Imidazolium-Based Ionic Liquid Binary Solvent System as an Extraction Medium in Enhancing the Rotenone Yield Extracted from *Derris elliptica* Roots

Zetty Shafiqa Othman, Nur Hasyareeda Hassan and Saiful Irwan Zubairi

Additional information is available at the end of the chapter

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**Abstract**

Rotenone is a biopesticide which can be isolated from *Derris* species roots. However, procuring significant amount of rotenone using green alternative solvent rather than harmful organic solvents for commercialization is a challenge to be faced. Therefore, an approach using imidazolium-based ionic liquids (ILs) as an extraction medium was employed in this study. Five different types of binary solvent systems comprising a combination of acetone and five respective ionic liquids (ILs) of (1) [BMIM] Cl; (2) [BMIM] OAc; (3) [BMIM] NTf2; (4) [BMIM] OTf; and (5) [BMPy] Cl were used in the normal soaking extraction (NSE) of rotenone for a 24-hour extraction. The yield of the rotenone, % (w/w), and its concentration (mg/mL) in the dried roots was quantitatively determined by means of the reversed-phase high-performance liquid chromatography (RP-HPLC) and thin-layer chromatography (TLC). The results showed that a binary solvent system of [BMIM] OTf:acetone was the best solvent system combination compared to other solvent systems \((p < 0.05)\). It contributed to the highest rotenone content of \(2.69 \pm 0.21\% \) (w/w) \((4.04 \pm 0.34 \) mg/ml) at the 14th hour of the exhaustive extraction time. In conclusion, a combination of certain ILs with a selective organic solvent has been proven to be able to increase a significant amount of bioactive constituents in the phytochemical extraction process.

**Keywords:** rotenone, *Derris* sp., binary solvent system, imidazolium-based, ionic liquids

**1. Introduction**

The destructive effects of numerous pests from the time immemorial led to a large decline in crop yield. Through the advent of chemical pesticides, this crisis was resolved to a great extent.
However, an overdose, overdependence on and uncontrolled usage of synthetic pesticides eventually created pest resistance which simultaneously led to frequent applications, application of bulk quantity of pesticide and a high cost [1]. In addition, the violative pesticides’ residues had contributed to food safety concern among consumers. Therefore, an eco-friendly alternative is needed to overcome the drawbacks of synthetic pesticides. As agricultural-industrial tools, biopesticides demonstrate exemplary benefits over chemically synthesized pesticides through harnessing the natural capabilities of organisms and their molecular constituents in minimizing the crop and plant damages from pests, affording the opportunity for protection, maintenance of biodiversity, and commerce-strengthening alternatives for organic farming and safe guarding of human health. Rotenone is one of the biopesticides that can be extracted from *Derris* and *Lonchocarpus* plants’ roots [2]. It exhibits a strong pesticidal activity due to its strong paralysis action (knock-down effect) on cold-blooded animals. Besides, its high degradability, exceptionally selective and poor absorption across the gut and skin of humans enhances its eco-friendly usage [3–7]. In accordance with that, the extraction process plays a major role in optimizing the extraction of the yield of rotenone compound. Conventionally, organic solvents were used as an extraction medium and the selection of solvent systems largely depended on the specific nature of the bioactive compound from natural products. Rotenone is an isoflavonoid and it does not dissolve in water but it dissolves in organic solvents. According to John and Ron [4], the solubility of pure rotenone in acetone is 0.066 g/ml, ethanol, 0.002 g/ml and chloroform, 0.47 g/ml. However, a research completed by Zubairi [8] showed that acetone extracted more rotenone and other bioactive constituents compared to a high-polarity solvent as it could extract rotenone from from 39.5% up to 72.8% compared to chloroform and ethanol.

Although conventional organic solvents have been used for so long as the extraction medium, their drawbacks such as volatility, toxicity, and flammability that lead to several human risks and environmental problems, limit its usage as an extractant. Taking all these into account, there have been several studies conducted on the exploration of ionic liquids (ILs) compatibility as green solvents for plant extraction. According to Fu et al. [9], ILs can be used as an alternative green solvent to replace volatile organic solvents. ILs are organic salts in the liquid state under ambient temperature that comprise a normally charge-stabilized organic cation paired with an organic or inorganic anion. They display a wide range of unique properties such as high thermal stability, nonflammability, insignificant vapor pressure, and low chemical reactivity. In addition to that, they also have fine tunable density, viscosity, polarity, and miscibility with other common solvents through the change of the cation and anion [10]. Some of ILs are also immiscible with organic solvents which define their polar alternative with nonaqueous nature for two-phase systems and ILs can be recycled that this enhances their green properties. The viscosity of ILs also plays a crucial role in the extraction and separation of bioactive compounds from plants. Their viscosity is affected by a range of intermolecular interactions such as electrostatic, van der Waals forces, and hydrogen bonding interaction [11]. The increase in temperature and asymmetry of ILs’ anions lead to the decrease in their viscosity. For instance, using the imidazolium cationic species, the viscosity can be intensified by increasing the substituted alkyl chain length or branching due to more van der Waals interactions between the ions themselves [11–14]. Some ILs also are immiscible in water (formation of
biphasic systems) and the organic species have a high solubility in these ILs, making them ideal solvents for bioactive compound extraction from plants and as mobile phase modifiers to improve liquid chromatography separation of bioactive targets. The usage of ILs as plant bioactive constituent extractants has a great impact and potential as they alleviate the environmental pollution and improve the selectivity and extraction yields of interesting compounds in sample pretreatment process compared to conventional organic solvents.

