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The Effects of Raw Material Particles Size, Types of Solvents and Solvent-to-Solid Ratio on the Yield of Rotenone Extracted from *Derris elliptica* Roots

(Kesan Saiz Partikel Bahan Mentah, Jenis Pelarut dan Nisbah Pelarut-kepada-Pepejal terhadap Keberhasilan Rotenon Diekstrak daripada akar *Derris elliptica*)

SAIFUL IRWAN ZUBAIRI*, MOHAMAD ROJI SARMIDI & RAMLAN ABDUL AZIZ

ABSTRACT

Currently, bio-pesticide is relatively harmless to human and environment and thus desirable for its use in the control of insect vectors. Bio-pesticide has been increasingly important 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. The study was carried out to investigate the appropriate processing parameters with the aimed to acquire high yield of rotenone (mg) and concentration (mg/mL) of the exhaustive extraction process by evaluating the kinetics of the normal soaking extraction (NSE) method. The raw plants were collected from Kota Johor Lama, Johor and sorted to collect the root. The roots were sorted into 2 types of raw material particles size (mm in diameter) prior to the extraction process. Rotenone from the root part was extracted by using a NSE method at ambient temperature of 27±1°C. Three types of solvents and 2 solvent-to-solid ratios were utilized throughout the extraction process. The extraction was carried out for 50 h and the fractions of the liquid crude extract were collected for each interval time (2 h/mL/fraction) and further cleaned up to remove any fine debris prior to the determination of rotenone content (mg) and its concentration (mg/mL) via reverse-phase high performance liquid chromatography (RP-HPLC). From the kinetics result obtained, it was found that the fine* Derris elliptica *roots with particles size of 0.5 - 2 mm in diameter and solvent-to-solid ratio of 10 mL/g of acetone solvent system were considered the best processing parameters to procure high yield of rotenone and its concentration.*

Keywords: Derris elliptica; *exhaustive extraction; liquid crude extract; normal soaking extraction; rotenone; yield*

ABSTRAK

Pada masa ini, secara relatifnya bio-pestisid tidak mendatangkan sebarang kesan berbahaya kepada manusia dan alam sekitar dan ia sesuai untuk kegunaan mengawal vektor serangga. Kepentingan bio-pestisid telah pun meningkat pada skala pertanian berkomersial dan kelompok kecilan serta kehidupan dalam pertanian sedia ada. Salah satu sumber untuk bio-pestisid adalah pokok tuba yang dikenali sebagai Derris elliptica. Derris elliptica *mengandungi bahan bio-aktif yang dikenali sebagai rotenone ($C_{23}H_{33}O_6$) dan ia tidak berbahaya kepada tumbuhan, sangat toksik kepada kebanyakan serangga dan secara relatifnya tidak berbahaya kepada mamalia. Kajian ini dijalankan untuk mengenal pasti parameter pemprosesan tertentu dengan objektif untuk mendapatkan keberhasilan rotenon dan kepekatan yang tinggi oleh pengekstrakan menyeluruh dengan menilai fasa kinetik kaedah pengekstrakan celuran norma (NSE). Sumber pokok mentah dikumpulkan dari Kota Johor Lama, Johor dan diasingkan untuk pengumpulan akar. Akar pokok diasingkan mengikut 2 jenis saiz partikel bahan mentah (diameter dalam mm) sebelum proses pengekstrakan. Rotenon daripada bahagian akar diekstrak menggunakan kaedah NSE pada suhu bilik iaitu 27±1°C. Tiga jenis pelarut dan 2 nisbah pelarut-kepada-pepejal digunakan sepanjang proses tersebut. Proses pengekstrakan dijalankan selama 50 jam dan fraksi daripada cecair ekstrak mentah dikumpulkan bagi setiap sela masa tertentu (2 jam/mL/fraksi) dan seterusnya ditapis untuk mengeluarkan sebarang serbuk halus sebelum analisis kandungan rotenon (mg) dan kepekatannya (mg/mL) dengan menggunakan fasa terbalik cecair kromatografi berprestasi tinggi (RP-HPLC). Daripada keputusan uji kaji kinetik, didapati bahawa akar* Derris elliptica *halus bersaiz 0.5 - 2 mm dalam diameter dan nisbah pelarut-kepada-pepejal 10 mL/g bagi sistem pelarut aseton boleh dianggap sebagai parameter pemprosesan yang terbaik untuk mendapatkan keberhasilan dan kepekatan bahan aktif rotenone yang tinggi.*

