Chapter 5

Modification Techniques for Rotenone Extraction

5.1 Introduction

Rotenone and rotenoids are the bioactive compounds occurred in derris plants. These toxicants are prospective candidates as broad-spectrum pesticides considering as additional tools for conventional chemical control alternatives. The proper isolation and determination, including the extraction techniques for the bioactive compounds is the most crucial steps and of great importance in the commercial development of the botanical pesticide products. There are several rotenone extraction and modified methods have been practiced by several investigators in previous years: i.e. ethanol extraction methods, Soxhlet and stirring soaking (Visetson and Milne, 2001); pressurized liquid extraction (PLE) at 50°C and pressure at 2000 psi (Sae-Yun *et al.*, 2006); supercritical carbon dioxide (SC-CO_2) extraction at 60°C and pressure at 44 MPa (D'Andrea *et al.*, 2007); Zeng *et al.* (2002) suggested the application of UV spectra with the detection wavelength of 240 nm is more adequate for the analysis of a complex of rotenoids. The suitable solvents for the extraction are acetonitrile, benzene, carbon tetrachloride, chloroform, ethanol, ether, ethyl acetate, methanol, trichloroethylene and etc., or the like (Meijer and Koolhaus, 1940; Smith, 1994). Botanical insecticides compared to conventional synthetic insecticides may be secured for the environment are generally less expensive and easily to processed, administered, and adopted by most farmers and small industries. Although, certain methods with varying details may not provide entirely satisfactory for all root sample analyses, but the most popular derris root extraction methods are water or alcohol extraction methods. Hence, the current study is aimed to modify conventional maceration extraction technique in combination with certain volumes of ethanol that makes its possible to maximize obtainable rotenone and its derivatives for effectiveness of the economic insect pest management and encourage the national sufficiency economy policy by building up the local capability for self-reliant production of Thai farmers.

5.2 Material and Methods

Experiment 1 Enhancing Effectiveness of Rotenone Water Extraction

Obtaining 0.5 gm of each of derris fresh root, derris root powder, and derris root precipitate for water extraction by 100 ml of distilled water as conventional maceration. In addition, prepared another sets of the samples and macerating with 10 ml of 95% ethanol for one hour before adding water to the volume of 100 ml and remacerated for another 24 hour in an agitator at the speed of 50 rpm. Both treatments

were replicated 4 times. Measurement of the rotenone content in the extract samples by HPLC method was employed.

Experiment 2 Preparing Derris Fresh Root Precipitate for Rotenone Water Extraction

Ten gram of finely crushed derris fresh root were introduced into an agitator with the speed of 50 rpm for filtering out the dirt residue. The obtained liquid was centrifuged with the velocity of 7000 rpm for 60 minutes. Introduced the precipitate separated from the liquid was transferred into Petri dish and dried indoor. Determination of the rotenone concentrations detected in the precipitate, the precipitate separated liquid, and the fresh root residues was analyzed by the HPLC instruments with the circumstances as mentioned in Chapter 3.

Analyzation of the precipitate by the GCMS (Gas Chromatography Mass Spectrum) instruments at Science and Technology Service Center, Chiang Mai University (STSC-CMU) to examine the type and content of substances in the precipitate with the following instrument applications:

1. GC 6890 Agilent Technologies
   Intel : 270^{o}C
   Oven : 80^{o}C – 10^{o}C / min 260^{o}C (42 min)
   Carrier : Helium 1.0 ml/ min
   Column : HP – 5 MS 30 m. x 0.25 mm ID x 0.25 µm film thickness.
2. MSD 5973 (EI) Hewlett Packard
   MS Quadruple 150^{o}C MS Source 230^{o}C

Experiment 3 Relationship between Rotenone Concentrations Extracted from Derris Fresh Root and Light Absorbance

One gram of finely crushed 1 cm diameter derris fresh root by sugarcane crusher was introduced and macerated in 10 ml of 95% ethanol for one hour, adding 90 ml of water into the solution and remacerated for another 24 hours. Residue was filtered and the clear supernatant was diluted with water to obtain the concentration of 100, 75, 50, 25, 12.5, and 6.25%. Rotenone concentration in each solution was measured by HPLC instruments (diluted with 10-fold Dioxane) and by measure light absorbance of the solution using Spectrophotometer instruments at 245 nm wavelength (diluted with 100-fold Dioxane).

