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A PRELIMINARY STUDY ON MOSQUITO LARVICIDAL EFFICACY OF ROTENONE EXTRACTED FROM MALAYSIA *Derris* sp.

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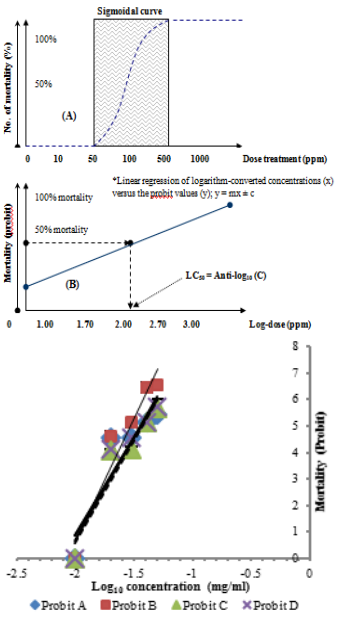
Graphical abstract



Abstract

Rotenone is a bio-active compound extracted from *Derris elliptica* (locally known as 'Tuba' plant). It has long been used as bio-pesticide, which is more environmental friendly than the commercially available pesticides and has the potential to be used in eliminating mosquito larvae. Therefore, the objective of this study is to determine the mosquito larvicidal activity (LC_{50}) through the usage of liquid crude extract of *Derris* plant root. The rotenone liquid crude extract was extracted using normal soaking extraction (NSE) method. Two different solvent ratios were used to extract rotenone namely: (A) methyl chloride: methanol (1:1) and (B) methyl chloride: methanol (1:9). The extracts were concentrated using rotary evaporator at 40 °C with vacuum pressure of 800 mbar prior to the reverse-phase high performance liquid chromatography analysis (RP-HPLC) and biological activity (LC_{50}) study. Next, the diluted extracts were subjected to the biological activity treatment for 6 hrs. The results showed that the concentrated liquid crude extracts of methyl chloride: methanol (1:1) which contained the highest rotenone content produced the lowest treatment concentration of 0.024 mg/ml to achieve 50% mortality within 3 hrs of treatment ($p<0.05$). The rapid mortality (as indicated by the LC_{50} value) of the mosquitoes' larvae against rotenone extracted from *Derris* plant roots has proven that it has the potential to be used as larvicide to control vector-borne diseases especially from mosquitoes.

Keywords: *Derris elliptica*, rotenone, liquid crude extract, mosquito larvae, biological activity, mortality

Abstrak

Rotenon adalah kompaun bio-aktif yang diekstrak daripada *Derris elliptica* (nama tempatannya dikenali sebagai pokok Tuba). Ia telah lama digunakan sebagai bio-pestisid yang mana lebih bersifat mesra alam berbanding pestisid komersial serta berpotensi untuk membunuh larva nyamuk. Oleh yang demikian, objektif kajian ini adalah untuk menentukan aktiviti kematian larva nyamuk (LC_{50}) melalui penggunaan hasil ekstrak mentah akar pokok *Derris*. Cecair ekstrak mentah rotenon diekstrak menggunakan kaedah pengekstrakan rendaman norma (NSE). Dua jenis nisbah pelarut digunakan bagi mengekstrak rotenone iaitu: (A) metil klorida: metanol (1:1) dan (B) metil klorida: metanol (1:9). Hasil ekstrak dipekatkan menggunakan penyejat berputar pada suhu 40 °C pada tekanan vakum 800 mbar sebelum analisis kromatografi cecair berprestasi tinggi fasa berbalik (RP-HPLC) dan kajian aktiviti biologi (LC_{50}) dijalankan. Seterusnya, beberapa cecair ekstrak mentah yang telah dicairkan menjalani rawatan aktiviti biologi selama 6 jam. Keputusan hasil kajian menunjukkan cecair ekstrak mentah pekat dengan nisbah pelarut metil klorida: metanol (1:1) yang mana mengandungi kandungan rotenon tertinggi menghasilkan kepekatan terendah iaitu 0.024 mg/ml untuk menghasilkan 50%

mortaliti dalam tempoh 3 jam rawatan ($p<0.05$). Mortaliti larva nyamuk yang cepat (sepertimana yang ditunjukkan pada nilai LC_{50}) terhadap rotenone yang diekstrak daripada akar pokok *Derris* telah membuktikan ia mempunyai potensi untuk digunakan sebagai larvisid bagi mengawal penyakit bawaan vektor nyamuk.