Kata kunci: Derris elliptica*; rotenone; pengekstrakan celuran norma; pengekstrakan menyeluruh; cecair ekstrak mentah; keberhasilan*

INTRODUCTION

Derris elliptica or 'Tuba' as it is locally known is an insecticidal plant in Malaysia that has been used for the purpose of bio-pesticide production. 'Tuba' plant is a kind of woody creeper plant and climber. It needs at least 75% soil moisture content and the surround temperature should be 25 to 30°C to obtain high content of the rotenone during its development. A calm area with low acidity soil content will enhance the production of rotenone (Grinda & Gueyne 1986). 'Tuba' is a member of the *Leguminosae*, Fabaceae family, which comprises 200 genera and 68 species including 21 species of *Tephrosia*, 12 of *Derris*, 12 of *Lonchocarpus*, 10 of *Millettia* and several of *Mundula* (John & Ron 1944). Three species were found in Malaysia, which were *Derris elliptica*, *Derris malaccensis* and *Derris uliginosa* (Gaby 1986; Zubairi et al. 2014a). *Derris* is a climbing plant of Southeast Asia and its roots contain rotenone (one of the secondary metabolites in isoflavonoids group), a strong insecticide that is commonly used by the aborigines/local peoples to kill insects that infested their vegetables and fruits (John & Ron 1944). *Derris elliptica* and *Derris malaccensis* contain 4 to 5% (w/w) rotenone while *Lonchocarpus utilis* and *Lonchocarpus urucu* contain 8 to 10% (w/w) rotenone in dry roots (Kole et al. 1992). The variation of bio-active constituents (specifically rotenone) is depending on how it is extracted and handled after the extraction process. In order to obtain high yield of bio-active compounds from herbal plant, the important processing parameters during the herbal extraction process (raw material particles size (mm in diameter), types of solvents and solvent-to-solid ratio (mL/g) need to be studied in details.

Herbal extraction processes are used to produce herbal extracts from the herbal raw material in several forms. These include the extracts which contain the soluble constituents, oleoresins which contain the volatile and non-volatile constituents and essential oils which only contain the volatile constituents from the plant material (Manuel & James 1985; Vickery & Vickery 1981). Herbal extract could be defined as a compound mixture obtained from the fresh or dried plant or parts of the plant such as leaves, flowers, seeds, roots and barks by different extraction procedures. Normally, the active constituents were obtained together with other materials present in the vegetal mass such as resins, fats, waxes, chlorophyll and colouring materials (Zubairi et al. 2014b, 2014c). Moreover, the extraction of bio-active components from the vegetal materials is an essential part of the nutraceuticals, pharmaceuticals, cosmeceuticals and phytochemical bio-pesticide industry (Mircea 2001; Pinelo et al. 2006; Rice 1995). Therefore, the key objective of this study was to determine the appropriate processing parameters with the aimed to produce high yield of rotenone (mg) and concentration (mg/mL). Therefore, it is important to understand the background of the herbal extraction processes and to discover the correlation between the operating conditions and the yield of the active ingredient obtained.

MATERIALS AND METHODS

PLANT COLLECTION

Derris elliptica was collected in the state of Johor (Kota Johor Lama), Malaysia.

RAW MATERIAL PARTICLE SIZE SEPARATION AND EXTRACTION PROCESS

The roots of *Derris elliptica* plant were dried at room temperature (27±1°C) and subsequently ground with a knife mill. The ground dried roots were separated into 2 types of raw particles size: (a) coarse (2 - 5 mm in diameter) and (b) fine (0.5 - 2 mm in diameter) by using mesh separator machine (Zubairi et al. 2014a). The normal soaking extraction (NSE) was carried out in 3 different types of solvents (acetone, chloroform and ethanol + oxalic acid + H_2O) by soaking 30 g of dried fine/coarse roots in a different volume of extraction (in accordance to 2 different sets of solvent-to-solid ratio: 3.3 mL/g and 10 mL/g) for 50 h at 27±1°C (Tables 1, 2 and 3). The fractions of rotenone liquid crude extract (LCE) were collected intervally (2 h min/mL/fraction) using micropipette. The sample were further cleaned up to remove any fine debris of dried root through an organic sample clarification kit (Waters™ Assoc.) containing 0.45/0.5 µm polytetrafluoroethylene (PTFE) filter and directly into 5 mL of dark vial prior to the determination of rotenone extraction yield, mass content and concentration by means of reverse-phase high performance liquid chromatography (RP-HPLC).

