

A STUDY INTO THE EFFECT OF CONCENTRATION PROCESS ON THE YIELD OF ROTENONE FROM THE EXTRACT OF LOCAL PLANT SPECIES (*Derris elliptica*)

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ABSTRACT

Bio-pesticides are becoming increasingly important as pest management tools in various cropping systems in the tropics essentially to remedy problems associated with the indiscriminate use of 'hard' inorganic pesticide and interest in organic agriculture. In the few decades, many bio-pesticidal products, both microbial-based (bacteria, fungi, microsporida, entomopathogenic nematodes, viruses) and plant-based botanicals (rotenone and azadirachtin) had been studied for use against insect pests in the tropics. In this paper, the effect of the concentration process towards the yield of rotenone; mg and its concentration; mg/ml are studied extensively. The raw plants were collected from Kota Johor Lama, Johor and sorted to collect the root and stem. Only the root and stem were utilized as a raw material of the extraction process. The root and stem were extracted using the Normal Soaking Extraction (NSE) at 28 °C to 30 °C with 95 % (v/v) of acetone as a solvent and the solvent-to-solid ratio of 10 ml/g). The extraction was carried out for 24 hours. The liquid crude extract was concentrated further (the solvent removed under reduced pressure) using the rotary evaporator at 50 °C and 80 mbar of vacuum pressures. The fractions of the liquid crude extract were collected for each interval time (15 mins/1.0 ml/fraction). Each fractions were diluted 1/100 with acetone and further cleaned up prior to determination of rotenone content; mg and concentration; mg/ml by using the High Performance Liquid Chromatography (HPLC). Significant effect of the concentration process against the yield of rotenone; mg was recorded and shows a significant thermal degradation or dissipation of rotenone content at higher operating temperature. The possibilities for better exploitation and identification of the effective operating parameters will be discussed.

Keywords: Bio-pesticides; *Derris elliptica*; rotenone; concentration process; thermal degradation.

INTRODUCTION

Derris elliptica or 'Tuba' as it is known locally is an insecticidal plant in Malaysia that has been used for the purpose of bio-pesticide production. 'Tuba' plant is a kind of woody creeper plant and climber. It needs at least 75 % soil moisture content and the surround temperature should be 25 °C to 30 °C to obtain high content of the rotenone during its development. A calm area with low acidity soil content will enhance the production of rotenone (Grinda *et al.*, 1986). 'Tuba' is a member of the *Leguminosae, Fabaceae* family, which comprises 200 genera and 68 species including 21 species of *Tephrosia*, 12 of *Derris*, 12 of *Lonchocarpus*, 10 of *Millettia* and several of *Mundula* (John, 1944). Three species are found in Malaysia, which are *Derris elliptica*, *Derris malaccensis* and *Derris uliginosa* (Gaby Stroll, 1986). *Derris* is a climbing plant of Southeast Asia and its roots contain rotenone, a strong insecticide (Hutchison Encyclopaedia, 2000). *Derris elliptica* and *Derris malaccensis* contains 4.0 % (w/w) to 5.0 % (w/w) rotenone while *Lonchocarpus utilis* and *Lonchocarpus urucu* contain 8.0 % (w/w) to 10.0 % (w/w) rotenone in dry roots (Kole *et al.*, 1992). Many uses have been found for these insecticides. In addition to their effectiveness for both piercing-sucking insects, such as aphids and red bugs and chewing insects, especially caterpillars upon plants, they make excellent dusts for external parasites of animals such as fleas and lice. The toxic principles all deteriorate rapidly into *dihydrorotenone* (non-toxic substance) and water when exposed to sunlight and air; spray and dusts usually lose their effectiveness within a week after application (Schnick, 1974).

They are more effective when used during periods of cloudy weather, and the dusts should be applied to foliage moist with dew. The outstanding advantages of this group of poisons are that they are harmless to plants (phyto-toxic), relatively non-toxic to man and act as both contact and stomach poisons to insects (John, 1944).

METHODOLOGY

Plant collection - *Derris elliptica* is collected in the state of Johor; Kota Johor Lama, Malaysia.