Kata kunci: *Derris elliptica*, rotenon, ekstrak cecair mentah, larva nyamuk, aktiviti biologi, mortality

1.0 INTRODUCTION

Mosquitoes are the principal vector of a variety of serious diseases, including malaria, yellow fever, dengue and encephalitis. Alone, malaria is estimated to kill between around 700 to 800 thousand people every year.[1] Despite significant advances in the techniques used to control their population (by affecting the development of eggs and larvae of mosquitoes) in these decades, mosquitoes continue to cause serious public problems.[2] The use of insecticides (mainly from the organic synthetic based-products) for vector control such as organochlorides, organophosphates, carbamates and pyrethroids are quite ineffective, not to mention the potential to significantly alter the ecosystems.[3]

It is also toxic to humans, concentrated in the food chain and responsible in the development of insect resistance and environmental pollution due to continued accumulation of slowly degradable toxic compounds.[4] Alternatively, for the past two decades, the use of rotenone as bio-pesticide has been historically recorded by plant, fruit and vegetable growers worldwide. For that reason, the reintroduction of this bio-active compound as an environmental friendly bio-pesticide in organic fruit and vegetable plantations and also in controlling disease vector has now been considered as an alternative approach to the commercially available synthetic pesticides. One of the sources of bio-pesticides is rotenone which is extracted from *Derris elliptica*. *Derris elliptica* or also locally known as 'Tuba' plant is a kind of leafy woody plant that is a creeper which grows along the ground and climbs onto other plants. It needs at least 75% (w/w) of moisture content and temperature of 25 °C to thrive.

Up to now, the commercially important plants like *Derris elliptica* and *Derris malaccensis* contain approximately 4 - 5% (w/w) rotenone in the form of rotenoids resin produced using a normal soaking extraction process.[5] However, the yield of rotenone could be increased up to 14% (w/w) by employing the method of supercritical fluid extraction (SFE). Besides, the main bio-active compound (rotenone) extracted from *Derris elliptica* and other important constituents of *Derris* roots (deguelin and β-rotenolone) have been discovered to be toxic to several household insecticides and for eradicating non-native fish populations in lake and enclosed waters.[6] Due to its effective toxicity against specific insects, we hypothesize that the rotenone liquid crude extract will be an effective toxicant that is able to exterminate and control mosquitoes' larvae at low rotenone concentration. The results could be an indicator of the toxicity of the rotenone against mosquitoes' larvae and it is anticipated to be used as larvicides to control the spread of diseases originating from the mosquitoes.

2.0 EXPERIMENTAL

2.1 Plant Collection

Derris elliptica was collected in the state of Johor; Kota Johor Lama, Malaysia.[7]

2.2 Mosquito Larvae

The larvae were collected around residential area in the state of Johor (Taman Universiti, Skudai), Malaysia. The larvae were randomly collected either from their early stage to fourth instar larvae. All larvae were kept in a humidified and dark cage at room temperature at 27 °C overnight prior to the bioassay (mosquito larvicidal activity (LC_{50}) of rotenone liquid crude extracts).