TABLE 1. Independent processing parameters involved

Factor names	Factor levels
[a]Types of solvent	Chloroform, ethanol + oxalic acid and acetone
[b]Solvent-to-solid ratio	10 mL/g and 3.3 mL/g
[c]Raw material particles size	Fine and coarse particles size (mm in diameter)
Extraction duration	50 h (sampling every 2 h)

[a]Purity of the solvents were 95% (v/v).
[b]The solvent-to-solid ratio of 3.3 mL/g and 10 mL/g were selected to evaluate the significant effect on the response variables as compared to the ratio of 5.5 mL/g used by Grinda & Gueyne (1986) which produced high yield of rotenone -14% (w/w).
[c]Source: Pagan & Hageman 1949: (a) Fine; 0.5 to 2 mm in diameter and (b) Coarse; 2 to 5 mm in diameter

TABLE 2. Control independent parameters

Factor names	Factor levels
Weight of raw material	30 g of dried roots
Extraction temperature	Ambient (27±1°C)

TABLE 3. Experimental design of studying the effects on different processing parameters on the kinetics of the normal soaking extraction of rotenone from *Derris* dried roots

Solvent/particles size/solvent-to-solid ratio	3.3 mL/g		10 mL/g	
	Coarse	Fine	Coarse	Fine
[a]Acetone	A1	A2	A3	A4
[a]Ethanol + oxalic acid + H_2O	B1	B2	B3	B4
[a]Chloroform	C1	C2	C3	C4

[a]Purity of the solvents were 95% (v/v)

ANALYSIS OF LIQUID CRUDE EXTRACT

The filtered liquid crude extract was subjected to a quantitative analysis by using a reverse-phase high performance liquid chromatography (RP-HPLC) with UV (Photodiode Array - PDA) detection at 294 nm. The analysis of the extract solutions were 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 particles size of 10 µm (3.9 mm I.D × 150 mm lengths) was utilized. 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 amplitude unit full scale (AUFS) of 2.

RESULTS & DISCUSSION

Figures 1, 2 and 3 show the kinetics of the normal soaking extraction of rotenone from *Derris elliptica* roots using ethanol + oxalic acid solution, chloroform and acetone, respectively. All figures show a profile of the effect on the raw material particles size (mm in diameter) and solvent-to-solid ratio (mL/g) with respect to the concentration and yield of rotenone. The yield of rotenone in dried roots, % (w/w) and rotenone concentration, mg/mL was observed for 2 h intervally using an external standard method of reversed-phase high performance liquid chromatography (RP-HPLC).

Figure 1(a) indicates that by using the ethanol + oxalic acid solution with a solvent-to-solid ratio of 3.3 mL/g and fine particles size, the highest concentration of rotenone was obtained at 3.81 mg/mL after 50 h of the extraction process (B2). A highly exposed surface area of fine particles size was expected to give the highest concentration of rotenone. However, the small amount of rotenone (Figure 1(b)) in the liquid crude extract (LCE) was due to the rapid solvent evaporation which affected diffusivity of the active ingredient (rotenone) as more solvent is needed to penetrate deep inside the vegetal cell structures and to extract the bio-active constituents (Mircea 2001). The optimum time of extraction and the highest yield of rotenone were observed to be around 18 to 20 h and 0.8 to 0.9% (w/w), respectively (B3) (Figure 1(b)) (Table 4). The orders of the highest to the lowest rotenone concentration (mg/mL) produced using ethanol + oxalic acid as a solvent are as follows: B2 (fine particles size, 3.3 mL/g) > B3 (coarse particles size, 10 mL/g) > B1 (coarse particles size, 3.3 mL/g) > B4 (fine particles size, 10 mL/g). The orders of the highest to the lowest rotenone yield in dried roots are as follows: B3 (coarse particles size, 10 mL/g) > B4 (fine particles size, 10 mL/g) > B2 (fine particles size, 3.3 mL/g) > B1 (coarse particles size, 3.3 mL/g).

