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Characterization of rutin extracted and purified by different methods from leafy by-products of asparagus (Asparagus officinalis)

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Abstract

Valorization of asparagus leafy by-products as a potential source of rutin through selected extraction and purification protocols was investigated. Protocol resulting in the highest extraction yield was first selected. Crude extract was subject to purification via multiple liquid-liquid back extraction using ethanol, methanol or water as a solvent; selection of the most appropriate purification solvent was made, based on rutin solubility. The proposed purification protocol yielded yellow-color crystals, which were characterized by fluorescence microscopy, Fourier-transform infrared spectroscopy and liquid chromatography mass spectrometry to confirm them as rutin. Purity of rutin was confirmed by ultra-performance liquid chromatography at 97.6%; yield of the purified rutin was determined to be 78.2%. The remaining rutin (21.8%) was found in the liquids collected at various stages of purification; such liquids could be recycled using the same purification process. The proposed protocols are simple, yet effective for rutin extraction and purification from asparagus leafy by-products.

1. Introduction

Asparagus (*Asparagus officinalis*) is regarded as “king of vegetables” for its fully nutritious but low-calorie nature. Asparagus indeed ranks top among common vegetables due to its higher antioxidant activities, which are contributed to various phenolic phytochemicals, including rutin (Lee et al., 2014; Pellegrini et al., 2003). Unfortunately, as much as 75% of the asparagus plant after processing becomes low-value by-products in various forms, including leaves, hard stems and roots; only 25% of the plant is identified as edible spears. These by-products are nevertheless known to contain various compounds; the contents are in fact as much as or even higher than those in the edible spears (Fan et al., 2015; Zhang et al., 2019).

Proper valorization of asparagus by-products would imply both a higher profit margin for the asparagus processing industry and a more sustainable use of the produce. Several attempts have indeed already been made along this line of thought. Hard-stem by-products, for example, have been utilized to produce juices (Sun et al., 2005), cookies (Liu et al., 2016b; Liu et al., 2017; Liu et al., 2016a), yoghurts (Sanz et al., 2008) and dietary fibers (Fuentes-Alventosa et al., 2009). Studies on the utilization of leafy by-products, however, are still very limited, especially when it comes to systematic scientific or technical studies. This may be due to the fact that leafy by-products are hard and fibrous, making them difficult to be incorporated in any mainstream products. Through communication with the asparagus processing industry in China, asparagus leaves have been used as animal fodders or left on the field for next season farming (Chitrakar et al., 2019).

Based on the few available studies (Chitrakar et al., 2020; Chitrakar et al., 2022a,b), extraction of rutin, which is rich in the leafy by-products, represents an interesting alternative use of this otherwise asparagus waste (Chitrakar et al., 2022a).

Rutin extraction has been conducted from various plant materials, including buckwheat (*Fagopyrum esculentaum* Moench) seeds (Kreft et al., 1999), Japanese pagoda tree (*Sophora japonica*) flowers (Paniwnyk et al., 2001; Xi & Yan, 2017), paper bush (*Edgeworthia chrysantha* Lindl.) flowers (Tong et al., 2009), pot-marigold (*Calendula officinalis*) flowers (Martins et al., 2014), little-leaf linden (*Tilia cordata*) inflorescences (Oniszczuk & Podgórski, 2015), Chinese scholar-tree (*Styphnolobium japonicum*) flowers (Xi & Luo, 2015), purple amaranth (*Amaranthus paniculatus*) leaves and seeds (Kraujalis et al., 2015), Sichuan pepper (*Zanthoxylum bungeanum* Maxim) leaves (He et al., 2016), banana (*Musa balbisiana*) leaves (Yingyuen et al., 2020) as well as cassava (*Manihot esculenta* Crantz) leaves (Chahyadi & Elfahmi, 2020). These studies used either methanol or ethanol or their mixture as the solvents (Kraujalis et al., 2015) to extract rutin. Various extraction methods, e.g., ultrasound- and microwave-assisted extraction (Chahyadi & Elfahmi, 2020; Martins et al., 2014; Périno-Issartier et al., 2011) as well as partitioning with solvents like NaH_2PO_4, petroleum ether, ethyl acetate, *n*-butanol and salt/ethanol (He et al., 2016; Tong et al., 2009; Chong et al., 2020) were employed. Purification of crude extracts, on the other hand, was attempted by such simple methods as chilling (Chahyadi & Elfahmi, 2020) and aqueous two-phase systems (Velho et al., 2020) or advanced methods as column chromatography in combination with reverse-phase high-performance liquid chromatography (Yingyuen et al., 2020) and high-speed counter-current chromatography (Tong et al., 2009). The reported extraction yields ranged from 0.362–4.4 g per 100 g, while the purity was noted to be higher than 98% (Tong et al., 2009; Yingyuen et al., 2020), except for the case where chilling method was used and the purity was noted to be only 82% (Chahyadi & Elfahmi, 2020). No prior study is so far available, however, on the extraction and purification of rutin from asparagus leafy by-products.