Experiment 4 The Effect of Solvent and Evaporation on Rotenone Content in Crude Extraction from Derris Root Powder

The rotenone content which was extracted from 1 gm of derris root powder with varying volume of ethanol, 5, 10, 15, and 20 ml were introduced into an agitator with 50 rpm and macerated for 3 days. After filtration with no.1 filter paper, the rotenone contents in the obtained solutions were measured by HPLC instruments. The extracts from all treatments were evaporated and dried with vacuum evaporator. The dried extracts were then redissolved with 95 % ethanol to make 10 % (w/v) concentration for rotenone content measurement by HPLC method, the state of HPLC instruments as described in Chapter 3.

5.3. Result and discussion

Experiment 1 Enhancing Effectiveness of Rotenone Water Extraction

Means of rotenone content from derris fresh root, derris root powder, and derris root precipitate were 65.79, 30.54, and 92.43, respectively (Table 5.1, and Figures 5.1, and 5.2). Derris root precipitate provided the highest rotenone content due to the mass accumulation of the suspended rotenone substances in the derris root solution after water extraction process. Maceration the samples in 10 ml of 95% ethanol certainly ample dissolvability of the rotenone substances in the solutions, thus, after water reextraction process the means of rotenone content of the samples were enormously increased to 114.42, 211.94, and 535.67 ppm, respectively, or equivalent to 1.74, 6.93, and 5.79 folds of the amount obtained by water extraction alone. Normally, the mixture formulation of 200-300 gm rotenone fresh root extract with 20 liters of water provided effective control of most insect pests. Although, the modified conventional water extraction method by adding 10 ml of 95% ethanol in the treatment combination might be necessary to increase rotenone yield the ethanol cost of approximately 6.65 bahts per 1 liter of rotenone extract was just small amount (95% alcohol costs 66.5 bahts per liter). Nevertheless, the modified treatment could furnish even better rotenone quantity and plant protection effectiveness and feasibility, thus, encouraging the national sufficiency economy policy to all Thai farmers.

Table 5.1 Means rotenone concentrations detected from solutions of derris fresh root, derris root powder, and derris root precipitate extracted by 2 different treatments in 4 replications

Solutions	Rotenone (ppm)		Folds of rotenone content increasing
	Water	Ethanol + Water	
Derris fresh root	65.79 ± 2.16	114.42 ± 2.84	1.74
Derris root powder	30.54 ± 1.60	211.94 ± 1.63	6.93
Derris root precipitate	92.43 ± 1.67	535.67 ± 5.24	5.79

Experiment 2 Preparing Derris Fresh Root Precipitate for Rotenone Water Extraction

Water extraction of the derris fresh root by 100 ml of water produced 0.76 $\pm$ 0.3 gm (7.6%) of dry precipitate with rotenone content of 34.93 $\pm$ 3.41 %. The clear supernatant revealed no rotenone content while 2.61 $\pm$ 0.38 % of the toxicants obtained from the derris fresh root residues (Figures 5.1 and 5.2). Since the centrifuge equipment applied in the current derris fresh root extraction has very high cost and inapplicable for small farmers. Hence, recommendation for collecting large amount of rotenone precipitate by water extraction associated with application of the larger centrifuge equipment for commercial scale product could be assured.

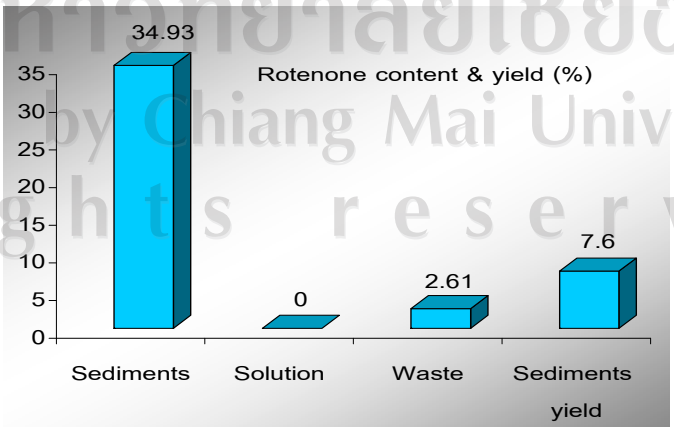

Figure 5.1 Percent rotenone content and weight of derris root precipitate from derris fresh root 10 gm/water 100 ml

Analyzation of the derris root precipitate by the GCMS demonstrated of 14 substances recorded in the precipitate; however, only 7 substances were determined as follow