Meanwhile, Figure 2(a) indicates that by using chloroform with a solvent-to-solid ratio of 3.3 mL/g and coarse particles size, the highest concentration of rotenone was obtained at 0.74 mg/mL after 50 h of the extraction process (C1). On the contrary, the fine particles size of 10 mL/g solvent-to-solid ratio produced the highest amount of rotenone (C4) (Figure 2(b)). The optimum time of extraction and the highest yield of rotenone were observed to be around 8 to 10 h and 0.15 to 0.16% (w/w), respectively (C4) (Figure 2(b)) (Table 4). The orders of the highest to the lowest rotenone concentration (mg/mL) produced using chloroform as a solvent are as follows: C1 (coarse particles size, 3.3 mL/g) > C2 (fine particles size, 3.3 mL/g) > C4 (fine particles size, 10 mL/g) > C3 (coarse particles size, 10 mL/g). The orders of the highest to the lowest rotenone yield in dried roots are as follows: C4 (fine particles size, 10 mL/g) > C3 (coarse particles size, 10 mL/g) > C1 (coarse particles size, 3.3 mL/g) > C2 (fine particles size, 3.3 mL/g).

Finally, Figure 3(a) signifies that the usage of acetone with a solvent-to-solid ratio of 3.3 mL/g and fine particles size procured the highest concentration of rotenone of 8.30 mg/mL after 50 h of the extraction process (A2). However, the finding was contrary with the yield of rotenone result wherein the fine particles size with the solvent-to-solid