The present study first comparatively determined the phenolic and flavonoid profiles of asparagus spears and leafy by-products to confirm that the latter is a rich source of rutin. Since rutin is slightly soluble in ethanol, but highly soluble in methanol (Serban et al., 2016), the latter solvent was chosen as the extraction solvent in this study. The extraction was carried out using three different methods, namely, single-step extraction at room temperature and at 50 ^{o}C as well as three-step extraction (one h for each step) at 50 ^{o}C. The most suitable extraction method, based on the rutin yield, was selected. Rutin purification was conducted using multiple liquid-liquid back-extraction with ethanol, methanol or extra-pure water as a solvent; selection of the most appropriate purification solvent was made, based on rutin solubility. Purified rutin was characterized via fluorescence microscopy, Fourier-transform infrared (FT-RI) spectroscopy and liquid chromatography mass spectrometry (LC-MS). Rutin purity was determined via ultra-performance liquid chromatography. Losses of rutin during the purification process in various solvents were also determined.

2. Materials And Methods

2.1 Raw materials

Asparagus leaves were supplied by Nantong Shuangyang Ecological Agriculture Technology Co., Ltd. (Nantong, Jiangsu, China), having a moisture content of 80.1 ± 0.7% (wet basis). They were washed with water after sorting out from unwanted debris. The surface water was absorbed using adsorbent paper; then, the leaves were made small pieces (Approximately 5 cm) before drying in a hot dryer at 50 ^{o}C till the moisture content was about 7% (w.b.). Dried leaves were powdered to an 80-mesh size (177 µm) using a grinder (Bo Ou Metal Products Co., Ltd., Zhejiang, China) at a speed of 34,000 rpm with 30 s RUN and 10 s STOP functions. The powder was used as the raw material for subsequent extraction experiments.

2.2 Chemicals

Methanol and acetic acid (Both HPLC-grade) as well as analytical-grade ethanol, aluminum chloride, sodium hydroxide and sodium nitrite were obtained from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China). Selected phenolic compounds standards, namely, rutin (> 95% purity), ferulic acid (> 98%), chlorogenic acid (> 98%), vanillic acid (> 99.5%), caffeic acid (> 98%), para-hydroxy benzoic acid (> 99%), and gallic acid (> 95%) were also supplied by Sinopharm Chemical Reagent Co., Ltd.

2.3 Extraction of rutin

Extraction of rutin was performed using methanol (70%, v/v) as the extraction solvent. The ratio of solid: solvent was tested at 1:10, 1:20 and 1:30 (solid: liquid, w/v) to find the optimum one. Total flavonoids content (TFC), which was assigned as an indirect indicator of rutin, was used as the criterion for selecting the most suitable ratio. Through our preliminary experiments (data not shown), 1:20 was noted as the optimal ratio. Noted that TFC was calculated following the suggested methods of Patel et al. (2010).

Three different methods of rutin extraction were compared in terms of the extracted rutin yield. As listed in Fig. 1, the first extraction method consisted of three steps (each step lasted for 1 h) at 50 ^{o}C (Fig. 1A). The second method involved single-step extraction for 3 h at 50 ^{o}C (Fig. 1B), while the third method was single-step extraction for 3 h at room temperature of around 25 ^{o}C (Fig. 1C). The resulting extracts were subject to ultra-performance liquid chromatography (UPLC) for rutin quantification.

