm and 36 hours of LC_{50} and LT_{50} for hexane while 250 ppm with 36 hours for methanol, respectively. *Murraya koenigii* showed the highest potential of

larvicidal activity when methanol as solvent applied compared to hexane. Hexane crude extract shows low efficiency since it has higher LC_{50} compared to methanol crude extract. It is the evident that the phytochemical constituents contained in the crude extract are the main reason of the effect on mortality rate. Phytochemical screening test determined the reason why mortality rate is higher in methanol extract than hexane extract. The finding are in line with literature studies by Harvae and Kamath [14] that showed the percentage of mortality of *A. aegypti* in 50 ppm of polar acetone crude extract and 100 ppm in non-polar petroleum ether crude extract. The concept also applied to methanol and hexane as polar and non-polar solvents, respectively. The presence of saponins and tannins in methanol leaves extract contribute to the susceptibility of mosquito larvae and act as a killing agent. Contrarily, hexane extract's low mortality effect on mosquito larvae is because of the absence of saponin and tannin molecules [15, 13].

Conclusion

The use of plant extracts in insect control is an alternative method for minimizing the non-toxic effect of pesticide compounds. The results suggest future investigation on *M. koenigii* in becoming the best alternative medicine for other aspect of life.

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