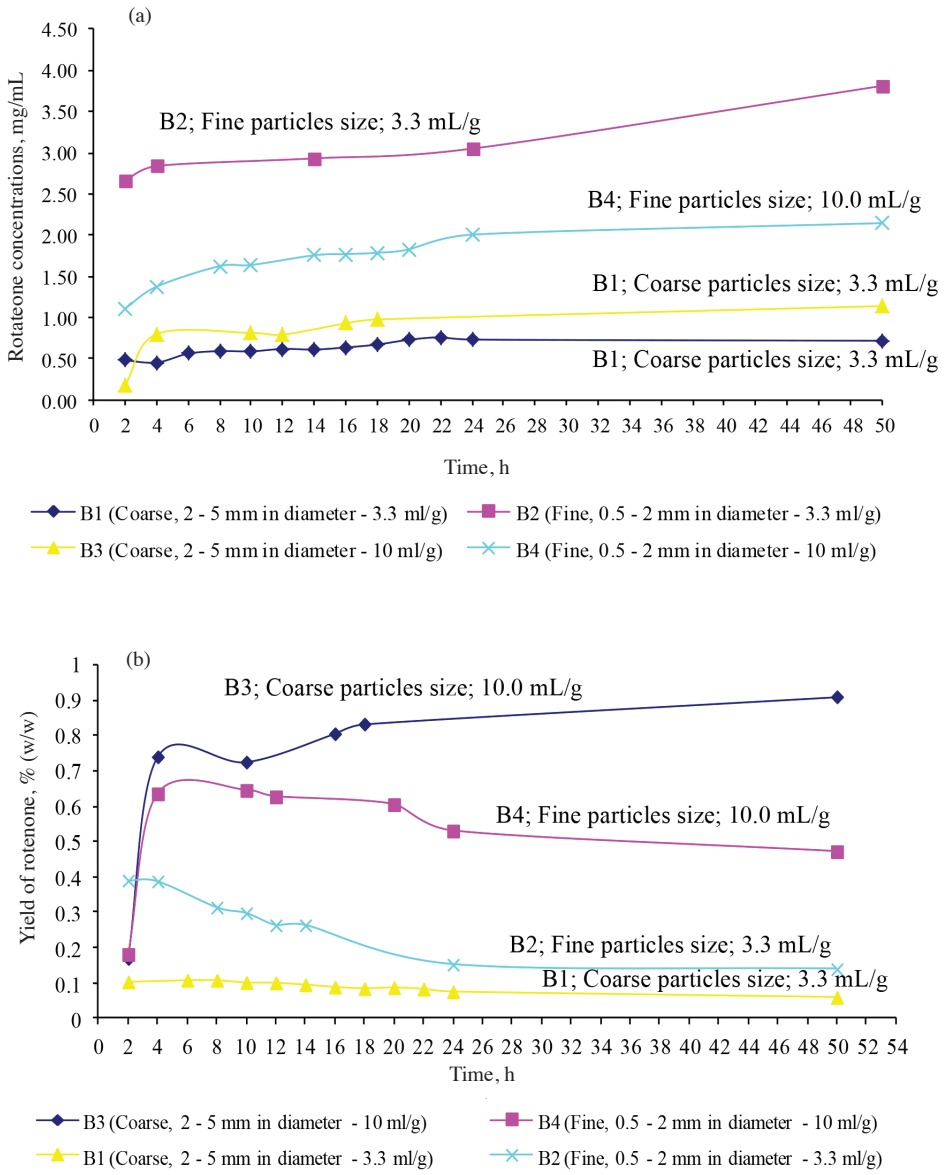

FIGURE 1. Kinetics of rotenone extraction process using ethanol + oxalic acid (a) Rotenone concentration, mg/mL and (b) yield of rotenone, % (w/w)

TABLE 4. The final yield of rotenone (% w/w) and optimal time of extraction for each type of extract used

Solvent/particles size/solvent-to-solid ratio	[b]Final yield (% w/w)				Optimal time (h)			
	3.3 mL/g		10 mL/g		3.3 mL/g		10 mL/g	
	C	F	C	F	C	F	C	F
[a]Acetone	0.08	0.19	0.84	[c]1.22	4	12	50	12-14
[a]Ethanol + oxalic acid + H_2O	0.11	0.21	[c]0.92	0.55	10-12	2-4	18-20	7-9
[a]Chloroform	0.07	0.01	0.06	[c]0.16	10	2	4-5	8-10

[a]Purity of the solvents were 95% (v/v), [b]Final yield after 50 h of extraction, [b]The best yield of rotenone in each respective solvent used. C = Coarse; F = Fine

ratio of 10 mL/g (A4) produced the highest amount of rotenone due to the large volume of solvent used (Table 4). The orders of the highest rotenone concentration (mg/mL) to the lowest produced using acetone as a solvent are as follows: A2 (fine particles size, 3.3 mL/g) > A4 (fine particles size, 10 mL/g) > A3 (coarse particles size, 10 mL/g) > A1 (coarse particles size, 3.3 mL/g). The orders of the highest of rotenone yield in dried roots to the lowest are

