EVALUATION OF KINETIC FOR THE EXTRACTION OF BIO-ACTIVE COMPOUND (ROTENONE) FROM *Derris elliptica* AND IDENTIFICATION OF OPTIMUM VARIABLES OF THE EXHAUSTIVE EXTRACTION PROCESS

[1]S. I., Zubairi*, [1]M. R., Sarmidi, [1]R. A., Aziz, [2]M. K. A., Ramli & [1]R., Latip.

[1]Chemical Engineering Pilot Plant,
Faculty of Chemical and Natural Resources Engineering,
81310 UTM Skudai, Johor Darul Takzim.
[2]Heritage Biotech (M) Sdn. Bhd, c/o Chemical Engineering Pilot Plant,
Faculty of Chemical and Natural Resources Engineering,
81310 UTM Skudai, Johor Darul Takzim.
✆ Tel: +607-5532595
✆ Fax: +607-5569706
e-mail: saiful@cepp.utm.my, saiful-z@utm.my

ABSTRACT

Currently Bio-pesticide is relatively harmless to human and environment and thus desirable for the use in the control of insect vectors. Bio-pesticide have been increasing importance in both scale commercial agriculture and small plot, subsistence farming. One of the sources for bio-pesticide is 'Tuba' plant, known as *Derris elliptica*. *Derris elliptica* contains bio-active compounds known as rotenone ($C_{23}H_{33}O_6$) which is harmless to plants, highly toxic to many insects and relatively innocuous to mammals. Research carried out was to investigate the optimum independent and dependent variables from the exhaustive rotenone extraction process by evaluating the kinetic equilibrium phase of the Normal Soaking Extraction (NSE) method. 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 by using the Normal Soaking Extraction (NSE) at ambient temperature of 28 ^{0}C to 30 ^{0}C with 95 % (v/v) of acetone as a solvent and the solvent-to-solid ratio of the extraction was (10 ml/1g). The extraction was carried out for 24 hours and the fractions of the liquid crude extract were collected for each interval time (30 mins/1.0 ml/fractions) and further cleaned up to remove the fine debris of root and stem prior to determination of rotenone content, % (w/w) and concentration, mg/ml by using the High Performance Liquid Chromatography (HPLC). From the result obtained, it was found that the optimum independent and dependent variables was 10 hours to 12 hours of extraction time, 1.65 % (w/w) rotenone extraction yield, 800 mg to 820 mg of rotenone content and 2800 ppm to 2950 ppm of rotenone concentration respectively.

Keywords: *Derris elliptica*; rotenone; Normal Soaking Extraction; independent and dependent variables.

INTRODUCTION

Rotenone and its derivatives, commonly referred to as rotenoids are well known for their insecticidal properties. They occur naturally as constituents of the roots, stems, and leaves of many leguminous species of the genera *Derris, Lonchocarpus*, *Tephrosia,* and *Amorpha*. The 'Tuba' plant is a woody plant which grows along the ground, crawling and climbing to other plant. It needs at least 75 % moisture and a temperature of 25 ^{0}C to 30 ^{0}C to live. 'Tuba' is known by its botanical name as *Derris elliptica*. Rotenone is the bio-active compound extracted from *Derris elliptica* and other important constituents of *Derris* root (deguelin and tephrosin) have been shown to be toxic to insects, however they are less active than rotenone (Waterman, 1980). Commercially important plants like *Derris elliptica* and *D. malaccensis* contains 4 % (w/w) to 5 % (w/w) rotenone (Parmar, 2001). For several centuries, these plants have been used to prepare hunting and fishing poisons. More recently, rotenone has come of interest because of its selectivity and low environmental hazard. Rotenone is highly toxic to insects but relatively non-toxic to plants and mammals. This moderate polar molecule is toxic towards cold blooded animals and when exposed to sunlight, it is easily biodegrades to form *dihydrorotenone* and water (H_2O). Rotenone is extremely active as contact and stomach poisons against many crop pests such as Mexican bean beetle, apple and pea aphids, corn borer and household pests.

