

NORMAL SOAKING EXTRACTION (NSE) OF ROTENONE FROM *Derris elliptica*

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ABSTRACT

Derris elliptica or the tuba plant contains rotenone, bio-active compound known that has the potential to be used as bio-pesticide. Bio-pesticide is the best-known alternative bio-pesticide which has the potential to replace the use of conventional pesticides, as it is more environmentally friendly. The main objective of the paper is to obtain the best extraction solvent for optimizing the yield in rotenone extraction. Rotenone was extracted from *Derris* root using three different parts of roots and three types of solvent in Normal Soaking Extraction (NSE). The types of *Derris* roots are: (1) Fine root (2) Coarse root (3) Stem. The three types of solvent system are: (1) Acetone 95 % (v/v), (2) Chloroform 99.9 % (v/v) and (3) a mixture of ethanol: H₂O (9:1) added with oxalic acid (1mg/ml). The liquid crude extracts were further cleaned up to remove the fine debris of roots. The presence of rotenone was confirmed using qualitative analysis Thin Layer Chromatography (TLC) and thereupon the determination of rotenone content was carried out using High Performance Liquid Chromatography (HPLC). From the results obtained, it was found that the Normal Soaking Extraction (NSE) using acetone 95 % (v/v) was the best method to extract the highest yield of rotenone; 1.14 % (w/w).

Keywords: *Derris elliptica*; rotenone; environmentally friendly; Normal Soaking Extraction (NSE)

INTRODUCTION

Rotenone and its derivatives 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 25 °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* contain 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 (H₂O). 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. Besides having low mammalian toxicity, they are reasonably safe to honeybees. (Opendor, 2001)

METHODOLOGY

Plant collection - *Derris elliptica* was collected in the state of Johor; Kulai and Kangkar Pulai, 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 °C to 30 °C). The cleaned raw

materials were sorted to collect the root and stem. Only root and stem were utilized. The dried roots that have been collected at Kangkar Pulai were categorized into 2 types of root (1) Fine root and (2) Coarse root and for the Kulai samples, the root and stem were kept in its originally condition. The root (fine and coarse) and stem were cut into small pieces prior to grinding.

Extraction apparatus and procedure - The extraction was carried out in 3 sets of solvent system by soaking 25 g of dried root (Fine and Coarse root) and stem in 250 ml solution which is (A) Acetone 95 % v/v, (B) Chloroform 99.9 % v/v and (C) a mixture of ethanol: H₂O (9:1) added with oxalic acid (OA: 1.0 mg/ml) 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 crude extracts/absolutes were analyzed using HPLC.

Analysis of crude extract - The extract solution 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 using external standard method (Rotenone PESTANAL[®], analytical grade, 96.2 % - SIGMA Aldrich[™]).

Apparatus and Reagents - Operating conditions for reverse phase HPLC: Flow rate: 1.0 ml/min for Genesis[™] (C18) stainless steel column with particles size of 4.0 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 Stopped Erlenmeyer flask and dissolved with 50 ml of acetonitrile on a Gyrotory shaker for 10 min. After shaking, the samples were 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 and Amplitude Unit Full Scale (A.U.F.S) was methanol-distilled water (80:20) and 2.0 respectively.

RESULT AND DISCUSSION

Normal Soaking Extraction (Acetone) result: As shown in Figure 1.0, fine root samples collected from Kangkar Pulai give the highest yield of 1.14 % (w/w) followed by Kangkar Pulai coarse root; 0.60 % (w/w), Kangkar Pulai stem; 0.13 % (w/w) and Kulai root; 0.33 % (w/w), Kangkar Pulai stem; 0.13 % (w/w) and Kulai stem; 0.13 % (w/w).

Normal Soaking Extraction (Ethanol+oxalic acid solution) result: As shown in Figure 1.0, fine root samples collected from Kangkar Pulai give the highest yield of 0.31 % (w/w) followed by Kangkar Pulai coarse root; 0.28 % (w/w), Kulai root; 0.24 % (w/w), Kulai stem; 0.23 % (w/w) and Kangkar Pulai stem; 0.18 % (w/w).

Normal Soaking Extraction (Acetone) result: As shown in Figure 1.0, fine root samples collected from Kangkar Pulai give the highest yield of 0.70 % (w/w) followed by Kangkar Pulai coarse root; 0.33 % (w/w), Kangkar Pulai stem; 0.15 % (w/w), Kulai root; 0.11 % (w/w) and Kulai stem; 0.06 % (w/w).

CONCLUSIONS

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

- 1) Acetone is the best solvent for the determination of rotenone content in fresh raw material as compared to the others two solvents.
- 2) Rotenone content in the root is always higher than in the stem.
- 3) Rotenone is being bio-accumulated at the root.
- 4) The fine roots tend to be more superior in rotenone content because of the resin cell tissue that contains the rotenoids (rotenone and its derivatives) was relatively abundant in roots of small and medium diameters (Waterman, 1980).
- 5) The condition in which the *Derris elliptica* is collected play vital part in ensuring high rotenone content. It is believed that if the production of rotenone by *Derris elliptica* can be understood, the optimum *Derris* plant can be cultivated and therefore increasing the overall yield of the extraction.

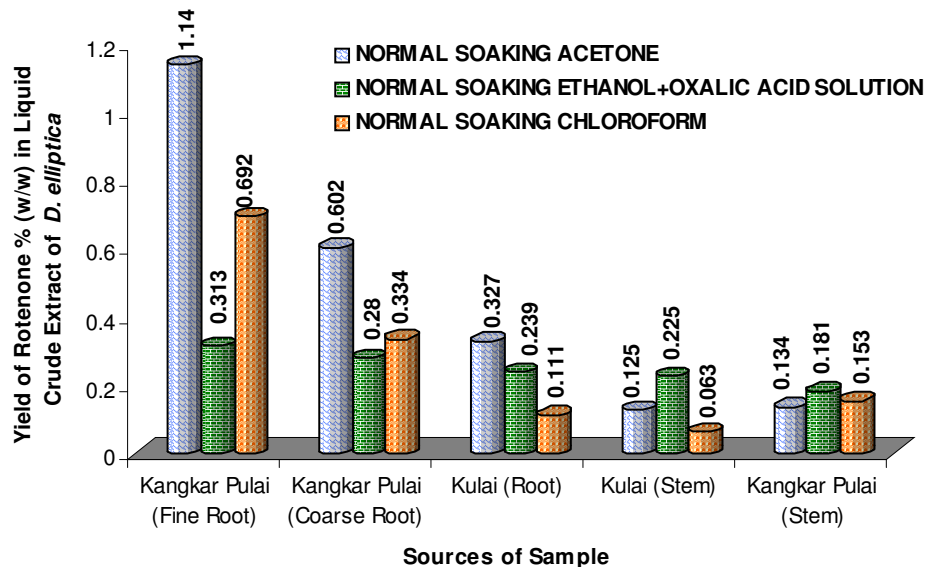


Figure 1.0: Yield of rotenone content using the Normal Soaking Extraction (NSE) method for different types of solvent

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