Precipitation of Rotenoids Resin Extracted from *Derris elliptica* Roots By Means of Clarifying Agents

1Saiful Irwan Zubairi, 2Mohamad Roji Sarmidi and 3Ramlan Abdul Aziz

1School of Chemical Sciences & Food Technology, Faculty of Science & Technology, The National University of Malaysia, 43600 UKM Bangi, Malaysia.
2Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Skudai, Malaysia.

**ABSTRACT**

Rotenone and its congeners have come into an 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 which primarily contain rotenone. The main objective of this work is to obtain the best combination of the main precipitator agent (aluminium sulfate: Al(SO$_4$)$_3$) with 4 different types of co-precipitator to completely solidify rotenone from liquid crude extract. Rotenone was extracted from *Derris elliptica* root using two solvent systems: (1) methanol 95% (v/v), and (2) a mixture of methanol and H$_2$O (8:2). After the extraction, the precipitation process was carried out using Al(SO$_4$)$_3$ (1.5M) with the addition of co-precipitator (clarifying agents) such as calcium hydroxide, sodium carbonate, sodium caseinate and kieselguhr (diatomaceous earth) with a concentration of 1M to 3M. The result has shown that natrium caseinate was the best co-precipitator with the highest yield of 38.65% (w/w) at the highest concentration of 3M. None of the rotenone was detected in the supernatant to indicate that all rotenone content have been precipitated adequately. Finally, the presence of rotenone was confirmed by using qualitative analysis of thin layer chromatography (TLC) method.

**INTRODUCTION**

Over the recent years, there is a growing concern in the public awareness regarding the problems posed by chemical pesticides to both humans and the environment. Hence, after many years of endless research, scientists have found bio-pesticides as an alternative to chemical pesticides [4]. ‘Tuba’ is a kind of woody plant that grows along the ground, leafy, crawling and climbing onto other plants. It needs at least 75% moisture content and temperature of 25 °C to live [6]. ‘Tuba’ plant is known by its botanical name as *Derris elliptica*. This commercially important botanical plant like *Derris elliptica* and *Derris malaccensis* contains around 4% (w/w) to 5% (w/w) rotenone [5]. Rotenone is the main bio-active compound extracted from *Derris elliptica* and other important constituents of *Derris* root (e.g., deguelin and tephrosin) have been shown to be toxic to insects. However, they are less active than rotenone [7]. The commercially available rotenone is sold in dispersible powder, emulsifiable concentrate and wettable powder formulations [1]. The easiest way to formulate a rotenone based products without using complicated formulations is by making a dispersible powder. To do so, precipitation process or fining was recommended through the usage of main precipitator and clarifying agent. Fining is to aid in producing a product that is nearly perfect in terms of color and clarity [3]. In definition, fining is the process where a substance (fining agent) is added to the liquid crude extract to create an adsorbent or ionic bond with the suspended particles, producing larger molecules and larger particles that will precipitate out of the liquid extract more readily and rapidly [2]. Fining could be a simple as letting nature takes its course through gravity, but by using 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 [3]. Rotenone has a negative charge, meaning that it can attract positively charged materials. Eventually, the combined positive and negative charge particles will completely strip the cloudy or haze solution.
MATERIALS AND METHODS

Plant collection:

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

Preparation of co-precipitator solution:
The concentrations for each of the co-precipitators (calcium hydroxide, sodium carbonate, sodium caseinate and kieselguhr) were set at 1M, 2M and 3M. All of these co-precipitators were dissolved in 300 ml of dionized water (DIW) in accordance to the respective molarity. The selection of the above mentioned solution concentration was based on the saturation point of each of the chemicals during the solution preparation where 3M was the highest concentration.

Raw material preparation, extraction and precipitation:
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 + H2O (8:2) for 24 hrs at room temperature (28 to 30 °C). The liquid crude extracts were filtered through 15 cm Whatman filter paper (number 2) directly into 1000 ml beakers. The ratios between all solutions are as follows: Rotenone liquid crude extract: distilled water: aluminium sulfate (1.5M); co-precipitators (e.g., natrium carbonate (1M)) = 1: 4: 3: 1 (100 ml: 400 ml: 300 ml: 100 ml). All those chemicals were mixed by using a magnetic stirrer. The mixed solution was allowed to precipitate for 24 hrs. The supernatant was siphoned (silicone tube) slowly by using 100 ml syringe to avoid from destroying the precipitate layer. Next, the precipitate was filtered through 15 cm Whatman filter paper (number 4) and allowed to be dried in the oven cabinet at 30 °C. The weight of dried solid precipitate was measured and recorded. The procedures were repeated for natrium carbonate with molarity of 2M and 3M as well as natrium caseinate, calcium hydroxide and kieselguhr.

Analysis of liquid extract supernatant:
The fractions of the liquid crude extract supernatant were subjected to quantitative analysis by using a reverse-phase high performance liquid chromatography (RP-HPLC) with UV (Photodiode Array - PDA) detection at 294 nm to determine the rotenone content. 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. (C18) liquid chromatography stainless steel column with particle size 10 μm (3.9 mm I.D. × 150 mm length) was utilized in the analysis. 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 the detection was 2.