However, due to their charged and asymmetric structures, ILs have a relatively high polarity [15], as they do not have a good affinity with weak-polar compounds, thus causing a reduction in the distribution of weak-polar compounds in the IL phase. The viscosity of ILs increases with longer alkyl chain of ILs in accordance with the strong electrostatic and hydrogen bonding interaction between ions [16, 17]. The high viscosity of ILs will hinder the mixing and transferring of properties in the extraction process by influencing the dissolution of the compounds in ILs. In exchange, the mixture of ILs and polar molecular solvents as an extraction medium could be an effective approach to solve their flaws. Besides creating a wide-adjusted range of solvent polarity, hydrophobicity, hydrogen-bond acidity, and basicity [18, 19], the addition of miscible molecular solvents as co-solvents helps to break the microscopic hydrogen-bond network and the aggregation of ILs, which significantly reduces the viscosity and improves the mixing and transferring process in their mediated extraction [20, 21]. The rotenone compound is an acidic isoflavonoid compound that consists of ketonic chemical groups (R–C(=O)−R) [22] which has the potential of interacting with intermediate-polar solvents. It can be easily dissolved in moderate-polar organic solvents (e.g., methanol, chloroform, and acetone) [23] and is sparingly soluble in water [24]. For that reason, a combination of any ILs with a moderate polarity of organic solvents would perhaps increase the chances of extracting a high rotenone content due to its low viscosity and mediate polarity property and the high tendency of interaction between rotenone compound with the anion and cation of ILs. The previous study indicated that solubility of flavonoids and their derivatives can be increased by using ILs as the of flavonoids are greatly anion-dependent [25]. The anionic potency of both organic solvents and ILs in extracting a large amount of bioactive compounds (e.g., rotenone) and moving into solvent systems is significantly undeniable as both chemicals facilitate the extraction process via salvation power and multiple interactions (e.g., hydrogen bonding, polarity, ionic/charge-to-charge, and π-π, π-σ) with the analytes [26, 27].

2. Imidazolium-based ionic liquids as a green extraction medium

2.1. Structural features of ionic liquids

Ionic liquids (ILs) are organic salts in the liquid state under ambient temperature that comprise a normally charged stabilized organic cation paired with an organic or inorganic anion. The widely used cations (Figure 1) are ammonium, sulfonium, imidazolium, pyridinium, pyrrolidinium, tetraalkylammonium, phosphonium, picolinium, and the functionalized cations with different substitutions [28, 29]. On the other hand, anions are weakly basic inorganic or organic compounds that have a diffuse or protected negative charge [28]. ILs based on halides such as [BF₄]⁻, or [PF₆]⁻ ions are not preferred due to their
unfavorable properties and they are also strongly hygroscopic [26]. The most preferred anions are the ones which are more complex, perfluorated anions such as bis(trifluoromethane sulfonyl) amide or trifluoromethanesulfonate or halogen-free ions such as dicyandiamide, tosylate, or n-alkyl sulfates (Figure 1) [30]. The environment constituted by ionic liquids is completely different from that of polar and nonpolar molecular solvents. In addition to the existing interactions in conventional organic solvents such as hydrogen bonding, dipole-dipole, and van der Waals interactions, ionic liquids have strong electrostatic interactions.

2.2. Imidazolium-based ionic liquid characterization

Among all ionic liquids, the ionic liquid based on imidazolium cation is widely used and studied due to the stability of the imidazolium ring and its excellent liquescency [31] which is resulted from its electronic structure of the aromatic cation. With delocalized 3-centre-4-electron configuration across the N₁-C₂-N₃ moiety, a double bond between C₄ and C₅ at the opposite side of the ring, and a weak delocalization in the central region (Figure 2) [32], the hydrogen atoms C₂-H, C₄-H and C₅-H carry almost the same charge but carbon C₄ is positively charged owing to the electron deficit in the C=N bond, whereas C₄ and C₅ are practically neutral. The resulting acidic proton or hydrogen on the C₂ carbon is the key for understanding the properties of the ionic liquids (ILs) and it is presented that the hydrogen on the C₂ carbon (C₂-H) binds specifically with solute molecules [33, 34] or its counter ion [35] as a good hydrogen bond donor.

![Figure 1. Some typical cations and anions.](image1.png)

![Figure 2. Electronic structure of 1,3-dialkylimidazolium cation.](image2.png)
2.3. Structural organization of imidazolium-based ionic liquids

Long-range coulomb interaction may play a major role in ionic liquids which are composed solely of ions by creating the structure and dynamics that are unique to ionic liquids without being associated with molecular liquids [36]. From macroscopic point of view, ionic liquids can be considered as a continuum system characterized by their macroscopic constants such as boiling point, vapor pressure, density, and surface tension. However, from microscopic point of view, they are a discontinuum system consisting of individual, mutually interacting molecules characterized by molecular properties such as dipole moment, electronic polarizability, hydrogen-bond donor (HBD), and hydrogen bond acceptor (HBA) capability, electron pair donor (EPD) and electron pair acceptor (EPA). The types and degrees of these interactions control and determine the macroscopic properties of ionic liquids and their possibilities for various applications. Specifically, the ionic liquids’ structure exhibits a unique spatial heterogeneity due to their inherent polar/nonpolar phase separation. The underlying reason for the microphase segregation resulted from the interplay between electrostatic interaction (between polar imidazolium ring and anion) and van der Waals interaction with the nonpolar alkyl tails of the cation [37]. In fact, charge-charge distribution and anion size affect the nanostructural segregation of ionic liquids. As the ions’ size increases, the charge becomes more delocalized and the cation-anion interaction is reduced resulting in less charge ordering and nanostructural segregation [38]. On the other hand, divalent anions such as sulfate (SO₄²⁻), thiosulfate (S₂O₃²⁻), chromate (CrO₄²⁻), dichromate (Cr₂O₇²⁻), carbonate (CO₃²⁻), and oxalate (C₂O₄²⁻) increase the electrostatic interaction between cation and anion and enhance intermolecular structuring. Besides, it was observed that the size of structural heterogeneities depended on alkyl chain length [37, 39].

2.4. Physicochemical properties of imidazolium-based ionic liquids

Ionic liquids are constituted exclusively by ions and hence they experience a strong interionic interaction that yield a long-lived association of ions [40]. The nature and types of cation-anion interactions and intermolecular forces in bulk ionic liquids affect their physical and chemical properties and how they interact with other solutes [41]. The examples of conformational heterogeneity of cations and anions are the coexisting trans-trans and trans-gauche conformations of n-butyl chain in 1-butyl-3-methylimidazolium cation and bisimide [Tf₂N] anion conformational which forms trans- and cis conformers and this seems to be crucial in lowering their ionic liquid melting point [42]. Ionic liquids which comprise a low symmetry cation possess a low melting point than the one with a higher symmetry due to weak intermolecular interactions and good distribution of charge in the cation.