1. Oxacyclopentadec-6-en-2-one 0.12%
2. Rotenone A 19.79%
3. Rotenone B 8.26%
4. 3-Alpha, 5-cyclo-ergosta-7,22-dien-6-one 19.12%
5. Rotenone C 21.98%
6. Rotenone D 0.99%
7. Rotenone E 1.31%

There are several chemical substances composed in the precipitate, especially rotenone and its derivatives. All of these substances can be categorized into 5 groups; some exhibited different molecular structure, while some demonstrated similar molecular structure with minor variations, therefore illustrated varying consequences. Pitiyon and Songwanit (1997) analyzed the derris root extract by the HPLC instruments noted that the identified substances recorded were rotenone, deguelin, sumatrol (tephrosin), elliptone, and melacol. In addition, the structures of these substances were similar to the chemical substance structures detected by the GCMS of the current study. Besides, Fang and Casida (1999) also reported that cubé resin (*Lonchocarpus utillis*) furnished at least 28 rotenoids, including deguelin 22%, retenolone 6.7%, tephrosin 4.3%, and 25 other substances less than 0.5%.

Figure 5.2 Water derris extract (A), ethanol + water derris extract (B), derris root precipitate (C)

Experiment 3 Relationship between Rotenone Concentrations Extracted from Derris Fresh Root and Light Absorbance

The rotenone concentrations detected by HPLC instruments from Derris fresh root solution samples with the concentrations of 100, 75, 50, 25, 12.5, and 6.25%, appeared to be 255.69, 187.95, 116.64, 51.56, 19.50, and 6.08 ppm, respectively (Table

5.2). The linear equation between light absorbance and rotenone concentration was y = 814.20(X) – 79.128 and the R^2 was 0.9893 [X = light absorbance, y = rotenone concentration (ppm)] (Figure 5.4). This equation was applied to estimate rotenone content in water extraction of derris fresh root by mean of the Spectrophotometer instruments, which demonstrated less time-consuming and less expensive than application of the HPLC instruments. Derris root solutions extracted by modified water extraction appeared dimly white in color due to the crystallized substances and suspended rotenone substances in the solution. These crystallized substances were occurred in various forms and sizes as illustrated in Figure 5.3

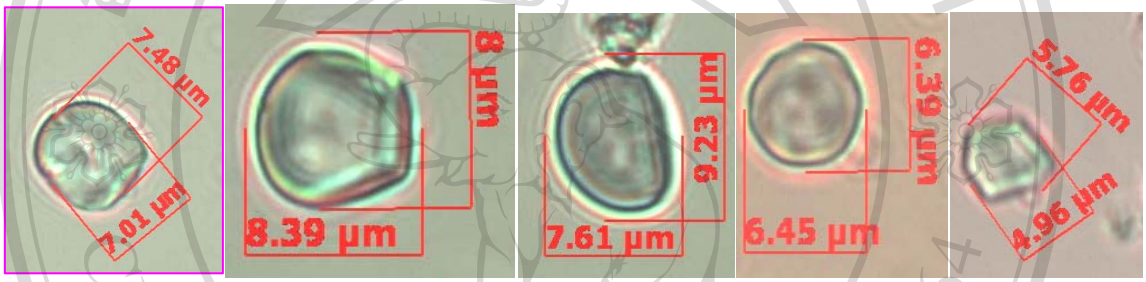

Figure 5.3 Derris crystals in forms and sizes detected in modified water extraction solutions