Product analysis:
Initially, 1 g of dried solid precipitate and 0.5 mg of rotenone standard powder (Rotenone PESTANAL®, analytical grade, 96.2% - Sigma-Aldrich™) were vortexed and dissolved with chloroform (analytical grade) for 10 mins to 15 mins. Next, the thin layer chromatography (TLC) was carried out to analyze the presence of rotenone in the precipitate which had been clarified using 4 different types of clarifying agents/co-precipitator. Petroleum ether and ethyl acetate with a ratio of 6:4 were used as a mobile phase in the development chamber. The separation of the bio-active compound was about 30 mins to 45 mins. The markers that appeared onto the TLC silica plate were viewed and illustrated using the ultra-violet (UV) lamp with the wavelength of 254 nm and 365 nm.

Statistical analysis:
The 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.

RESULTS AND DISCUSSION

Figure 1 shows the yield of precipitate, % (w/w) versus different concentration of co-precipitator of 2 types of solvent system. Both of the employed extract solvents (methanol 95% (v/v) and methanol + H2O (8:2)) produced nearly an equal yield of precipitate on different chemical concentrations except for the calcium hydroxide of 2M and 3M (p<0.05). Overall, with the highest concentration of all co-precipitators (3M), natrium caseinate produced the highest yield of sediment followed by kieselguhr, natrium caseinate and natrium carbonate of both employed extraction solvents (p<0.05).
Fig. 1: The yield of precipitate, % (w/w) versus different concentration of co-precipitator of 2 types of solvent system. Results shown were means ± S.D. in triplicate (n = 3). (*) p<0.05 comparison of the yield of precipitate with the other extraction solvent. (Ψ) p<0.05 comparison of the yield of precipitate with the other co-precipitators at concentration of 3M.

Figure 2 shows that the presence of rotenone in the precipitate of the highest yield (natrium caseinate (3M)) and the availability of rotenone markers was confirmed using TLC. The Rf values of rotenone markers and standard solution are shown in Table 1. The values confirmed the availability of rotenone inside the precipitate by means of markers visualization under the UV lamp. The quantitative analysis (RP-HPLC) was not carried out due to the fact that the precipitate contains a lot of coarse particles that might block the HPLC column. Meanwhile, the precipitation process using 4 different types of co-precipitator (calcium hydroxide, sodium carbonate, sodium caseinate and kieselguhr) coupled with the main precipitator was done to induce flocculation of the bio-active compounds especially rotenone in the liquid crude extract of Derris roots. The main precipitation agent of this study was aluminum sulfate (1.5M). However, around 5.3 ± 1.4% (w/w) yield of precipitate was attained only by the use of Al₂(SO₄)₃. For that reason, the usage of the clarifying agents was needed so that the cloudy or haze solution could be reduced up to 95% invisibility.

Fig. 2: The presence of rotenone on the thin layer chromatography (TLC) silica plate. Petroleum ether and ethyl acetate with a ratio of 6:4 was used as a mobile phase. The markers were visualized under UV light with wavelength (λ) of 254 nm (dₛ = migration distance of solvent front; dᶜ = migration distance of substance; STD = rotenone standard).
Table 1: Rotenone Rf values of natrium caseinate co-precipitator in the precipitate as compared to the rotenone standard. Results shown were means ± S.D. in triplicate (n = 3).

<table>
<thead>
<tr>
<th>Methanol 95% (v/v)</th>
<th>Methanol: H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>StdRf</td>
<td>StdRf</td>
</tr>
<tr>
<td>0.63 ± 0.12</td>
<td>0.67 ± 0.08</td>
</tr>
<tr>
<td>Rf (1M)</td>
<td>Rf (1M)</td>
</tr>
<tr>
<td>0.62 ± 0.11</td>
<td>0.66 ± 0.13</td>
</tr>
<tr>
<td>Rf (2M)</td>
<td>Rf (2M)</td>
</tr>
<tr>
<td>0.61 ± 0.14</td>
<td>0.62 ± 0.25</td>
</tr>
<tr>
<td>Rf (3M)</td>
<td>Rf (3M)</td>
</tr>
<tr>
<td>0.62 ± 0.10</td>
<td>0.63 ± 0.30</td>
</tr>
</tbody>
</table>

In theory, by adding the co-precipitator, it reduces the haze solution by attracting the negative charges and neutralizing the particle surface of rotenone as it flocks and forms colloidal particles [3]. These colloidal particles agglomerate with each other and form a much bigger colloid until it flocculates through gravitational movement [2].

Conclusion:

Based on the study, it can be concluded that the higher the concentration of co-precipitator involved, the higher the amount of sedimentation is likely to occur. All concentration of natrium caseinate, calcium hydroxide and kieselguhr give an indication (as illustrated on the TLC plate) of capturing rotenone inside the precipitate. None of the rotenone was detected in the supernatant to indicate that all rotenone content had been precipitated adequately. The RP-HPLC is highly recommended to be used for analyzing the amount of rotenone in the precipitate as thin layer chromatography will only give a visual indication. However, the precipitate needs to be purified beforehand by means of the vacuum-liquid chromatography (VLC) so that the accurate measurement of rotenone content could be further quantified as per gram of dispersible powder form product.

ACKNOWLEDGEMENT

All thanks are due to the Malaysia Ministry of Science, Technology & Environment (MOSTE) for the financial assistance under RM8 IRPA 04-01-06-SF0077 and the Universiti Teknologi Malaysia (UTM) for providing the research facilities throughout the study.

REFERENCES