Raw material - An important aspect of the phytochemical processing is the pre-processing of the herbal material prior to extraction. The treatment of the herbal material affects the viability of the phytochemical as well as the extraction yield. The procured *Derris* roots were immediately undergoes cleaning process to remove dirt and soil. The procured *Derris* roots were kept and dried into oven for overnight at room temperature (28 °C to 30 °C) and sorted to collect the root and stem. Only root and stem were utilized. The root and stem were cut into small pieces prior to grinding.

Extraction and concentration procedure - The extraction was carried out by soaking 50 g of dried root and stem in 500 ml of acetone 95 % (v/v) with a solvent-to-solid ratio of 10 ml/g for 24 hours at room temperature (28 °C to 30 °C). The liquid crude extracts were filtered through 15 cm Whatman no.4 filter paper directly into 500 ml beakers after 24 hours of extraction. The liquid crude extract was concentrated further (the solvent removed under reduced pressure) using the rotary evaporator at 50 °C and 80 mbar of vacuum pressures. The fractions of the liquid crude extract were collected for each interval time (15 mins/ 1.0 ml/fraction). Each fractions were diluted 1/100 with acetone and further cleaned up to remove the fine debris of root and stem through organic sample clarification kit (Waters™ Assoc.) containing 0.45 µm/0.5 µm directly into 5.0 ml of dark vial prior to determination of rotenone content; mg and concentration; mg/ml by using the High Performance Liquid Chromatography (HPLC).

Analysis of the rotenone liquid crude extract - The fractions of the liquid crude extract were subjected to quantitative analysis by using a reverse phase High Performance Liquid Chromatography (HPLC) to determine the rotenone content with UV (Photodiode Array - PDA) detection at 294 nm. The analysis of extract solutions were carried out by using the external standard method (Rotenone PESTANAL®, analytical grade, 96.2 % - SIGMA-Aldrich™ as an external standard solution).

Apparatus & reagents for the analysis of rotenone liquid crude extract - Waters™ Corp. (C18) liquid chromatography stainless steel column with particles size of 10 µm (3.9 mm I.D × 150 mm Length), analytical grade of rotenone standard with known purity (PESTANAL®, analytical grade, 96.2 % - SIGMA-Aldrich™), analytical grade of acetonitrile; 99.9 % (v/v) and deionized water (DOW). For the preparation of rotenone standard solution, about 20 mg rotenone standard powder (Rotenone PESTANAL®, analytical grade, 96.2 % - SIGMA-Aldrich™) was weighed into 125-ml glass Stoppered Erlenmeyer flask and dissolved with 50 ml of acetonitrile on a Gyrotory shaker for 10 mins. After shaking, the standard solution was filtered through 15 cm Whatman no. 2 filter paper directly into 50 ml beakers. About 10 ml of the standard solution was re-filtered through an organic sample clarification kit (Waters™ Assoc.) containing 0.45 µm/0.5 µm filters to remove impurities during the preparation of the solution. The isocratic solvent system was implemented as shown in Figure 1.0 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 (A.U.F.S) of the detection was 2.0 (Rodney & Ralph, 1976; AOAC, 2000).

Operational parameters of High Performance Liquid Chromatography (HPLC) - The conditions given below is typical values and may have to be adjusted to obtain optimum results from the given apparatus (AOAC Official Method, 2000). Table 1.0 shows the operating conditional for reversed-phase HPLC isocratic system.

Column temperature	Ambient
Flow rate	0.7 ml/min
Wavelength	294 nm
Injection volume	5.0 μ L