Generally, ionic liquids (ILs) are denser than water. The density (ρ) of ionic liquids (ILs) decreases with the increase in organic cation bulkiness and anion selection affects ILs’ density. Normally, the density of ILs varies in the range of 1.05–1.36 g/cm³ at ambient temperature [43, 44]. In terms of ILs’ thermal stability, a research conducted had observed that thermal stability was dependent on both the cation and the anion of ILs. Ngo et al. [45] reported that imidazolium-based cations exhibited a higher thermal stability than tetraalkylammonium cations based on thermal gravimetric analysis (TGA) and differential scanning calorimetry.
(DSC). In addition, imidazolium-based ILs have a thermal stability that increases in the following order: $[\text{Cl}]^-$, $[\text{Br}]^-$, $[\text{I}]^-$ $< [\text{BF}_4^-] < [\text{CF}_3\text{SO}_3^-] < [\text{NTf}_2^-] < [\text{PF}_6^-]$ and Ngo et al. [45] also reported that IL-based organic anions have a higher thermal stability than that of those based on inorganic anions.

The viscosity of ILs also plays a crucial role in the extraction and separation of bioactive compounds from plants. Their viscosity is affected by a range of intermolecular interactions such as electrostatic, van der Waals forces, and hydrogen bonding interaction [11]. The increase in temperature and asymmetry of IL anions leads to the decrease in their viscosity. For instance, using imidazolium cationic species, the viscosity can be increased by increasing the substituted alkyl chain length or branching due to higher van der Waals interactions between the ions themselves [12–14]. Ionic liquids also have their own polarity values according to Kamlet-Taft parameters such as dipolarity or polarizability ($\pi^*$), hydrogen bond basicity ($\beta$), and hydrogen bond acidity ($\alpha$) [46]. Although measurement has not been made for a large number of ionic liquids, the general trend suggests that the $\pi^*$ values for ionic liquids are higher than that of alkyl chain alcohol, while the $\alpha$ values are less than those of water and alkyl chain alcohols and also the magnitude of $\beta$ is determined by the anion of ionic liquids. Tables 1 and 2 display the summary of some physicochemical properties of ILs and Kamlet-Taft parameters for some ionic liquids.

### Tables 1 and 2

| Cation | Anion       | Density (g/ml) | Viscosity (cP) |
|--------|-------------|----------------|----------------|
| [EMIM]$^+$ | [BF$_4$]^- | 1.248          | 66             |
|        | [PF$_6$]^- | 1.373          | 450            |
| [BMIM]$^+$ | [BF$_4$]^- | 1.208          | 233            |
|        | [PF$_6$]^- | 1.373          | 400            |
|        | [Br]^-      | 1.134          | Solid          |
|        | [Cl]^-      | 1.12           | Solid          |
|        | [CF$_3$SO$_3$]^- | 1.29   | 90             |
|        | [(CF$_3$SO$_3$)$_2$N]^- | 1.42   | 52             |
|        | [NTf$_2$]^- | 1.404          | 48             |
| [AMIM]$^+$ | [BF$_4$]^- | 1.213          | 321            |
| [HMIM]$^+$ | [BF$_4$]^- | 1.075          | 211            |
|        | [PF$_6$]^- | 1.304          | 800            |
| [OMIM]$^+$ | [BF$_4$]^- | 1.11           | 440            |
|        | [Cl]^-      | 1              | 16,000         |
| [MPPyr]$^+$ | [NTf$_2$]^- | 1.44           | 39             |
| [C$_2$H$_5$NH$_3$]$^+$ | [HCOO]^- | 0.99            | 11.5           |
| [BMPyrrol]$^+$ | [NTf$_2$]^- | 1.4             | 71             |

**Table 1.** Summary of some physicochemical properties of ILs at 25°C.
| ILs                                    | α     | β     | π*    |
|----------------------------------------|-------|-------|-------|
| [OMIM][BF₄] [49]                       | 0.62  | 0.41  | 0.98  |
| [OMIM][NTf₂]                           | 0.60  | 0.29  | 0.96  |
| [HMIM][NTf₂] [48]                      | 0.65  | 0.26  | 0.97  |
| [HMIM][Cl] [49]                        | 0.48  | 0.94  | 1.02  |
| [HMIM][Br] [49]                        | 0.45  | 0.74  | 1.09  |
| [BMIM][BF₄] [49]                       | 0.77  | 0.39  | 1.04  |
|                                        | 0.63  | 0.37  | 1.05  |
| [BMIM][NTf₂]                           | 0.72  | 0.24  | 0.90 [47] |
|                                        | 0.64  | 0.25  | 0.97 [48] |
|                                        | 0.61  | 0.23  | 0.99  |
| [BMIM][C₆]OSO₃ [49]                    | 0.69  | 0.79  | 0.89  |
| [BMIM][PF₆]                            | 0.68  | 0.21  | 1.02 [49] |
|                                        | 0.65  | 0.25  | 1.02  |
|                                        | 0.63  | 0.24  | 1.02 [49] |
|                                        | 0.63  | 0.19  | 1.04  |
| [BMIM][SBF₆]                           | 0.62  | 0.15  | 1.04  |
| [BMIM][OTf]                            | 0.62  | 0.49  | 1.00  |
| [BMIM][MeCO₂]                          | 0.57  | 1.18  | 0.89 [50] |
|                                        | 0.43  | 1.05  | 1.04 [51] |
|                                        | 0.48  | 1.20  | 0.96  |
| [BMIM][N(CN)₂]                         | 0.54  | 0.60  | 1.05  |
| [BMIM][MeSO₄]                          | 0.53  | 0.66  | 1.06  |
| [BMIM][PrCO₂] [51]                     | 0.51  | 1.23  | 0.92  |
| [BMIM][HO]C₅CO₂] [51]                  | 0.44  | 0.87  | 1.12  |
| [BMIM][EtCO₂] [51]                     | 0.48  | 1.16  | 0.94  |
| [BMIM][Me₃PO₄]                         | 0.45  | 1.13  | 0.98  |
| [BMIM][MeSO₃]                          | 0.44  | 0.77  | 1.02  |
| [BMIM][O₂CCH₂CH(OH)CO₂] [51]           | 0.41  | 1.00  | 1.10  |
| [BMIM][O₂CCH₂CH₂CO₂] [51]              | 0.39  | 1.08  | 1.09  |
| [BMIM][O₂CCCHCHCO₂] [51]               | 0.34  | 1.02  | 1.11  |
| [BM2IM][BF₄]                           | 0.39  | 0.36  | 1.08  |
| [BM2IM][NTf₂]                          | 0.38  | 0.26  | 1.02  |
| [EMIM][NTf₂]                           | 0.71  | 0.23  | 0.98 [48] |
|                                        | 0.42  | 0.10  | 1.02 [47] |
|                                        | 0.63  | 0.23  | 1.00  |
| [EMIM][C₆]SO₄                           | 0.65  | 0.71  | 0.98  |
2.5. Ionic liquid binary solvent system as phytochemical extractant