METHODOLOGY

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

Raw material - The collected raw materials immediately undergo cleaning process to remove dirt and soil. They were kept and dried into oven for overnight at room temperature (28 ^{0}C to 30 ^{0}C). The cleaned raw materials were 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 apparatus and procedure - The extraction was carried out by soaking 50 g of dried root and stem in 500 ml of acetone 95 % (v/v) with solvent-to-solid ratio of 10 ml/g for 24 hours at room temperature (28 ^{0}C to 30 ^{0}C). The fractions of the liquid crude rotenoids extract were collected for each interval time (30 mins/ 1.0 ml/ fractions) 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 the determination of rotenone extraction yield, mass content and concentration using the High Performance Liquid Chromatography (HPLC).

Analysis of the liquid crude rotenoids extract - The extract solutions were subjected to quantitative analysis using 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™).

Apparatus and reagents for the HPLC system operation - Operating conditions for reverse phase HPLC: Flow rate: 0.7 ml/min for Genesis™ (C18) stainless steel column with particles size of 4 µm - (3.9 mm I.D × 120 mm Length). For the preparation of rotenone standard solution, about 20 mg rotenone standard powder (Rotenone PESTANAL®, analytical grade, 96.2 % - SIGMA-Aldrich™) were weighed into 125-ml glass Stoppered Erlenmeyer flask and dissolved with 50 ml of acetonitrile on a Gyratory 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 filtrate were re-filtered through an organic sample clarification kit (Waters™ Assoc.) containing 0.45 µm/0.5µm filters. The solvent system (mobile phase) and Amplitude Unit Full Scale (A.U.F.S) was acetonitrile-distilled water (60:40) and 2.0 respectively (Rodney & Ralph, 1976; AOAC, 2000).

RESULT AND DISCUSSION

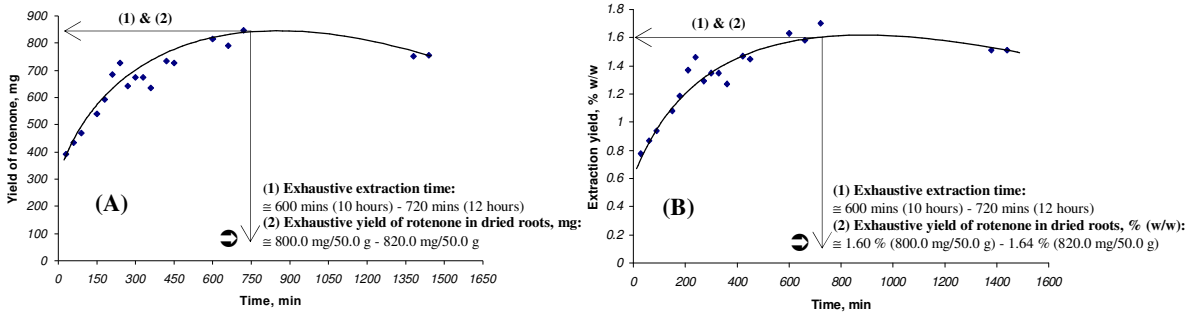
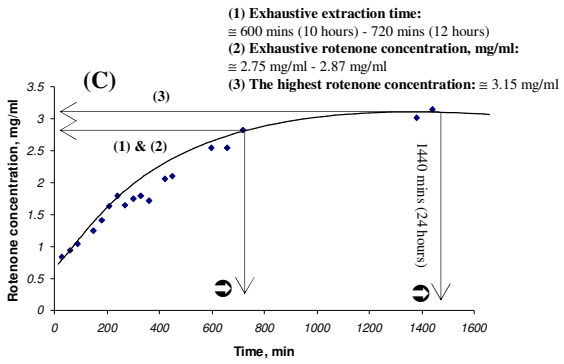


Figure 1.0: Kinetic profiles of Normal Soaking Extraction (NSE) process of *Derris elliptica* roots: **(A)** Rotenone content (mg); **(B)** Yield of extraction % (w/w); **(C)** Rotenone concentration (mg/ml).