2.3 Extraction Process

The roots of the *Derris elliptica* plants were dried at room temperature and later ground to become fine raw particles (2 mm in diameter) using a knife mill. The normal soaking extraction (NSE) was carried out in 2 sets of solvent system ratios by soaking 12 g of dried fine roots in 250 ml for 24 hrs at room temperature (≅ 28 °C).[8-9] Next, the liquid crude extracts were divided into 2 conditions which were the concentrated and unconcentrated. The liquid crude extract conditions prior to the bioassay are as follows: (A) unconcentrated extract solution of methyl chloride: methanol (1:1), (B) concentrated extract solution of methyl chloride: methanol (1:1), (C) unconcentrated extract solution of methyl chloride: methanol (1:9) and (D) concentrated extract solution of methyl chloride: methanol (1:9). The concentrated extract solutions of set (B) and (D) were concentrated by using a rotary evaporator at 40 °C[10] with a vacuum pressure of 800 mbar to remove approximately 90% (w/w) of the solvent volume. Three

replications were made on each of the solvent system compositions (n = 3).

2.4 Analysis of Liquid Crude Extract

The filtered liquid crude extract was subjected to a quantitative analysis by using a reverse-phase high performance liquid chromatography (RP-HPLC) with UV (Photodiode Array - PDA) detection at 294 nm to determine rotenone concentration (mg/ml). The analysis of the extract solutions was carried out by using an external standard method (Rotenone PESTANAL®, analytical grade, 96.2% - Sigma-Aldrich™ as an external standard solution). The Waters™ Corp. (C_{18}) liquid chromatography stainless steel column with particle size of 10 μm (3.9 mm I.D × 150 mm length) was utilized. The isocratic solvent system was implemented throughout the whole analysis using acetonitrile and deionized water with a ratio of 60:40 as a mobile phase and the amplitude unit full scale (AUFS) of 2.[11]

2.5 Biological Activity (Mosquitoes' Larvicidal Activity (LC_{50}))

Each unconcentrated and concentrated liquid crude extract was diluted to some appropriate concentrations which were of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml. Eight larvae were pipetted into each *Petri* dish containing 20 ml of distilled water. For each *Petri* dish, 1 ml of each concentration was pipetted and the mortality was counted and recorded every 15 mins of treatment. Larvae were considered dead or moribund if they stopped moving for a prolonged period even after some gentle probing with a small spatula[12]. The negative (-ve) and positive (+ve) control of larvae in distilled water and solvents (methyl chloride: methanol of both ratios) respectively, with no rotenone liquid crude extract treatment were observed as well to produce a correct mortality (if any) of probit values. Subsequently, the mortality of mosquitoes' larvae was evaluated to determine the LC_{50} values using the probit analysis.[13,14] It was based on the dose-response curves as shown in Figure 1 (A). By plotting the probit values against $\log_{10}$ dose of rotenoids resin (ppm) as shown in Figure 1 (B), a least-squares simple linear regression of logarithm could be obtained and the LC_{50} could be calculated by anti-$\log_{10}$ of the utilized concentration (ppm) [Anti-$\log_{10}$ (ppm)]. Three replications were made on each of the rotenone concentrations (n = 3).

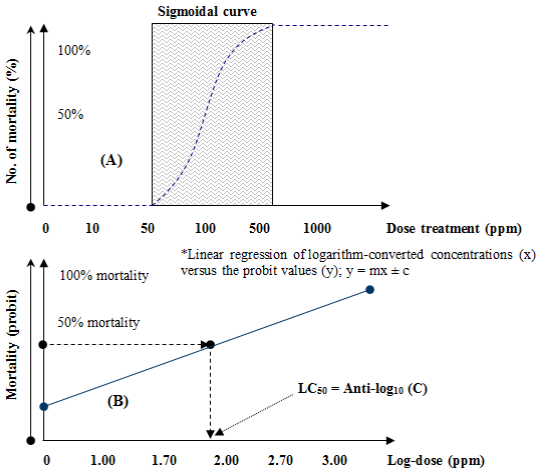


Figure 1 Mortaliy of mosquito larvae when exposed to the extracts of *Derris elliptica*: (A) dose response curve[15], (B) probit analysis curve.[13,16]

2.6 Statistical Analysis

Data was presented as mean ± standard deviation (SD) of mean. Statistical comparisons were performed using Students *t*-test (PASW version 17.0 IBM Co.). A p<0.05 was considered statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 Biological Activity (LC_{50}) of Rotenone Extract

The analysis of rotenone liquid crude extract concentration was carried out by using an external standard method of RP-HPLC (Rotenone PESTANAL®, analytical grade, 96.2% Sigma-Aldrich™). The concentrations (mg/ml) of each set of rotenone liquid crude extracts are shown in Table 1.