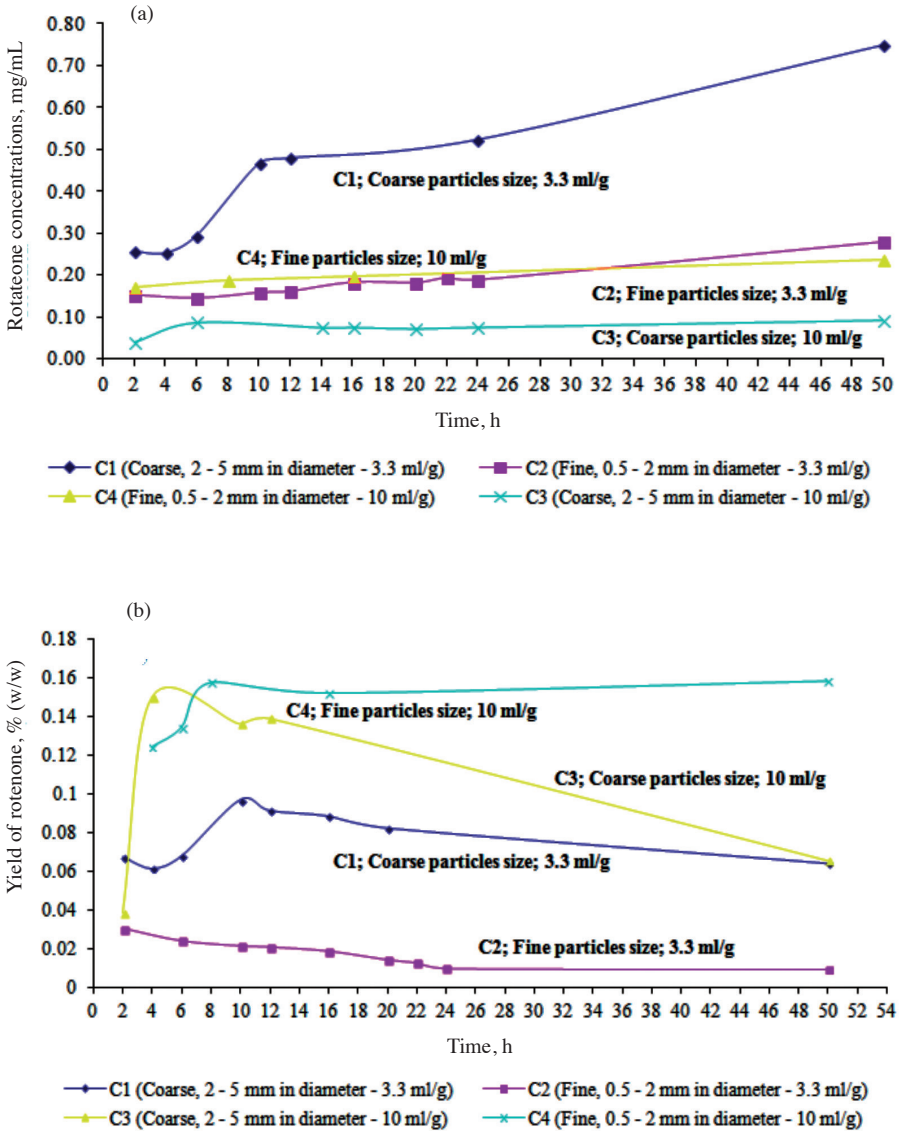

FIGURE 2. Kinetics of rotenone extraction process using chloroform. (a) Rotenone concentration, mg/ml; (b) yield of rotenone, % (w/w)

as follows: A4 (fine particles size, 10 mL/g) > A3 (coarse particles size, 10 mL/g) > A2 (fine particles size, 3.3 mL/g) > A1 (coarse particles, 3.3 mL/g).

All results shown in Figures 1, 2 and 3 indicate that the raw material particle size (mm in diameter) affects the extraction rate by increasing the total mass transfer area when the particle size is reduced (Schwartzberg & Chao 1982). Theoretically, it was expected that the fine raw material particles size produced the highest yield of rotenoids resin as well as the amount of rotenone. The results showed that 2 out of 3 different types of solvent were used, the highest yield of rotenone was obtained from fine particles size (0.5 - 2 mm in diameter) at 10 mL/g solvent-to-solid ratio. For that reason, the fine *Derris* roots with particles size of 0.5 - 2.0 mm in diameter was considered the best raw material size to extract high amount of rotenone.

For type of solvent used, the best parameters of acetone extract (10 m/g solvent-to-solid ratio, fine roots) show a dramatic increase of 26 - 91% with respect to the other solvents of different solvent-to-solid ratios and raw material particle size (Table 4). It has been expected from the previous study reported by Grinda and Gueyne (1986) in which acetone was considered as the best solvent to separate rotenone and other essential constituents from rotenoids resin efficiently. Meanwhile, a prolong extraction time was considered to affect the yield of rotenone. A sudden reduction of the yield on certain parameters combination was observed. Rapid solvent evaporation due to the inappropriate closed system extraction setting might have reduced the solvent volume and diffusivity of rotenone from vegetal cell structures into bulk volume.

Finally, there was an increased profile of the yield of rotenone in dried roots; % (w/w) with the increased of the solvent-to-solid ratio. The acetone, ethanol + oxalic acid solution and chloroform extract were observed to have an increase in the amount of rotenone as the solvent-to-