Table 1.0: Operating conditions for the isocratic solvent system



Figure 1.0: HPLC equipment - Isocratic solvent system

RESULT AND DISCUSSION

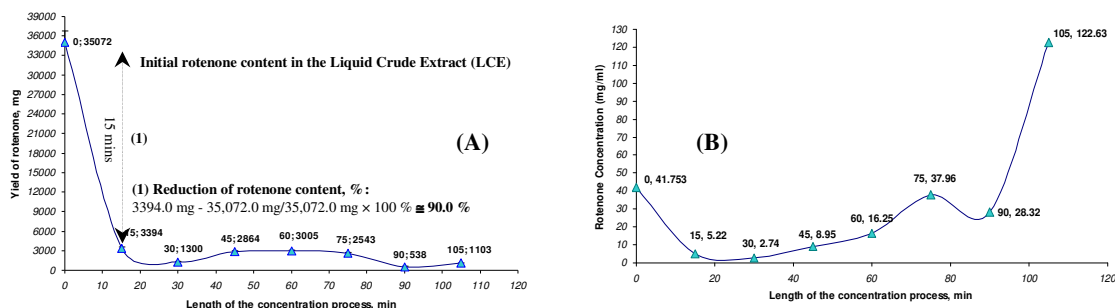


Figure 2.0: (A) The dissipation profile of rotenone content (mg) in the extract; (B) The concentration profile of rotenone (mg/ml) during the concentration process at 50 °C & 80 mbar of operating temperature and vacuum pressures respectively

Figure 2.0 (A) indicates that there is a significant effect of concentration process on the yield of rotenone; mg. Based on the degradation of the rotenone during concentration process, it appears that rotenone is strongly affected by the temperature above 40 °C. The initial concentration of the liquid crude extract is 41.75 mg/ml (rotenone content is 35,072 mg) and the concentration increases gradually as the time increases until the highest concentration (122 mg/ml) occurred at 105 mins with remained only 1,108 mg of rotenone. The amount of rotenone is reduced tremendously by 90 % for the first 15 mins of the process and constantly reduced up to 97 % until 105 mins. Grinda *et al.* 1986 claims that they have been used the operating temperature up to 45 °C for half an hour and they succeed to retain the rotenone content up to 14 % (w/w) in the finely crushed *Derris* powder. Therefore, these anomalies of the study are possibly due to the improper working condition such as over exposure to room lighting and the vacuum pump used during the experiment were not working sufficiently under the reduced pressure condition and consequently dissipated or deteriorated rotenone up to 90 % at the first 15 mins process.

The main constraint in this study is the ability to identify an appropriate extraction temperature to retain as much as possible rotenone content as rotenone is a light and heat sensitive compound. Therefore, when exposed to light and air, rotenone decomposes into *dihydrorotenone* and water resulting non-insecticidal bio-active compounds (Schnick, 1974). Further study by Cheng *et al.* 1972, using photo-degradation, identified that rotenone decomposes to at least 20 degradation products, most of which are rotenoids (rotenone and its derivatives). They reported that only one product, 6 α β , 12 α β -rotenolone, is toxic. The fact that the other 19 or more degradation product is not toxic is reason why rotenone is justifiable uses.

On top of that, based on the secondary data provided by Grinda *et al.* 1986; rotenone usually decomposed and detoxify within one or two weeks and it is difficult to predict in any given condition on how long the toxicity will remain. In general, high alkalinity (more than pH 8 to 9), high temperature, abundant light and air and lower concentrations favour rapid dissipation of rotenone. The half life ($t_{1/2}$) of rotenone is predicted to be 3 ½ hours when exposed to bright sunlight (\approx 30 °C to 40 °C) but accordingly to Pagan, (1949), the rotenone content of the roots of all treatments (e.g. effect of direct sunlight and oven drying) does not have any significant effects on the reduction of rotenone content and toxicological values.

CONCLUSION

After extensive studies have been done, there are several conclusions can be drawn:

- a) Rotenone is strongly affected by the operating temperature above 40 °C.
- b) Although there is no data and any extensive research have been done yet for the dissipation of the rotenone content, the operating temperature either for the extraction and concentration process should not be surpassing 40 °C to retain the rotenone content as well as to minimize any thermal degradation.
- c) Theoretically on the extraction process, as the extraction temperature is increased; it increases the rate of extraction by increasing the internal diffusion as well as the mass transfer coefficient values and reduced the extraction time (Frank *et al.*, 1999). However, it should be noted that increasing the temperature beyond certain values led to a decrease in isoflavonoid compounds yield due to the high susceptibility of the isoflavonoid to high temperature (Cacace & Mazza, 2003).

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