Table 1 Concentration of each rotenone liquid crude extract analyzed using RP-HPLC

Solvent system ratios	Samples	[a]Rotenone concentration (mg/ml)
Methyl chloride: methanol 1:1	(A)	0.21 ± 0.08
	(B)	2.77 ± 0.14*
Methyl chloride: methanol 1:9	(C)	0.13 ± 0.05
	(D)	0.59 ± 0.11*

Results shown were means ± S.D. in triplicate (n = 3). (*) p<0.05: Results were considered statistically significant.

Set B had the highest rotenone concentration (mg/ml) of all and this was followed by set D (p<0.05) of a different extraction solvent system ratio. Each set of the extract of known concentration (mg/ml) was diluted to an appropriate concentration prior to the biological activity study. Different concentrations of *Derris elliptica* root extract were tested against randomly selected mosquitoes' larvae instar. Figure 2 shows the linear regression of the logarithm-converted concentrations (x) after 5 hrs of treatment versus the probit values (y) respectively.

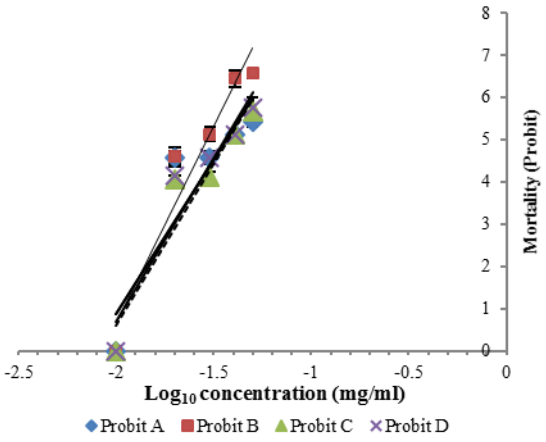


Figure 2 The biological activity (LC_{50}) of concentrated and unconcentrated rotenone liquid crude extracts extracted using 2 different solvent system ratios. Linear regression of logarithm-converted concentrations (x) versus the probit values (y). Results shown are means ± S.D. in triplicate (n = 3). Probit A: unconcentrated extract solution of methyl chloride: methanol (1:1), probit B: concentrated extract solution of methyl chloride: methanol (1:1), probit C: unconcentrated extract solution of methyl chloride: methanol (1:9) and probit D: concentrated extract solution of methyl chloride: methanol (1:9).

The results showed that the concentrated liquid crude extracts of a solvent ratio of methyl chloride: methanol (1:1) of set (B) was the most effective extract to affect the mosquitoes' larvae. Additionally, the (B) extract was considered the most active solution as compared with the other extracts (p<0.05) as indicated by the mortality (LC_{50}) value at a lower concentration value of 0.024 mg/ml (24 ppm). In fact, it accomplishes 50% mortality mark of approximately 2 hrs earlier than the other treatments (p<0.05) (Table 2).

Evidently, the manipulation of the solvent extraction system ratios and the extracts condition via heat treatment (concentrated/unconcentrated) has affected the larvicidal activity (LC_{50}) as it represented the amount of the extracted rotenone and other bio-active constituents available in the extracts. The least chemicals involved, the better the results observed as the concentrated extracts would represent a highly concentrated of bio-active components to affect the larvicidal activity. The results were fully supported by both -ve and +ve control data in which it demonstrates the survival strength of the mosquito's larvae against organic solvents with no observed mortality. This phenomenon would possibly be due to ability of the mosquitoes' larvae by secreting a layer of non-cellular material which separated the food from the epithelial cells of the gut as part of its defensive mechanism. This layer is called peritrophic matrix (PM). The PM acts as a protective barrier against various chemical, physical and microbial food components.[1,17] In this study, the larval mortality (LC_{50}) caused by the unconcentrated/concentrated methyl chloride: methanol (1:1/1:9) extracts appeared to be related to the disruption of PM structure which could rupture the midgut epithelium cells and eventually kill the cells. The disruption of PM could actually facilitate the transport and enhance the insecticidal activity of different agents such as virus, bacteria, protozoa, toxic proteins and plant secondary metabolites.