The usage of ILs as plant bioactive constituents’ extractants has a great impact and potential as they alleviate the environmental pollution and improve the selectivity and extraction yields of interesting compounds in the sample pre-treatment process compared to conventional organic solvents. However, ILs have a relatively high polarity due to their charged and asymmetric structures [15], which cause them not to have a good affinity with weak-polar compounds and thus this gives rise to a reduction in the distribution of weak-polar compounds in the IL phase. Although a longer alkyl chain of ILs has a lower polarity, their viscosity is large in accordance with the strong electrostatic and hydrogen bonding interaction between the ions [16, 17]. This drawback impairs the mixing and transferring properties in the extraction process by influencing the dissolution of the compounds in ILs. In exchange, the mixture of ILs and organic molecular solvents as an extraction medium could be an effective approach to solve their flaws. Besides creating a wide-adjusted range of solvent polarity, hydrophobicity, hydrogen-bond acidity, and basicity [18, 19], the addition of miscible molecular solvents as cosolvents helps to break the microscopic hydrogen-bond network and the aggregation of ILs, which significantly reduces the viscosity of ILs and improves the mixing and transferring process in their mediated extraction [20, 21].

3. Materials and methods

3.1. Sample collection and preparation

*Derris elliptica* roots were first collected from Ladang 2, Faculty of Agriculture, Universiti Putra Malaysia, UPM, Malaysia. The collected roots (Figure 3a) were cleaned and cut into smaller parts prior to rapid drying. The cleaned parts of the roots were placed in the freezer to maintain their freshness and dried using a vacuum oven at the temperature of 28 ± 2°C for 24 days.
hours. Once dried, the roots were ground into smaller particles of the size of approximately 0.86 ± 0.20 mm (Figure 3b). The selected sieved, ground samples were weighed prior to the normal soaking extraction process (NSE).

3.2. Preparation of binary solvent system

The binary solvent system comprises five selected ILs which are listed as follows: (1) 1-butyl-3-methylimidazolium chloride, [BMIM] Cl; (2) 1-butyl-3-methylimidazolium acetate, [BMIM] OAc; (3) 1-butyl-3-methylimidazolium bis(trifluorosulfonyl)imide, [BMIM] NTf₂; (4) 1-butyl-3-methylimidazolium trifluoromethanesulfonate, [BMIM] OTf; and (5) 1-butyl-1-methylpyrrolidinium chloride, [BMPy] Cl. The binary solvent systems were prepared by adding 2 ml of respective ILs into a round bottom flask (with stopper) containing 18 ml of organic solvent (acetone) with the ratio of 1:9. To avoid any moisture absorption due to the hygroscopic properties of some ILs, the ILs’ collection was carried out in the glove box. The mixtures were stirred by using a magnetic stirrer for 5–6 hours to homogenize the combined solvents. The ratio of 1:9 was based on the exploratory experiment results (data not shown). The mixing of the ILs in acetone was considered homogeneous if no apparent residue appeared in the flask.

3.3. Normal soaking extraction (NSE)

The extraction process was conducted at room temperature (28 ± 2°C) by using a combination of five different types of ILs and acetone with a mixing ratio of 1:9. The optimized parameters were utilized in accordance to protocols of Zubairi et al. [52, 53] as presented in Table 3. The extraction process was carried out by soaking 0.50 g of dried roots in 10 ml of the solvent systems for 24 hours with the solvent-to-solid ratio of 10 ml/g (n = 3). The liquid crude extract was collected twice at the 14th hour and 24th hour prior to the reversed-phase high-performance liquid chromatography (RP-HPLC) and thin-layer chromatography (TLC) analysis.
3.4. Liquid crude extract collection

The liquid crude extracts were collected at the 14th hour and 24th hour and placed in the labeled vials. There were 18 samples in total of five different types of solvent system used in three replicates ($n = 3$). Acetone was used as a control solvent. Later, the collected samples were placed in a freezer (−18°C) to prevent any thermal degradation.

3.5. Preparation of fine debris-free liquid crude extract

The collected liquid crude extracts were diluted using analytical grade acetonitrile, Sigma-Aldrich, 95% (v/v) with the dilution factor (DF) of 20. Then, the extracts were filtered by using polytetrafluoroethylene (PTFE—0.45 µm pore size) vacuum filtration to remove any fine debris. A 2-ml vial was used to store the extracts prior to the qualitative and quantitative analyses.

3.6. Qualitative analysis using thin-layer chromatography

MERCK Silica gel 60 F254 TLC aluminum sheet was used as a stationary phase to observe the presence of rotenone in the liquid crude extracts ($n = 3$). The migrations of rotenone markers were compared with rotenone standard. The markers and their migrated distance were visualized and determined under UV light of 254 and 365 nm wavelengths, respectively. In the development chamber, chloroform and n-hexane were combined and utilized as a mobile phase system with the ratio of 70:30. The retardation factors ($R_f$) of each extract were calculated by using Eq. (1).

\[
R_f = \frac{\text{Migration distance of substance}}{\text{Migration distance of solvent front}}
\]  

3.7. Quantitative analysis using reversed-phase high-performance liquid chromatography

Approximately 21.80 mg of rotenone standard Dr. Ehrenstorfer GmbH, 93.80% (w/w) was diluted with 50 ml of acetonitrile in a volumetric flask. The stock solution was filtered using Whatman™ filter paper no. 2 with 8 µm pore size. The quantitative analysis was completed by using Symmetry® C18 5 µl column, Waters with the internal diameter of 4.6

| Table 3. Processing parameters used in the rotenone extraction process. |
|-----------------------------------|--------|
| Solvent-to-solid ratio (mg/ml)    | 10     |
| Weight of raw material (g)       | 0.50   |
| Raw material particle size (mm)   | 0.86 ± 0.20 |
| Temperature (°C)                 | 28 ± 2 |
| ILs-to-acetone ratio             | 1.9    |
| Extraction time (hour)           | 14     |

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and 150 mm in length. The physical parameters involved in the RP-HPLC are as follows:
(1) 0.7 ml/min flow rate; (2) injection volume of 20 µl; (3) mobile phase of acetonitrile and deionized water with the ratio of 60:40 and (4) photodiode array detector (PDA) wavelength of 294 nm.