2.4 Purification of extracted rutin

Rutin extraction was performed as per the selected method listed in Section 2.3 (three-step extraction at 50 ^{o}C; more explanation will be given in Section 3.3). After extraction, the extract was subject to purification via multiple liquid-liquid back extraction using either ethanol, methanol or extra-pure water as a solvent, with rutin solubility in each solvent as the criterion.

Figure 2 shows a step-by-step experimental flow diagram of the purification process. The concentration of crude extract was done in a rotary vacuum evaporator (RE-5203, Shanghai Yarong Biochemical Instrument Factory, Shanghai, China) at 50 ^{o}C until precipitation started to be observed in dark-color concentrated extract (Fig. 2C). Absolute ethanol was added to solubilize both the colored and non-rutin compounds; rutin being insoluble in ethanol, remained as precipitates within the mixture. These

precipitates were recovered after filtering through Whatman filter paper (No. 1); the filtrate was named as discarded liquid-1 (DL-1), which was collected to analyze for rutin content via UPLC. The precipitates thus obtained were solubilized in methanol (70%, v/v) to obtain yellow-color liquid, which was also filtered using the same Whatman filter paper (No. 1) to remove insoluble residues. The clear yellow-color liquid was once again concentrated in the rotary evaporator until yellow-color crystals were obtained. The crystals were harvested after centrifugation at 5,600× g for 10 min, while the clear liquid was decanted and collected as discarded liquid-2 (DL-2) for rutin content analysis via UPLC. The crystals thus obtained was washed with chilled extra-pure water for three times to remove water-soluble impurities. The water used for such washing was decanted after centrifugation and was collected (named DL-3, DL-4 and DL-5, respectively) for further analysis of rutin content. The final collected crystals were subject to freeze drying in a freeze dryer (SCIENTZ-10ND Freeze Dryer, Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China) for 16 h to obtain dried crystals for further analysis.

2.5 Fluorescence microscopy of rutin crystals

A fluorescence microscope was used to observe rutin crystals (Axio Vert.A1, Zeiss, Oberkochen, Germany). A slide was prepared with rutin crystals dyed with fluorescent diacetate (50 mg/L). Observation was made at an excitation wavelength of 320–380 nm. An image at an appropriate location was captured using a computer connected with the microscope.

2.6 Fourier transform infrared (FTIR) spectroscopy of rutin

After preparing into a transparent disk with KBr, a freeze-dried sample was scanned for 16 times using an FTIR spectrometer (Nicolet™ iS™ 10, Thermo Fisher Scientific, Waltham, MA, USA) between the wavenumbers of 4000 and 400 cm^{-1}. A sample was mixed with KBr powder at a ratio of 1:100; appropriate amount of the mixture was then placed in a mold to prepare a disk under high hydraulic pressure. Background scanning was performed without a sample slot to remove any inferences.

2.7 Liquid chromatography-mass spectrometry (LC-MS) of purified rutin

The LC-MS analysis was done using a mass spectrometer (Waters MALDI SYNAPT QToF, Milford, MA, USA), equipped with a column named BEH C18 (2.1 mm × 150 mm; 1.7 µm). The mobile phases used were (A) formic acid (0.1%) and (B) acetonitrile (100%). A linear gradient program was followed: From 0 to 40 min, 100% A; 40 to 45 min, 70% A; 45 to 50 min, 20% A; 50 to 55 min, 0% A; and finally, 55 to 60 min, 100% A. 5 µL sample was injected into the column; the column temperature was set at 45 ^{o}C, while the flow rate was set at 0.3 mL/min. Each chromatogram was created by the liquid chromatograph with photodiode array detector (Waters AQUITY PDA) using a range of wavelength (200–700 nm). The equipment was set at the negative ion mode to conduct mass spectrometry at 6 eV collision energy and 400 ^{o}C capillary temperature; nitrogen gas flow was maintained at 700 mL/h. Scanning was performed over an *m/z* range of 50 to 1000 (Chitrakar et al., 2022a).