Table 2 Biological activity (LC_{50}) of different extraction solvent system ratio

Solvent system ratios	Samples	[a]Lethal concentration (LC_{50})	Time of LC_{50}
Methyl chloride: methanol 1:1	(A)	0.036 ± 0.003 mg/ml	5 hrs ± 30 mins
	(B)	0.024 ± 0.001* mg/ml	3 hrs ± 15 mins*
Methyl chloride: methanol 1:9	(C)	0.037 ± 0.001 mg/ml	5 hrs ± 15 mins
	(D)	0.032 ± 0.002 mg/ml	5 hrs ± 30 mins
Positive control (methyl chloride: methanol 1:1)	(+ve)	ND	ND
Positive control (methyl chloride: methanol 1:9)	(+ve)	ND	ND
Negative control (distilled water)	(-ve)	ND	ND

Results shown were means ± S.D. in triplicate (n = 3). Probit A: unconcentrated extract solution of methyl chloride: methanol (1:1), probit B: concentrated extract solution of methyl chloride: methanol (1:1), probit C: unconcentrated extract solution of methyl chloride: methanol (1:9) and probit D: concentrated extract solution of methyl chloride: methanol (1:9). (*) p<0.05: Result was considered statistically significant as compared to A, C and D. [a]One ml of rotenone liquid crude extract (prepared in 5 different concentrations) in 20 ml of distilled water containing 8 mosquitoes' larvae. ND - Not determined (100% survival).

Overall, the extract of *Derris elliptica* has already been the subject of phytochemical studies and has been shown to contain insecticidal isoflavonoid-type compounds known as rotenone and rotenoids (deguelin and β-rotenolone).[18] It is recommended that a biological activity-guided chemical fractionation protocol should be conducted in order to identify the exact larvicidal components in the extracts (other

important bio-active constituents apart from rotenone: deguelin and β-rotenolone), mainly those responsible for PM disruption. Moreover, these secondary metabolites extracted from *Derris elliptica* roots have the potential to be used as part of a novel strategy for insect control especially for the vector diseases by inhibiting the larvae's development at the beginning of its growth cycles.

4.0 CONCLUSION

The results of the experiment suggested that methyl chloride: methanol (1:1) is the best solvent system to exhibit a high rotenone content (mg) as compared to the ratio of 1:9 ($p<0.05$). Moreover, the concentrated liquid crude extracts of methyl chloride: methanol (1:1) which contained the highest rotenone content produced the lowest treatment concentration of 0.024 mg/ml to achieve 50% mortality within 3 hrs of treatment ($p<0.05$). The rapid mortality (as indicated by the LC_{50} value) of the mosquitoes' larvae against rotenone extracted from *Derris* plant roots has proven that it has the potential to be used as larvicide to control vector-borne diseases especially from mosquitoes.

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References

[1] World Health Organization. World Malaria Report 2010. <*http://www.who.int/malaria/world_malaria_report_2010/en/index.html*>; 2011 [Accessed: 18.01.2014].

[2] Mace, K. E., M. F. Lynch, J. R. MacArthur, S. P. Kachur, L. Slutsker, R. W. Steketee, T. Popovic. 2011. Grand Rounds: The Opportunity for and Challenges to Malaria Eradication. *Morbidity and Mortality Weekly Report* CDC. 60(15): 476-80.

[3] Cantrell, C. L., F. E. Dayan and S. O. Duke. 2013. Natural Products as Sources for New Pesticides. *J. Nat. Prod.* 75(6): 1231-1242.