4. Results and discussion

4.1. Solubility of ionic liquids (ILs) in acetone

To enhance the rotenone extraction capacity, several ionic liquids were selected preliminarily. However, there was a drawback with regard to their solubility in organic solvents. The solubility of the selected ILs in acetone was observed by conducting a normal mixing with the ratio of 1:9. An exploratory experiment implemented revealed that the higher the amount of ILs used in the mixture, the higher the tendency of the ILs to produce undissolved solid residue (data not shown). Of the five selected ILs, only 1-butyl-3-methylimidazolium acetate, [BMIM] OAc, 1-butyl-3-methylimidazolium bis(trifluorosulfonyl)imide, [BMIM] NTf₂, and 1-butyl-3-methylimidazolium trifluoromethanesulfonate, [BMIM] OTf could be easily homogenized. In contrast, 1-butyl-3-methylimidazolium chloride, [BMIM] Cl and 1-butyl-1-pyrrolidinium chloride, [BMPy] Cl required a longer time to be homogenized (with the aid of heating at 80°C prior to mixing with acetone) which this could be possibly due to their physical properties (solid form at ambient temperature) and a specific range of organic solvent polarity, so that the solubility of the ILs in the organic solvent could be achieved. Theoretically, ionic liquids are miscible with an organic solvent of a medium to a high dielectric constant (ε) and they become immiscible with a low dielectric constant (ε) [54, 55]. Thus, the solubility of all of the selected ILs was considered satisfactory as there was no apparent residue appeared in the flask due to the high dielectric constant of acetone that aided the solubility of both chemicals.

4.2. Qualitative analysis of rotenone

The qualitative analysis of thin-layer chromatography (TLC) was performed to identify the existence of rotenone in the extracts of all of the binary solvent systems used. Figure 4 displays the images of rotenone markers visualized under the UV light of 254 nm. All samples exhibited the presence of rotenone markers in the extracts. On the other hand, Table 4 shows the migration distance (cm) and retardation factor (Rf) of rotenone in a standard solution and liquid crude extracts. The results indicated that the rotenone’s Rf value in a standard solution and all extracts (including control) were determined to be insignificantly different when compared to each other (p > 0.05). However, there were still a lot of impurities (unknown markers left behind rotenone) as presented in Figure 4. For that reason, a purification process of the liquid crude extracts via high-vacuum pressure liquid chromatography (VLC) is highly recommended as to increase the accuracy of rotenone and its derivative compounds’ identification [54, 56].
4.3. Quantitative analysis of rotenone content

Table 5, Figures 5 and 6 show the concentration (mg/ml) and yield of rotenone, % (w/w), respectively, from five different types of binary solvent systems extracted at the 14th hour of extraction. The markers were visualized on the alumina-based TLC plate under the UV light of 254 nm. (a) Acetone; (b) [BMIM] Cl; (c) [BMIM] Oac; (d) [BMIM] NTF2; (e) [BMIM] OTF and (f) [BMP] Cl. The dark circled markers of STD and R (R1, R2, and R3) represent the rotenone standard and replication of each of the binary solvent systems used.

| Binary solvent system       | 14 hour | 24 hour | 14 hour | 24 hour |
|-----------------------------|---------|---------|---------|---------|
| Rotenone standarda          | 1.80 ± 0.05 | 2.60 ± 0.03 | 0.45 ± 0.04 | 0.65 ± 0.03 |
| Control (acetone)           | 2.03 ± 0.06 | 2.60 ± 0.01 | 0.51 ± 0.02 | 0.65 ± 0.04 |
| [BMIM] Cl + acetone         | 1.93 ± 0.06 | 2.20 ± 0.02 | 0.49 ± 0.01 | 0.55 ± 0.01 |
| [BMIM] Oac + acetone        | 1.53 ± 0.06 | 1.40 ± 0.04 | 0.39 ± 0.01 | 0.35 ± 0.01 |
| [BMIM] NTF2 + acetone       | 3.20 ± 0.01 | 2.17 ± 0.06 | 0.80 ± 0.01 | 0.54 ± 0.01 |
| [BMIM] OTF + acetone        | 3.20 ± 0.08 | 1.70 ± 0.07 | 0.80 ± 0.08 | 0.43 ± 0.06 |
| [BMP] Cl + acetone          | 3.10 ± 0.00 | 2.93 ± 0.06 | 0.78 ± 0.00 | 0.74 ± 0.01 |

*a* Rotenone standard was prepared in acetone + ILs.

Table 4. Qualitative analysis of rotenone via TLC on varied binary solvent systems.

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**Figure 4.** Visualization of rotenone migration markers of five different types of binary solvent systems used at the 14th hour of extraction. The markers were visualized on the alumina-based TLC plate under the UV light of 254 nm. (a) Acetone; (b) [BMIM] Cl; (c) [BMIM] Oac; (d) [BMIM] NTF2; (e) [BMIM] OTF and (f) [BMP] Cl. The dark circled markers of STD and R (R1, R2, and R3) represent the rotenone standard and replication of each of the binary solvent systems used.
and 24th hour. The dependent variables were calculated based on the external standard method of RP-HPLC. The retention times of the rotenone in the standard solution (8.83 mins) and liquid crude extract (8.82 mins) are shown in Figures 7 and 8, respectively. Overall, it was observed that the binary solvent system of 1-butyl-3-methylimidazolium trifluoromethanesulfonate, [BMIM] OTf produced the highest rotenone concentration of 4.04 ± 0.34 and 4.19 ± 0.48 mg/ml as compared to the others ionic liquids and control solvent (acetone) (p < 0.05) at the 14th hour and 24th hour, respectively. The highest yield of rotenone ((2.69 ± 0.21% (w/w) and 2.03 ± 0.11% (w/w) in dried roots) was also determined in the 1-butyl-3-methylimidazolium trifluoromethanesulfonate, [BMIM] OTf solvent system at the 14th hour and 24th hour, respectively. However, both of the optimized processing parameters and the control extract (acetone) reported in the previous study resulted in only 2.44 ± 0.02% (w/w) [8] and 2.44 ± 0.09% (w/w) with the concentration of 3.65 ± 0.13 mg/ml respectively. The results were approximately 10.25% lower than that of the yield of rotenone extracted using a combination of [BMIM] OTf: acetone (p < 0.05).