2.8 UPLC of rutin and other phenolic compounds

UPLC of rutin and other phenolic compounds was conducted using the method of Nour et al. (2012) with slight modification. (A) acetic acid (0.1%) and (B) methanol (100%) were used as mobile phases under a linear gradient program as follows: From 0 to 27 min, 90% A; from 27 to 55 min, 90% − 60% A; from 55 to 60 min, 60% A; from 60 to 63 min, 60% − 56% A; from 63 to 71 min, 56% A; from 71 to 72, 56% − 90% A; finally, from 72 to 75 min, 90% A to come to a normal condition before the next analysis. 10 µL sample was injected into the column (4.6 mm × 250 mm, 5 µm; SunFire C18 column; Waters, Milford, MA, USA); the column temperature was set at 30 ^{o}C, while the flow rate was set at 1 mL/min.

The equipment selected 254 nm, 278 nm, and 300 nm as the detection wavelengths. The retention time of standards were matched with those of the sample peaks to recognize them, while the compounds were quantified using the standard curve method through the peak areas at different concentrations; the linearity of standard curves were > 98%. Each analysis was performed in triplicate.

2.9 Statistical analysis

Results were presented as mean with standard deviation after ANOVA (One-way analysis of variance) testing. The significance among results were tested through Duncan's new multiple range test (95% confidence level; $p < 0.05$) through SPSS 23.0 (SPSS Inc. Chicago, IL, USA). OriginPro 2016 b9.3.226 (OriginLab Corporation, Northampton, MA, USA) was used to prepare the UPLC chromatograms. MassLynx 4.2 SCN 982 (Waters) was used to prepare LC-MS chromatograms, including TIC and diode array chromatograms.

3. Results And Discussion

3.1 Phenolic compositions of edible spears and leafy by-products

For the analysis of phytochemicals in edible spear and leafy by-products of asparagus, seven phenolic and flavonoid standards, namely, rutin, ferulic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-hydroxy benzoic acid, and gallic acid were used; such selections were based on the published literature on which compounds are dominantly available in asparagus (Chitrakar et al., 2019; Fuentes-Alventosa et al., 2008; Kobus-Cisowska et al., 2019). UPLC was first performed on each individual standard to determine its retention time. Then, a mixture of the seven standards was run to obtain a chromatogram of the mixed standards. A chromatogram for the mixed standards is shown in Fig. 3. Then, the extracts from both the edible spears and leafy by-products were assayed. The obtained chromatograms, in comparison to that of the mixed standards, are presented in Fig. 4.

Phytochemical compounds contents in different parts of asparagus are listed in Table 1. Rutin was noted to be the dominating compound. Rutin has indeed been reported to be the major bioactive compound in asparagus, constituting 60 to 80% of its phenolic phytochemicals (Fuentes-Alventosa et al., 2008;

Fuentes-Alventosa et al., 2013). Our results were in line with the previously reported results. It is interesting to note that the leafy by-products were very rich in rutin (29.7 mg/g); the content was around 12 times higher than that found in the edible spears (2.5 mg/g). Similar results of around 14 times higher amount of rutin in the asparagus leafy by-products than that in the edible spears were reported (Kwon et al., 2020). Such results indicate that asparagus leaves are indeed a rich source of rutin, showing that rutin extraction and purification are a verified appropriate valorization route for the asparagus leafy by-products.

Table 1
Contents of phenolics and flavonoids in different parts of asparagus

Sample	Phenolics and flavonoids contents (mg/g)						
	GA	p-HBA	CA	VA	ChA	FA	Rutin
ES	1.2 ± 0.03	ND	0.04 ± 0.006	0.03 ± 0.003	0.02 ± 0.003	0.04 ± 0.01	2.5 ± 0.04
LB	0.7 ± 0.09	ND	0.03 ± 0.01	0.09 ± 0.04	1.2 ± 0.16	1.2 ± 0.13	29.7 ± 0.5

Ferulic acid, chlorogenic acid and gallic acid were the minor noted compounds (0.74–1.25 mg/g) in the leafy by-products, whereas gallic acid was the only minor compound (1.19 mg/g) found in the edible spears. The other compounds were only found in trace amounts, i.e., less than 0.1 mg/g.