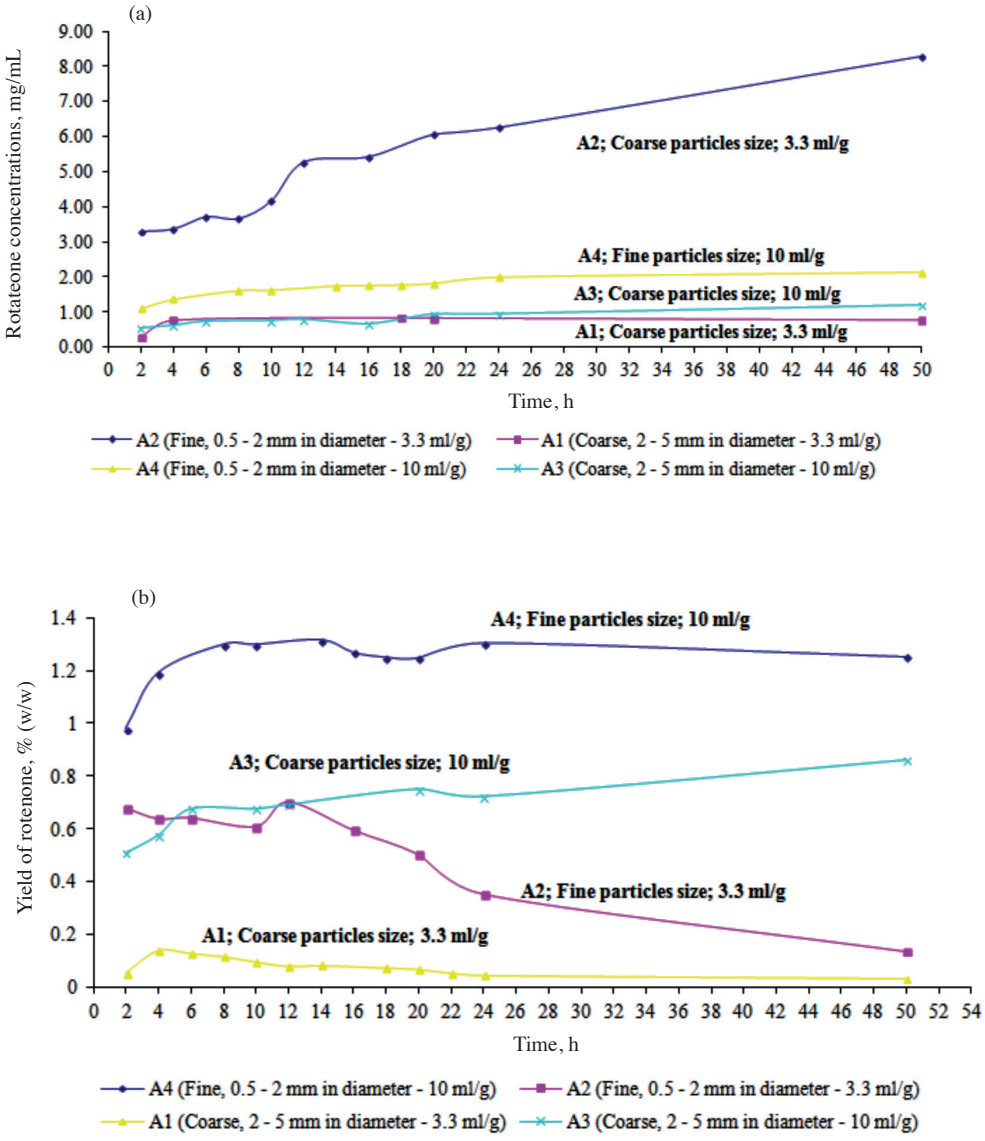

FIGURE 3. Kinetics of rotenone extraction process using acetone. (a) Rotenone concentration, mg/mL and (b) yield of rotenone, % (w/w)

solid ratio increased. The increase of rotenone yield with respect to a different solvent-to-solid ratio (mL/g) is in accordance to the mass transfer principles (Cacace & Mazza 2003). It has been observed that the driving force during mass transfer within the solid is considered to be the concentration gradient, which is greater when the high solvent-to-solid ratio are used, resulting in an increase of the diffusion rate. However, the solvent-to-solid ratio (mL/g) did not affect diffusivity at the point wherein the extractions are stopped when the equilibrium is reached. Therefore, it is recommended to modify the solubility and solute-solvent interactions by means of ultrasonic, agitation and stirring to give a significant increase in the amount of the extracted bio-active ingredient. On top of that, the bio-active compound's solubility is affected by changes in the activity coefficient, which varies with the temperature and composition of the solution (Frank et al. 1999). Interactions of the compounds with the solvent could have modified the activity coefficient and thus the solubility of the bio-active compounds.

CONCLUSION

The results suggested that acetone is the best solvent system to exhibit high yield of rotenone in dried roots with a solvent-to-solid ratio of 10 mL/g utilized. Given that the accumulation of rotenone in *Derris* roots are largely existed in the cell tissues (in the form of milky sap) of dried roots, the used of 0.5 - 2 mm in diameter of dried roots particle size have accommodated large surface area for an efficient mass transfer into bulk volume and increased bio-active constituents solubility throughout the extraction process.

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Saiful Irwan Zubairi*
School of Chemical Sciences & Food Technology
Faculty of Science & Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor D.E.
Malaysia

Mohamad Roji Sarmidi & Ramlan Abdul Aziz
Institute of Bioproduct Development
Universiti Teknologi Malaysia
81310 UTM Skudai, Johor D.T.
Malaysia

*Corresponding author; email: saiful-z@ukm.edu.my

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