This phenomenon can be explained from the perspective of the types of ILs. Of the five different ILs, four of them have the same cation but different anions ([BMIM] OTf, [BMIM] OAc, [BMIM] NTf2, [BMIM] Cl), and one with the same anion but a different cation ([BMPy] Cl). The increase of the rotenone yield is related to the anion and cation of ILs as ILs have different polarities depending on the anion and cation presence. ILs’ polarity is referred to Kamlet-Taft parameters such as polarity (π*), hydrogen bond basicity (β) and hydrogen bond acidity (α) [46]. ILs’ hydrogen bond basicity (β) depends on anion, while hydrogen bond acidity (α) depends on the cation. [BMIM] OTf has lower hydrogen bond basicity compared to [BMIM] OAc and [BMIM] Cl, but higher than that of [BMIM] NTf2 ([OAc]−>[Cl]−>[OTf]−>[NTf2]−) and has hydrogen bond acidity higher than that of [BMPy] Cl.

The rotenone compound is an acidic isoflavonoid compound that consists of ketonic chemical groups (R−C(=O)−R) [22] which has the potential of interacting with intermediate-polar solvents. It can be easily dissolved in moderate-polar organic solvents (e.g., methanol, chloroform, and acetone) [23] and sparingly soluble in water [24]. These are the factors that lead to the increase in the rotenone yield extracted when [BMIM] OTf with mid polarities is used as the extraction medium. The abundant presence of anion [OTf]− helped to attract more hydroxyl groups of rotenone to form more hydrogen bonds [25, 26]. With respect to the impact of the ILs’ cation, [BMIM] Cl and [BMPy] Cl on rotenone extraction, it was discovered that the rotenone yield extracted was high when the cation [BMIM]+ was used. This was due to the presence of an acid proton in the imidazole ring [32], which had the potential to form the hydrogen bond with oxygen of the rotenone compounds. The significant increase in the rotenone content can be explained in relation to several aspects as follows: (1) the trend of functional groups of rotenone toward foreign charge and (2) the capacity of the IL in the vicinity of various charges. For that reason, any combination of ionic liquids and middle-polar organic solvents does not only optimize the absorption of solute into the solvent due to the low viscosity but is also even able to increase the opportunities for extracting higher rotenone compounds.
The previous study also revealed that the solubility of flavonoids and their derivatives could be increased by using ILs, as the components were greatly an anion-dependent [25]. The anionic potency of both organic solvent and ILs in extracting a large amount of bioactive compounds (e.g., rotenone) and moving into the solvent systems was significantly undeniable as both chemicals facilitated the extraction process via salvation power and multiple interactions (e.g., hydrogen bonding, polarity, ionic charge-to-charge, and $\pi-\pi$, $\pi$-$n$) with the analysis [26, 27].

| Solvent system type       | Concentration (mg/ml) (±SD) | Yield (%, w/w) (±SD) |
|---------------------------|-----------------------------|----------------------|
|                           | 14 hour                     | 24 hour              | 14 hour                     | 24 hour              |
| Acetone (control)         | 3.65 ± 0.13                 | 3.50 ± 0.02          | 2.44 ± 0.09                 | 1.73 ± 0.09          |
| [BMIM] Cl:Acetone         | 2.73 ± 0.00                 | 2.57 ± 0.02          | 2.00 ± 0.06                 | 1.55 ± 0.01          |
| [BMIM] OAc:Acetone        | 2.51 ± 0.02                 | 2.69 ± 0.14          | 1.60 ± 0.01                 | 1.18 ± 0.06          |
| [BMIM] NTf2:Acetone       | 3.70 ± 0.11                 | 3.80 ± 0.03          | 2.42 ± 0.14                 | 2.07 ± 0.07          |
| [BMIM] OTf:Acetone        | 4.04 ± 0.34                 | 4.19 ± 0.48          | 2.69 ± 0.21                 | 2.03 ± 0.11          |
| [BMPy] Cl:Acetone         | 2.66 ± 0.20                 | 2.37 ± 0.26          | 1.78 ± 0.08                 | 1.27 ± 0.16          |

Table 5. Rotenone quantitative analysis using RP-HPLC using different binary solvent systems at the 14th hour and 24th hour of extraction time.

Figure 5. Concentration of rotenone (mg/ml) with respect to five different types of binary solvent systems. $^\psi p < 0.05$—[BMIM] OTf: acetone was the best binary solvent system to procure the highest rotenone concentration (mg/ml) ($n = 3$). $^* p < 0.05$—[BMIM] NTf2: acetone and acetone extract produced a high concentration as compared to the [BMIM] OAc, [BMPy] Cl and [BMIM] Cl.
Figure 6. Yield of rotenone, % (w/w) in dried roots with respect to five different types of binary solvent systems. *p < 0.05 — [BMIM] OTf : acetone was the best binary solvent system to procure the highest rotenone content in dried roots (n = 3).
5. Conclusion

In conclusion, the best ionic liquid to assist the organic solvent (acetone) extraction system was 1-butyl-3-methylimidazolium trifluoromethanesulfonate, [BMIM] OTf. The selected binary solvent system had contributed to the highest rotenone content of 2.69 ± 0.21% (w/w) with a concentration of 4.04 ± 0.34 mg/ml at the 14th hour (the time of the exhaustive extraction as reported in the previous study). The rotenone content was 10.25% higher than the optimized parameter of the acetone extract (control) \((p < 0.05)\). Therefore, the addition of certain ionic liquids to the organic solvent will potentially give rise to a significant increase in the amount of bioactive constituent in the phytochemical extraction process. Further study is required to optimize several processing parameters especially on the mixing ratio between the ILs and organic solvent in order to verify the increase in the rotenone content as the solubility problem between both chemicals, is relatively prominent.

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Figure 8. Chromatogram of [BMIM] OTf: acetone binary solvent system at the 14th hour.
Author details

Zetty Shafiqa Othman, Nur Hasyareeda Hassan and Saiful Irwan Zubairi*

*Address all correspondence to: saiful-z@ukm.edu.my

Faculty of Science and Technology, School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia

References

[1] Stoll, G. Natural Crop Protection Based on Local Farm Resources in the Tropics and Subtropics, Weikersheim, Margraf Publishers Scientific Books, Germany. 1988.