3.2 Extraction of rutin by different methods

Figure 5 shows the chromatograms of the leafy by-products extracts from the different methods; the extraction yields of rutin are also illustrated. Three-step extraction at 50 ^{o}C (Fig. 5A) was noted to be most promising. With its two consecutive extractions, the yield was as high as 96.4%. Single-step extractions at 50 ^{o}C and at room temperature were inferior, resulting in the yields of 79.8% and 30.6%, respectively (Fig. 5B and 5C).

Higher temperature is expected to accelerate mass transfer, hence enhancing the extraction process. However, excessive temperature may result in thermal destruction of a heat-sensitive extracted compound. Use of the extraction temperature of 50 ^{o}C in the present study might be mild enough to avoid thermal destruction of rutin when compared to the practices of other previous reports (He et al., 2016; Yingyuen et al., 2020). Multiple extraction was also noted to be superior to single extraction. This is due to the repeated use of fresh solvent, which helped increase the extraction efficiency.

3.3 Characterization of purified crystals

Based on the fact that rutin is only slightly soluble in ethanol, addition of absolute ethanol in a concentrated crude extract solubilized the colored and non-rutin compounds. Yellow precipitates were then seen to float in the liquid (Fig. 2C). Upon filtration, yellow-color solids, which were suspected to be

rutin, were obtained. Such solids were then solubilized in 70% (v/v) methanol, from where insoluble impurities were separated through filtration. Clear yellow-color liquid (Fig. 2G) was once again concentrated until yellow-color precipitates appeared (Fig. 2I). The precipitates were collected through centrifugation (Fig. 2J) and then freeze-dried to obtain yellow-color crystals (Fig. 2L). The crystals yield was noted to be 78.2% (24.4 mg/g), based on the total estimated amount in the initial extract. The rest (21.8%) was retained in the discarded liquids at various stages (DL-1 to DL-5). The harvested yellow-color crystals were further subject to various qualitative and quantitative analyses to confirm them as rutin crystals.

Although the proposed purification protocol is simple, it is interesting to note that comparable yield (78.2%) to those reported when using other more sophisticated protocols was obtained. For example, a yield of 78.2% was reported when H_2S decomposition with zinc precipitation was used to separate rutin from *Spilanthes paniculata* (Tag et al., 2017). A yield of 63.1% was noted when rutin was separated from *Euonymus alatus* through the use of AB-8 macroporous resin separation (Zhao et al., 2011). Nevertheless, it is necessary to consider that the amounts of rutin retained in the discarded liquids (DLs) were significant (21.8%). It may therefore be feasible to harvest more rutin through recycling DLs using the same purification protocol, hence improving the economics of the process.

3.3.1 Fluorescence microscopy of purified crystals

Figure 6 shows the microscopic view of the crystals, as observed via fluorescence microscopy. Only one kind of slender yellow-color crystals, indicated by the arrows, was noted. This implies that only one material with high purity was present. To confirm this observation, FTIR spectroscopy as well as LC-MS analysis were further conducted.

3.3.2 FTIR spectroscopy of purified crystals

FTIR spectrum of the purified crystals was compared with that of rutin standard (Fig. 7). Both spectra exhibited the same stretching and bending vibrations at various wavenumbers, including O-H stretching of alcohol at 3425 and 2936 cm^{-1} (intermolecular and intramolecular bonds, respectively); C = C stretching of alkene and conjugated alkene at 1655 and 1600 cm^{-1}, respectively; C-H bending of alkane at 1455 and 1365 cm^{-1}; C-O stretching of esters at 1295, 1205 and 1165 cm^{-1}; and C = C bending of alkene at 808 cm^{-1}. The matching of stretching and bending vibrations between the purified crystals and rutin standard indicate that the crystals was indeed rutin. This result was further confirmed by LC-MS analysis as will be discussed in the next section.

3.3.3 LC-MS of purified crystals

Results on LC-MS of the purified crystals are presented in Fig. 8. As shown in Fig. 8A, the total ionic chromatogram (TIC) showed only two peaks at 5.81 and 6.34 min, while the latter peaks at 12.73, 12.98 and 13.03 min were thought to be the peaks pertaining to the solvents and mobile phases used during the

analysis. To confirm this claim, diode array chromatogram of the purified compound was analyzed (Fig. 8B). Two major peaks at 5.77 and 6.32 min were noted; values within parentheses indicate their relative peak areas, showing their relative contents. The latter three peaks, which were supposed to be pertaining to the solvents and mobile phases in the TIC chromatogram (Fig. 8A), disappeared in the diode array chromatogram. This is because the diode array chromatogram shows only the phenolic compounds.