[4] Becker N., D. Petric, M. Zgomba, C. Boase, M. Madon, C. Dahl, A. Kaiser. 2010. *Mosquitoes and Their Control*. Springer, New York.

[5] Suraphon, V. and M. Manthana, 2001. Effects of root extract from *Derris* (*Derris elliptica* Benth) on Mortality And Detoxification Enzyme Levels in the Diamondback Moth Larvae (*Plutella xylostella* Linn.). *Am. Ent.* 8: 100-120.

[6] Radad, K., W.-D. Rausch, G. Gille. 2006. Rotenone Induces Cell Death In Primary Dopaminergic Culture By Increasing ROS Production And Inhibiting Mitochondrial Respiration. *Neurochem. Intern.* 49: 379-386.

[7] Zubairi, S .I., M. R. Sarmidi and R. A. Aziz. 2014. The Effects of Raw Material Particles Size, Types of Solvents and Solvent-To-Solid Ratio on the Yield of Rotenone Extracted From *Derris Elliptica* Roots. *Sains Malaysiana*. 43(5): 707-713.

[8] Zubairi, S. I., M. R. Sarmidi and R. A. Aziz. 2014. Identification of Bio-active Constituents from *Derris Elliptica* Liquid Crude Extract Using Vacuum Liquid Chromatography. *Adv. Environ. Biol.* 8(2): 437-440.

[9] Zubairi, S. I., M. R. Sarmidi and R. A. Aziz. 2014. A Preliminary Study of Rotenone Exhaustive Extraction Kinetic From *Derris Elliptica* Dried Roots Using Normal Soaking Extraction (NSE) Method. *Adv. Environ. Biol.* 8(4): 910-915.

[10] Zubairi, S. I., M. R. Sarmidi and R. A. Aziz. 2015. A Thermal Degradation (Thermolysis) Study of Rotenone Extracted from *Derris elliptica* Roots Using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC). *Sains Malaysiana*. 44(1): 161-166.

[11] Zubairi, S. I., M. R. Sarmidi and R. A. Aziz. 2014. Bio-active Constituents of Rotenoids Resin Extracted from *Derris Elliptica* Roots: Comparison Between Local Plant Extract and SAPHYR (France) Cube Resin. *Adv. Environ. Biol.* 8(4): 904-909.

[12] Armitage, P., G. Berry and J. N. S. Matthews. 2001. *Statistical Methods in Medical Research*. 4th Edition. Wiley-Blackwell Scientific Publications, Oxford.

[13] Finney, D. J. 2009. *Probit Analysis*. 1st Edition (digitally printed version). Cambridge University Press, New York.

[14] Zubairi, S. I., M. R. Sarmidi and R. A. Aziz. 2014. Biological Activity on the Extract of *Derris elliptica*: An Optimization Approach to Investigate the Effect of Processing Parameters on Mortality of *Artermia salina*. *Adv. Environ. Biol.* 8(10): 9018-924.

[15] Ottoboni, M. A. 1991. *The Dose Makes The Poison*. 2nd edn, Van Nostrand Reinhold, New York.

[16] Cheung, Y. K. 2011. *Dose Finding by the Continual Reassessment Method*. 1st Edition. Chapman & Hall/CRC Biostatistics Series.

[17] Farnesi, L. C., J. M. Brito, J. G. Linss, M. Pelajo-Machado and D. Valle. 2012. Physiological and Morphological Aspects of Aedes aegypti Developing Larvae: Effects of the Chitin Synthesis Inhibitor Novaluron. *PLoS ONE*. 7(1): e30363. DOI:10.1371/journal.pone.0030363.

[18] Cabizza, M., A. Angioni, M. Melis, M. Cabras, C. V. Tuberoso and P. Cabras. 2004. Rotenone and Rotenoids in Cube Resins, Formulations and Residues on Olives. *J. Agric. Food Chem.* 52: 288-293.