[2] Gingerich, W. H. Tissue distribution and elimination of rotenone in rainbow trout. Aquatic Toxicology. 1986; 8(1):27–40.

[3] Ray David, E. Pesticides derived from plants and other organism. In Handbook of Pesticide Toxicology, volume 2, Classes of Pesticides. Wayland J. Hayes & Edward R. Laws (eds.) Academic Press, NY. 1991.

[4] John, R. A. and Ron, R. K. Insect control. Journal of Economic Entomology. 1944; 37: 400–408.

[5] Andel, T. V. The diverse uses of fish-poison plants in Northwest Guyana. Economic Botany. 2000; 54(4): 500–512.

[6] Schnick, R. A. A review of literature on the use of rotenone in fisheries. United States Department of the Interior, Fish and Wildlife Service, Wisconsin. 1974.

[7] Bradbury, A. Rotenone and Trout Stocking n.p. Washington Department of Game Fisheries Management Division. 1986.

[8] Zubairi, S. I. The yield and biological activity (LC50) of rotenone extracted from Derris elliptica, Universiti Teknologi Malaysia, Faculty of Chemical and Natural Resources Engineering. 2006.

[9] Fu, X., Shugui, D. and Yu, Z. Comparison of extraction abilities between ionic liquids and dichloromethane. Chinese Journal of Analytical Chemistry. 2006; 34(5): 598–602.

[10] Bogdanov, M. G., Petkova, D., Hristeva, S., Svinyarov, I. and Kantlehner, W. New guanidinium-based room-temperature ionic liquids. Substituent and anion effect on density and solubility in water. Zeitschrift für Naturforschung. B, A Journal of Chemical Sciences. 2010; 65(1): 37.
[11] Endres, F. and El Abedin, S. Z. Air and water stable ionic liquids in physical chemistry. Physical Chemistry. 2006; 8(18): 2101–2116.

[12] Bonhote, P., Dias, A.-P., Papageorgiou, N., Kalyanasundaram, K. and Grätzel, M. Hydrophobic, highly conductive ambient-temperature molten salts. Inorganic Chemistry. 1996; 35 (5): 1168–1178.

[13] Handy, S. T. Room temperature ionic liquids: different classes and physical properties. Current Organic Chemistry. 2005; 9(10): 959–988.

[14] Rooney, D., Jacquemin, J. and Gardas, R. Thermophysical properties of ionic liquids. Topics in Current Chemistry 2009; 290: 185–212.

[15] Reichardt, C. Polarity of ionic liquids determined empirically by means of solvatochromic pyridinium N-phenolate betaine dyes. Green Chemistry. 2005; 7(5): 339–351.

[16] Huddleston, J. G., Visser, A. E., Reichert, W. M., Willauer, H. D., Broker, G. A. and Rogers, R. D. Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. Green Chemistry. 2001; 3(4): 156–164.

[17] Xie, L.-L., Favre-Reguillon, A., Wang, X.-X., Fu, X., Pellet-Rostaing, S., Toussaint, G., Geantet, C., Vrinat, M. and Lemaire, M. Selective extraction of neutral nitrogen compounds found in diesel feed by 1-butyl-3-methyl-imidazolium chloride. Green Chemistry. 2008; 10(5): 524–531.

[18] Mancini, P. M., Fortunato, G. G. and Vottero, L. R. Molecular solvent/ionic liquid binary mixtures: designing solvents based on the determination of their microscopic properties. Physics and Chemistry of Liquids. 2004; 42(6): 625–632.

[19] Mellein, B. R., Aki, S. N., Ladewski, R. L. and Brennecke, J. F. Solvatochromic studies of ionic liquid/organic mixtures. The Journal of Physical Chemistry B. 2007; 111(1): 131–138.

[20] Heintz, A. Recent developments in thermodynamics and thermophysics of non-aqueous mixtures containing ionic liquids. A review. The Journal of Chemical Thermodynamics. 2005; 37(6): 525–535.

[21] Gómez, E., González, B., Domínguez, Á., Tojo, E. and Tojo, J. Dynamic viscosities of a series of 1-alkyl-3-methylimidazolium chloride ionic liquids and their binary mixtures with water at several temperatures. Journal of Chemical and Engineering Data. 2006; 51(2): 696–701.

[22] Ahmad, F. and Raji, H. Kimia hasilan semula jadi dan tumbuhan ubatan. Dewan Bahasa dan Pustaka. 1993.

[23] Cabizza, M., Angioni, A., Melis, M., Cabras, M., Tuberoso, C. V. and Cabras, P. Rotenone and rotenoids in cube resins, formulations, and residues on olives. Journal of Agricultural and Food Chemistry. 2004; 52(2): 288–293.

[24] Dhauoudi, A., Monser, L. and Adhoum, N. Removal of rotenone insecticide by adsorption onto chemically modified activated carbons. Journal of Hazardous Materials. 2010; 181(1): 692–699.
[25] Guo, Z., Lue, B.-M., Thomasen, K., Meyer, A. S. and Xu, X. Predictions of flavonoid solubility in ionic liquids by COSMO-RS: experimental verification, structural elucidation, and solvation characterization. Green Chemistry. 2007; 9(12): 1362–1373.

[26] Anderson, J. L., Ding, J., Welton, T. and Armstrong, D. W. Characterizing ionic liquids on the basis of multiple solvation interactions. Journal of the American Chemical Society. 2002; 124(47): 14247–14254.

[27] Du, F.-Y., Xiao, X.-H. and Li, G.-K. Application of ionic liquids in the microwave-assisted extraction of trans-resveratrol from Rhizma Polygoni Cuspidati. Journal of Chromatography A. 2007; 1140(1): 56–62.

[28] Gonfa, G., Bustam, M., Man, Z. and Mutilib, M. A. Unique structure and solute–solvent interaction in imidazolium based ionic liquids: A review. Asian Transactions on Engineering. 2011; 1: 24–34.

[29] Geetanjali S. and Anil K. Ionic liquids: Physico-chemicals, solvent properties and their applications in chemical processes. Indian Journal of Chemistry. 2008; 47A: 495–503.

[30] Weingärtner, H. Understanding ionic liquids at the molecular level: facts, problems, and controversies. Angewandte Chemie International Edition. 2008; 47(4): 654–670.

[31] Nobuoka, K., Kitaoka, S., Iio, M., Harran, T. and Ishikawa, Y. Solute–solvent interactions in imidazolium camphorsulfonate ionic liquids. Physical Chemistry Chemical Physics. 2007; 9(44): 5891–5896.