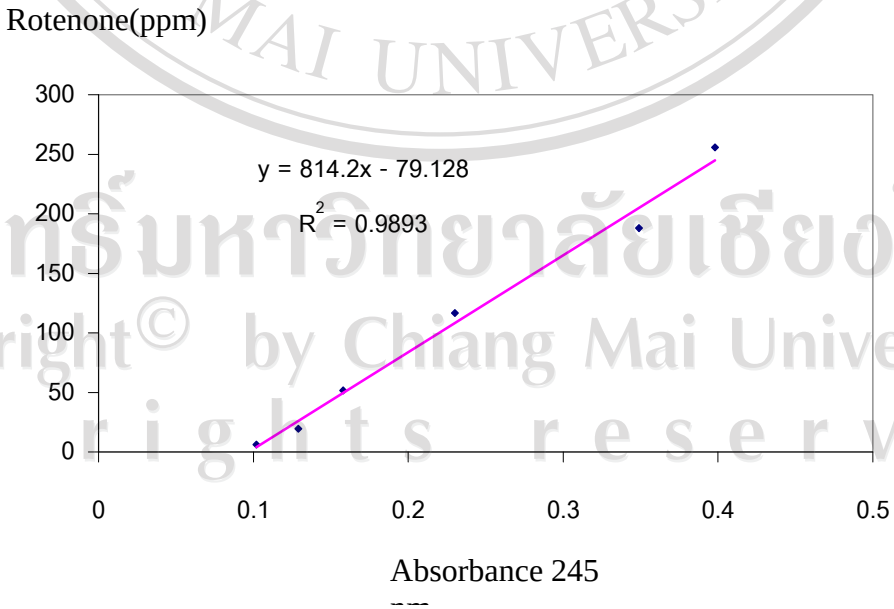

Figure 5.4 Linear regressions of rotenone concentration and absorbance for derris fresh root by modified water extraction

Table 5.2 Rotenone concentrations and absorbance determined by varying concentrations of derris fresh root solutions extracted with modified water extraction.

1gm/10ml Alc+90ml Water	Absorbance 245 nm	Rotenone (ppm)
100	0.398 ± 0.000	255.69 ± 18.25
75	0.349 ± 0.000	187.95 ± 10.06
50	0.230 ± 0.000	116.64 ± 5.36
25	0.158 ± 0.000	51.56 ± 3.67
12.5	0.129 ± 0.005	19.50 ± 0.25
6.25	0.102 ± 0.021	6.08 ± 0.58

Experiment 4 The Effect of Solvent and Evaporation on Rotenone Content in Crude Extraction from Derris Root Powder

The rotenone content extracted from derris root powder in non evaporate state with varying volumes of 5, 10, 15, and 20 ml of 95 % ethanol were 7.74, 6.89, 4.80, and 3.87 %, respectively. The rotenone content percentage demonstrated progressively decreased as ethanol volume in the solvent increased. The 5 ml of 95 % ethanol (200 gm/l of 95 % ethanol) provided the highest rotenone quantity of 7.74%. The rotenone contents detected from all treatments in evaporate state were not significantly different, the average rotenone content of 10% concentration (w/v) of crude extract was approximately 12 % (Table 5.3). Hence, varying volumes of 95% ethanol in the extraction process showed no effect to rotenone contents in the extracted solutions. Overall, the 5 ml of 95 % ethanol was convinced as the appropriate volume for modification of rotenone extraction. The obtained rotenone content still provided effective insect pest management and control with practicality and economy. On pilot plant level, Attanon (2006) reported the continuously extraction process produced the crude extract of 891.72 gms and rotenone content of 14.49% by triple extraction from 5 kgs of derris root powder with 60 liters of 95% ethanol.

Table 5.3 Rotenone content of evaporate and non evaporate of derris extracted by varying volume of 95% ethanol treatments.

Treatments	Rotenone (%)	
	non evaporate	Evaporate
Ethanol 5 ml	7.74	12.16
Ethanol 10 ml	6.89	12.11
Ethanol 15 ml	4.80	12.20
Ethanol 20 ml	3.87	12.64

5.4 Conclusion

Maceration the samples in 10 ml of 95% ethanol certainly ample dissolvability of the rotenone substances in the solutions, the means of rotenone content of the samples were enormously increased. Hence, the modified conventional water extraction method by adding 10 ml of 95% ethanol in the treatment combination could furnish better rotenone quantity. The Rotenone concentration (y) can be calculated from the value of light absorbance (X) using the linear equation of y = 814.20(X) – 79.128 and the R^2 was 0.9893. This equation was applied to estimate rotenone content in water extraction of derris fresh root by mean of the Spectrophotometer instruments, which demonstrated less time-consuming and less expensive than application of the HPLC instruments. There were 14 chemical substances occurred in derris root precipitate, however; only 7 compounds were verified. The rotenone quantities extracted from 1 gm of derris root powder in non evaporate state with varying volumes of 5, 10, 15, and 20 ml of 95 % ethanol were 7.74, 6.89, 4.80, and 3.87 %, respectively. The rotenone contents detected from all treatments in evaporate state were not significantly different, the average rotenone content of 10% concentration (w/v) of crude extract was approximately 12 %. The extraction of 1 gm derris root with 5 ml of 95 % ethanol (200 gm /1 liter of 95 % ethanol) was demonstrated to be the appropriate volume for ethanol supplement in modified water extraction of derris root powder.