Parameters involved in the studies:
INDEPENDENT VARIABLES:
☑ Extraction time (min).

DEPENDENT/RESPONSE VARIABLES:
☑ Rotenone content (mg).
☑ Yield of extraction % (w/w).
☑ Rotenone concentration (mg/ml)

CONSTANT VARIABLES:
☑ Temperature (28 ^{0}C to 30 ^{0}C).
☑ Solvent-to-solid ratio (10 ml/g).

DISCUSSION ON THE KINETIC PROFILES OF ROTENONE EXTRACTION PROCESS:

1) Figure 1.0 (B) shows that increasing the extraction duration/time, increases extraction yield. However, as shown in Figure 1.0 (A), 51.25 % to 52.44 % of extraction was achieved within 30 mins and 90 % within 8 hours. The steep rate of extraction at the beginning is possibly due to the washing (List & Schmidt, 1989) of solute from the ruptured cells rather than leaching alone, where the phtyochemicals released from within the cells by crushing or grinding are quickly dissolved into the bulk solution. In addition, it can be concluded that the exhaustive extraction of *Derris elliptica* occurs within 10 hours to 12 hours.
2) Accordingly to the Figure 1.0 (A), the optimum exhaustive time to produce the optimum yield of rotenone and mass of the rotenone was 600 mins (10 hours) to 800 mins (13 hours) and 800 mg/ 50 g to 820 mg/ 50 g respectively. Therefore, the optimum yield of extraction and concentration in the fine fresh roots and stem of *Derris elliptica* were 1.60 % (w/w) to 1.65 % (w/w) and 2.75 mg/ml to 2.87 mg/ml respectively.
3) The optimum concentration that can be obtained for the Normal Soaking Extraction (NSE) process was 3.15 mg/ml at the 24 hours of extraction time but the yield of rotenone at this period was only 760 mg (rotenone was decreased up to 60 mg (7.3 %)). These anomalies were due to the volume of solvent in the liquid crude rotenoids extract that rapidly decreased within 12 hours to 20 hours of extraction running. Consequently, the concentration of rotenone was increased tremendously hence, the solution became more concentrated with less amount of rotenone content (rotenone dissipation occurred due to the unstable ambient temperature). The most important consideration should be taken was the whole Normal Soaking Extraction (NSE) system should be insulated properly in order to minimize the dissipation of rotenone due to the fluctuation of ambient temperature, pressure and highly volatility of solvent (acetone). Rotenone is light and heat sensitive and with prolonged exposure to the bulk solution, a major loss of bio-active compounds and less effectiveness of insecticidal action will be undeniable.

ACKNOWLEDGEMENT

This research was supported by an IRPA grant 09-02-06-0083 EA261 under the Ministry of Science, Technology and Environment, Malaysia.

REFERENCES

Parmar, B. S., Walia, S., Opender, K. & Dhaliwal, G. S. (2001). Prospects and Problems of Phytochemical Bio-pesticide, *Phytochemical Biopesticides*. pp. 133-134**.**

Schmeltz, I. (1971). Nicotine and other tobacco alkaloids. In M., Jacobsan & D. G., Crosby (eds.), *Naturally Occurring Insecticides*, Marcel Dekker, New York, pp. 99-136**.**

Waterman, D. G. & Khalid, A. S. (1980). The major flavonoids of the seed of *Tephrosia* Phytochemistry, **19**, 909-915.

Rodney, B. & Alan, H. (1976). Determination of rotenone in pesticide formulations and the separations of six rotenoids by reversed-phase high performance liquid chromatography. *Journal of Chromatography*, 134 (1977) 210-215.

Ralph, I. & Ronald, L. Baron (1976). Separation of rotenoids by high performance liquid chromatography. *Journal of Chromatography*, 134 (1977) 207-209.

AOAC Official Method 983.06, Rotenone in Pesticide Formulations. Liquid Chromatographic Method. First Action 1983 & Final Action 1991. (2000) AOAC OFFICIAL METHOD OF ANALYSIS.