[32] Hunt, P. A., Kirchner, B. and Welton, T. Characterising the electronic structure of ionic liquids: An examination of the 1-butyl-3-methylimidazolium chloride ion pair. Chemistry: A European Journal. 2006; 12(26): 6762–6775.

[33] Aggarwal, A., Lancaster, N. L., Sethi, A. R. and Welton, T. The role of hydrogen bonding in controlling the selectivity of Diels–Alder reactions in room-temperature ionic liquids. Green Chemistry. 2002; 4(5): 517–520.

[34] Znamenskiy, V. and Kobrak, M. N. Molecular dynamics study of polarity in room-temperature ionic liquids. The Journal of Physical Chemistry B. 2004; 108(3): 1072–1079.

[35] Dieter, K. M., Dymek, C. J., Heimer, N. E., Rovang, J. W. and Wilkes, J. S. Ionic structure and interactions in 1-methyl-3-ethylimidazolium chloride-aluminium chloride molten salts. Journal of the American Chemical Society. 1988; 110(9): 2722–2726.

[36] Iwata, K., Okajima, H., Saha, S. and Hamaguchi, H.O. Local structure formation in alkyl-imidazolium-based ionic liquids as revealed by linear and nonlinear Raman spectroscopy. Accounts of Chemical Research. 2007; 40(11): 1174–1181.

[37] Raju, S. and Balasubramanian, S. Molecular dynamics simulation of model room temperature ionic liquids with divalent anions. Indian Journal of Chemistry-Section A: Inorganic, Physical, Theoretical and Analytical Chemistry. 2010; 49(5-6): 721–726.
[38] Deetlefs, M., Hardacre, C., Nieuwenhuyzen, M., Padua, A. A, Sheppard, O. and Soper, A. K. Liquid structure of the ionic liquid 1,3-dimethylimidazolium bis [(trifluoromethyl) sulfonyl] amide. The Journal of Physical Chemistry B. 2006; 110(24): 12055–12061.

[39] Triolo, A., Russina, O., Bleif, H.-J. and Di, C. E. Nanoscale segregation in room temperature ionic liquids. The Journal of Physical Chemistry B. 2007; 111(18): 4641–4644.

[40] Zhao, W., Leroy, F., Heggen, B., Zahn, S., Kirchner, B., Balasubramanian, S. and. Müller-Plathe, F. Are there stable ion-pairs in room-temperature ionic liquids? Molecular dynamics simulations of 1-n-butyl-3-methylimidazolium hexafluorophosphate. Journal of the American Chemical Society. 2009; 131(43): 15825–15833.

[41] Fernandes, A. M., Rocha, M. A., Freire, M. G., Marrucho, I. M., Coutinho, J. A. and Santos, L. M. Evaluation of cation–anion interaction strength in ionic liquids. The Journal of Physical Chemistry B. 2011; 115(14): 4033–4041.

[42] Hamaguchi, H. O., Saha, S., Ozawa, R. and Hayashi, S. Raman and X-ray Studies on the Structure of [bmim] X. In ACS Symposium Series Oxford University Press. 2005; 901: 68–78.

[43] Pringle, J. M., Golding, J., Baranyai, K., Forsyth, C. M., Deacon, G. B., Scott, J. L. and MacFarlane, D. R. The effect of anion fluorination in ionic liquids—physical properties of a range of bis (methanesulfonyl) amide salts. New Journal of Chemistry. 2003; 27(10): 1504–1510.

[44] Xu, W., Wang, L.-M., Nieman, R. A. and Angell, C. A. Ionic liquids of chelated orthoborates as model ionic glassformers. The Journal of Physical Chemistry B. 2003; 107(42): 11749–11756.

[45] Ngo, H. L., LeCompte, K., Hargens, L. and McEwen, A. B. Thermal properties of imidazolium ionic liquids. Thermochemistry Acta. 2000; 357: 97–102.

[46] Kamlet, M.J., Abboud, J. L. M., Taft, R. W. Progress in Physical Organic Chemistry. 1981; 13: 485.

[47] Coleman, S., Byrne, R., Minkovska, S. and Diamond, D. Thermal reversion of spirooxazine in ionic liquids containing the [NTf2]− anion. Physical Chemistry. 2009; 11(27): 5608–5614.

[48] Chiapeake, C. and Pieraccini, D. Determination of ionic liquids solvent properties using an unusual probe: The electron donor-acceptor complex between 4,4′-bis (dimethylamino)-benzophenone and tetracyanoethene. The Journal of Physical Chemistry A. 2006; 110(14): 4937–4941.

[49] Jeličić, A., García, N., Löhmannsröben, H.-G. and Beuermann, S. Prediction of the ionic liquid influence on propagation rate coefficients in methyl methacrylate radical polymerizations based on Kamlet-Taft solvatochromic parameters. Macromolecules. 2009; 42(22): 8801–8808.
[50] Doherty, T. V., Mora-Pale, M., Foley, S. E., Linhardt, R. J. and Dordick, J. S. Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. Green Chemistry. 2010; 12(11): 1967–1975.

[51] Wu, Y., Sasaki, T., Kazushi, K., Seo, T. and Sakurai, K. Interactions between spiropyrans and room-temperature ionic liquids: photochromism and solvatochromism. The Journal of Physical Chemistry B. 2008. 112(25): 7530–7536.

[52] Zubairi, S. I., Sarmidi, M. R. and Aziz, R. A. 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. Sains Malaysiana. 2014; 43(5): 707–713.

[53] Zubairi, S. I., Sarmidi, M. R. and Aziz, R. A. A preliminary study of rotenone exhaustive extraction kinetic from *Derris elliptica* dried roots using normal soaking extraction (NSE) method. Advances in Environmental Biology. 2014; 8(4): 910–915.

[54] Touchstone, J. C. Practice of Thin Layer Chromatography. John Wiley & Sons. 1992.

[55] Poole, C. F. and Poole, S. K. Extraction of organic compounds with room temperature ionic liquids. Journal of Chromatography A. 2010; 1217(16): 2268–2286.

[56] Zubairi, S. I., Sarmidi, M. R. and Aziz, R. A. Identification of bio-active constituents from *Derris elliptica* liquid crude extract using liquid chromatographic method coupled with high vacuum pressure. Advances in Environmental Biology. 2014; 8(2): 437–440.
