

# DETERMINATION OF SUITABLE CO-PRECIPIPARATOR (CLARIFYING AGENTS) FOR IDENTIFYING THE PRESENCE OF ROTENONE FROM *Derris elliptica*

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## ABSTRACT

Rotenone and its congeners have come into increasingly widespread usage as the active constituents of a large number of insecticides. The roots of *Derris elliptica* or tuba plant are used as a source of insecticidal compounds, primarily rotenone. The main objective of this work is to obtain the best co-precipitator and its optimal concentration in order to identify the appearances of rotenone in the extraction process. Rotenone was extracted from *Derris elliptica* root using two solvent systems: (1) Methanol 95 % (v/v) and (2) a mixture of methanol: H<sub>2</sub>O (8:2). The extraction was followed with the precipitation process using aluminium sulphate (1.5M) and the addition of co-precipitator (clarifying agents) such as calcium hydroxide, sodium carbonate, sodium caseinate and kieselguhr (diatomaceous earth) with 1M, 2M and 3M respectively. From the result obtained, it was found that calcium hydroxide was the best co-precipitator with the highest yield of 38.65 % (w/w) at an optimal concentration of 3M (mol/liter). The presence of rotenone was confirmed by using qualitative analysis of Thin Layer Chromatography (TLC).

*Keywords:* *Derris elliptica*; rotenone; co-precipitator; environmentally friendly, bio-pesticide.

## INTRODUCTION

Over the recent years, there is a growing trend in the public awareness regarding the problems posed by chemical pesticides to both human and environment. Therefore, after many years of endless research, scientist has found bio-pesticides as an alternative to chemical pesticides. 'Tuba' is a kind of woody plant that grows along the ground, leafy, crawling and climbing to other plant. It needs at least 75 % moisture and temperature 25 °C to live. 'Tuba' plant 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 *Derris malaccensis* contains 4 % (w/w) to 5 % (w/w) rotenone (Parmar, 2001). The object of fining is to aid in producing a product that is nearly perfect in terms of color and clarity. Fining could be a simple as letting nature takes its course through gravity, but by using a certain clarifying agents, the precipitation process can be enhanced tremendously. The array of clarifying agents available includes; activated carbon, casein, diatomaceous earth, sodium carbonate and calcium hydroxide. Fining agents work on the principle that all the particles responsible for the clouding or haze in the precipitation process solution have an electrical charge. Rotenone has a negative charge meaning that it can attract positively charged materials. Eventually, the combined desirable consequences such as completely stripped the cloudy or haze solution.

## METHODOLOGY

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

*Preparation for the co-precipitator solution* - The concentration for each co-precipitator were distributed for 1M, 2M and 3M. The solution concentration was based on the saturation process that occurred during the preparation. The 3M was the highest concentration in which saturation condition occurred for all co-precipitators.

*Preparation and Processing* - The roots of *Derris elliptica* plants were dried at room temperature and ground in a knife mill. The extraction was carried out by soaking 200 g of the dried powder root into 2500 ml of methanol 95% (v/v) and methanol:H<sub>2</sub>O (8:2) for 24 hours at room temperature (28 °C to 30 °C). The extracts were filtered through 15 cm Whatman no.2 filter paper directly into 1000 ml beakers. The solution for the precipitation process was prepared with a mixture of 100 ml of rotenone liquid crude extract, 400 ml of distilled water, 300 ml of aluminium sulfate (1.5M) and 100 ml of natrium carbonate (1M: Co-precipitator) and all those chemicals were mixed by using a magnetic stirrer. The solution was allowed to precipitate for 24 hours. The sediment was filtered through 15 cm Whatman no.4 filter paper and allowed to be dried in the oven cabinet at 30 °C to 40 °C. The weight of dried sediment was measured. The procedures were repeated for natrium carbonate with molarity of 2M and 3M as well as natrium caseinate, calcium hydroxide and kieselguhr (Rafizan, 2002)

*Product analysis* - 1.0 g of dried sediment and 0.5 mg of rotenone standard powder (Rotenone PESTANAL<sup>®</sup>, analytical grade, 96.2 % - SIGMA-Aldrich™) were dissolved and vortexed with chloroform (analytical grade) for 10 mins to 15 mins respectively. Thin Layer Chromatography (TLC) was used to analyze the presence of bio-active compound (rotenone) in the sediment that has been clarified using 4 kinds of different concentration clarifying agents. Petroleum ether and etil acetate with a ratio of 6:4 were used and putted as mobile phase in the development chamber. The development or separation was to be expected around 30 mins to 45 mins and the markers that appeared onto the TLC silica plate were viewed using the Ultra-Violet (UV) light for detection at 254 nm and 365 nm.