Through the use of the MSFINDER software, the two noted phenolic compounds were, respectively, rutin ($C_{27}H_{30}O_{16}$) and kaempferol rutinoside ($C_{29}H_{22}O_{14}$). Mass fragmentation pattern of rutin peak appeared at 5.797 min as the retention time is shown in Fig. 8C. In fact, rutin is represented, in LC-MS spectrum, with the base peak and a fragment peat at *m/z* value of 609 and 301, respectively (Wang et al., 2003); such kind of spectrum was observed in our study (Fig. 8C). These descriptions (Fig. 8A-8C) indicate that the purified crystals were rutin with high purity, containing minute quantity of non-rutin compounds (or, in other words, impurities).

To further determine the purity of the purified rutin, UPLC was carried out by using HPLC-grade rutin as the standard.

3.3.4 UPLC of purified crystals

Figure 9 shows UPLC chromatograms of the purified crystals and rutin standard, both exhibiting the same retention time. These chromatograms again confirm that the purified crystals were rutin of high purity. The purity of the purified rutin was noted to be 97.6% based on the comparison with the calibration curve with $R^2 > 0.997$. It is interesting to note that our purity value was within the reported values; for example, 82% in the case of rutin from cassava leaves (Chahyadi & Elfahmi, 2020), 96.5% in the case of rutin from *Edgeworthia chrysantha* flowers (Tong et al., 2009), and 98.4% purity in the case of rutin from banana leaves (Yingyuen et al., 2020). It is clear that the proposed simple and yet effective protocols of extraction and purification can be a promising method for the utilization of asparagus leaves as a source of rutin.

3.4 Rutin losses during purification process

During the purification process, liquids were discarded at filtration and centrifugation steps (as discussed above), where solids were harvested as the product of interest. Altogether, discarded liquids were collected at five locations, namely, the liquid discarded from the precipitation at the first rotary evaporation (DL-1) and at the second rotary evaporation (DL-2) as well as during the three washing steps (DL-3, DL-4, and DL-5); see Fig. 2D, 2J and 2K. Through UPLC of these discarded liquids, chromatograms with the peaks appearing at 63.2 min as the retention time were rutin peaks (Fig. 10). Rutin amounted in the first and second discarded liquids (DL-1 and DL-2) were 10.3% and 6.8%, respectively, whereas the last three washing liquids exhibited the amounts of 0.6%, 0.1% and 0.07%.

Based on the above-mentioned results, it is recommended to reuse the first two discarded liquids (DL-1, and DL-2) for further concentration and then extraction and purification. The remaining three discarded

liquids (DL-3, DL-4, and DL-5) containing minute quantities of rutin might not be economically feasible to be subject to the purification process.

4. Conclusion

The present study explored the valorization of asparagus leafy by-products as a potential source of rutin through its extraction and purification. Three methanolic extraction methods were first compared. Among them, three-step extraction was selected as the most suitable one, based on the extraction yield. Purification of the extracted rutin was then carried out through a newly proposed liquid-liquid back extraction method. Fluorescence microscopy, FTIR spectroscopy and LC-MS were used to confirm the purified yellow-color crystals as rutin; their purity was noted to be as high as 97.6%. Compared to previously reported methods, the proposed protocols are simple and yet capable of yielding comparable rutin yield of 78.2%, along with the high purity value. The proposed protocols therefore exhibit potential to serve as an economical means to valorize asparagus leafy by-products.