## RESULT AND DISCUSSIONS

*Precipitation process* - The precipitation process was done to precipitate the bio-active compounds such as rotenone from the extract solution. The major precipitation agent of this study was aluminum sulfate. Based on the previous study, the solution needed to be added with clarifying agents so that the cloudy or haze solution can be reduced up to 80 % visibility. By adding the co-precipitator, it can reduce the haze solution by attracting the negative charges or neutralize the particle surface of rotenone so that it can flock and form colloidal particles. These colloidal particles agglomerate with each others and form a much bigger colloid until it flocculates through gravitational movement. Figure 1.0 shows that methanol 95 % (v/v) produced more yield of sediment compared to the methanol: H<sub>2</sub>O (8:2) for natrium hydroxide and natrium casienate but slightly lower for kieselguhr and natrium carbonate. From the result, calcium hydroxide (CaOH) produced the highest yield of sediment; 38.65 % (w/w), followed by kieselguhr; 23.55 % (w/w), natrium caseinate; 16.45 % (w/w) and natrium carbonate; 2.58 % (w/w) for the methanol 95 % (v/v) extract at 3M of co-precipitator concentration. The higher the concentration of co-precipitator involved, the more sedimentation was occurred and apparently more rotenone was captured in the sediment. Figure 2.0 shows that the presence of rotenone in the sediment of CaOH (3M) with a solvent system of methanol 95 % (v/v) which is confirmed using TLC. The R<sub>f</sub> value gives the indication for further developing and investigation on the quantitative analysis using HPLC.

Table 1: R<sub>f</sub> value for calcium hydroxide co-precipitator sediment

Methanol 95 % (v/v)		Methanol: H <sub>2</sub> O	
Std R <sub>f</sub>	0.63	Std R <sub>f</sub>	0.67
R <sub>f</sub> (1M)	0.60	R <sub>f</sub> (1M)	0.75
R <sub>f</sub> (2M)	0.60	R <sub>f</sub> (2M)	0.75
R <sub>f</sub> (3M)	0.60	R <sub>f</sub> (3M)	0.75

$$R_f = \frac{\text{Distance traveled by the compound (dc)}}{\text{Distance traveled by the solvent front (ds)}}$$

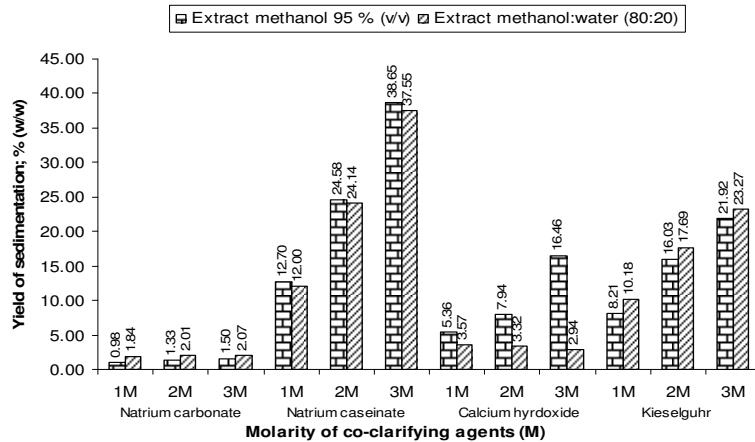
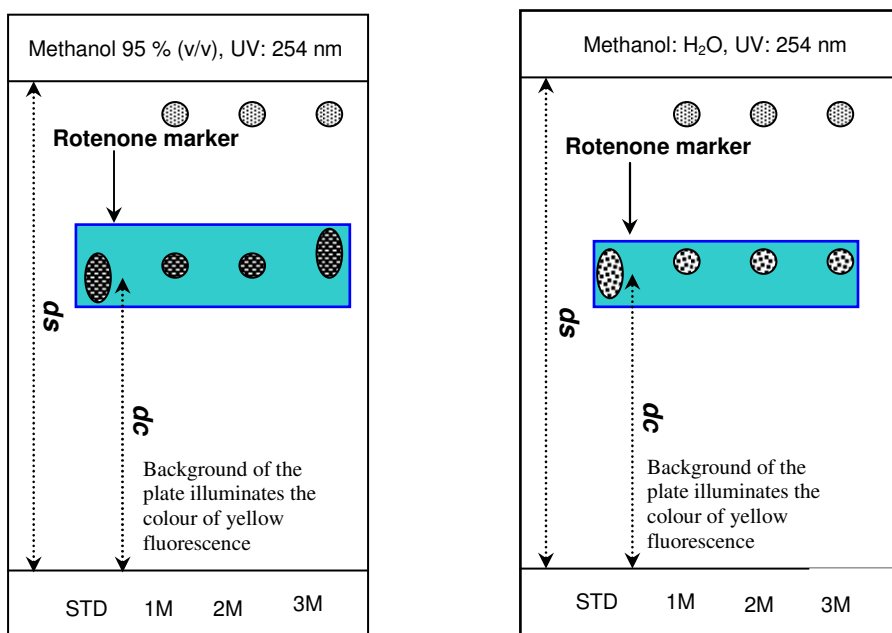


Figure 1.0: The yield of sediment (%) versus concentration of co-precipitator for 2 types of solvent system.

Figure 2.0: The presence of rotenone under UV light of 254 nm



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