Declarations

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Authors Contribution: Bimal Chitrakar: Conceptualization, methodology, investigation, data curation, writing original draft; Yakun Hou: Conceptualization, methodology, data curation, writing – review and editing; Sakamon Devahastin: Methodology, writing – review and editing, supervision; Min Zhang: Writing – review and editing, supervision; Yaxin Sang: Resources, writing – review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

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Figures

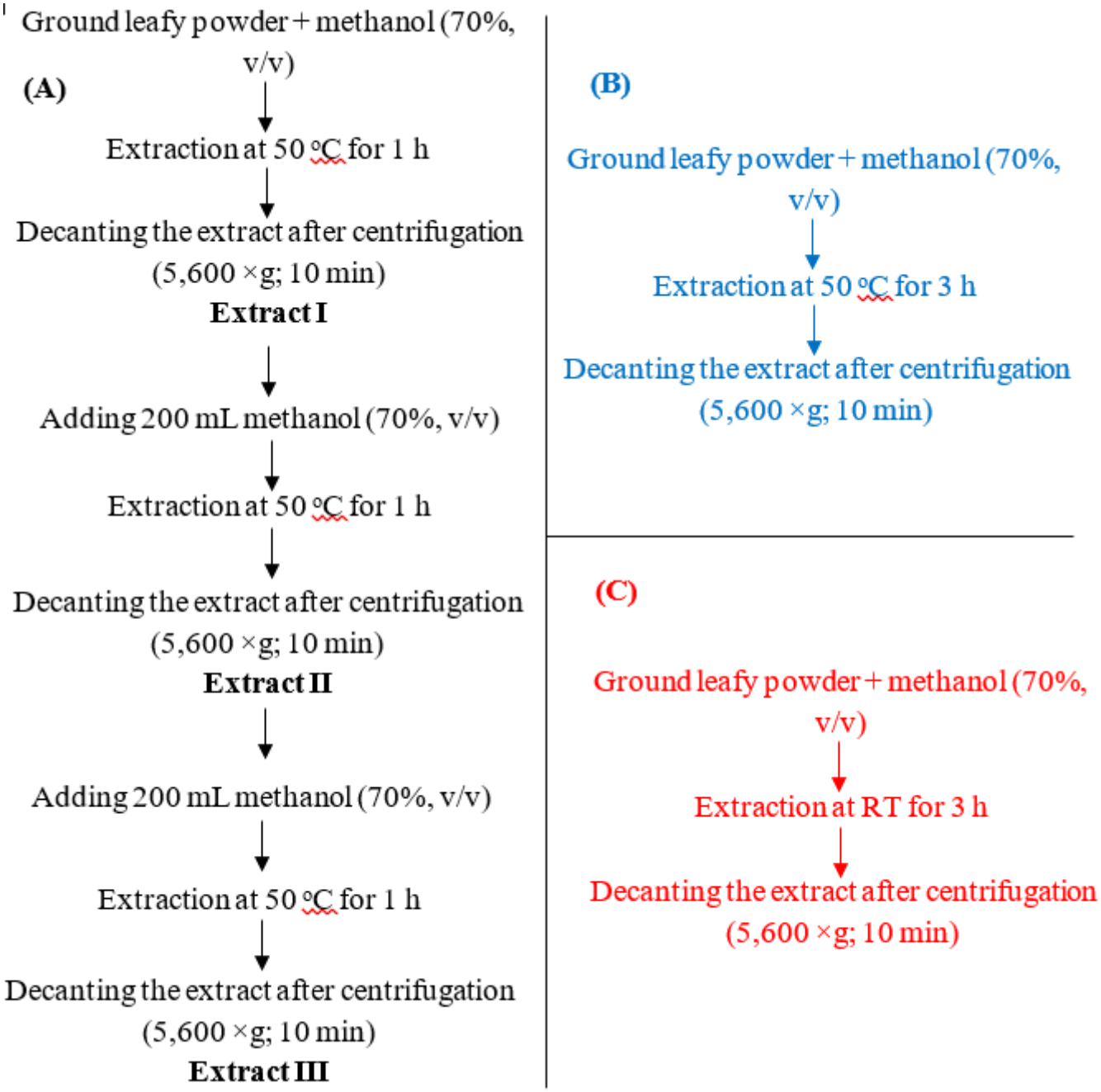


Figure 1

Differently tested methods of rutin methanolic extraction. (A) Three-step extraction at 50 °C (1 h each step); (B) Single-step extraction at 50 °C for 3 h; (C) Single-step extraction at RT for 3 h. Note: RT = room temperature.

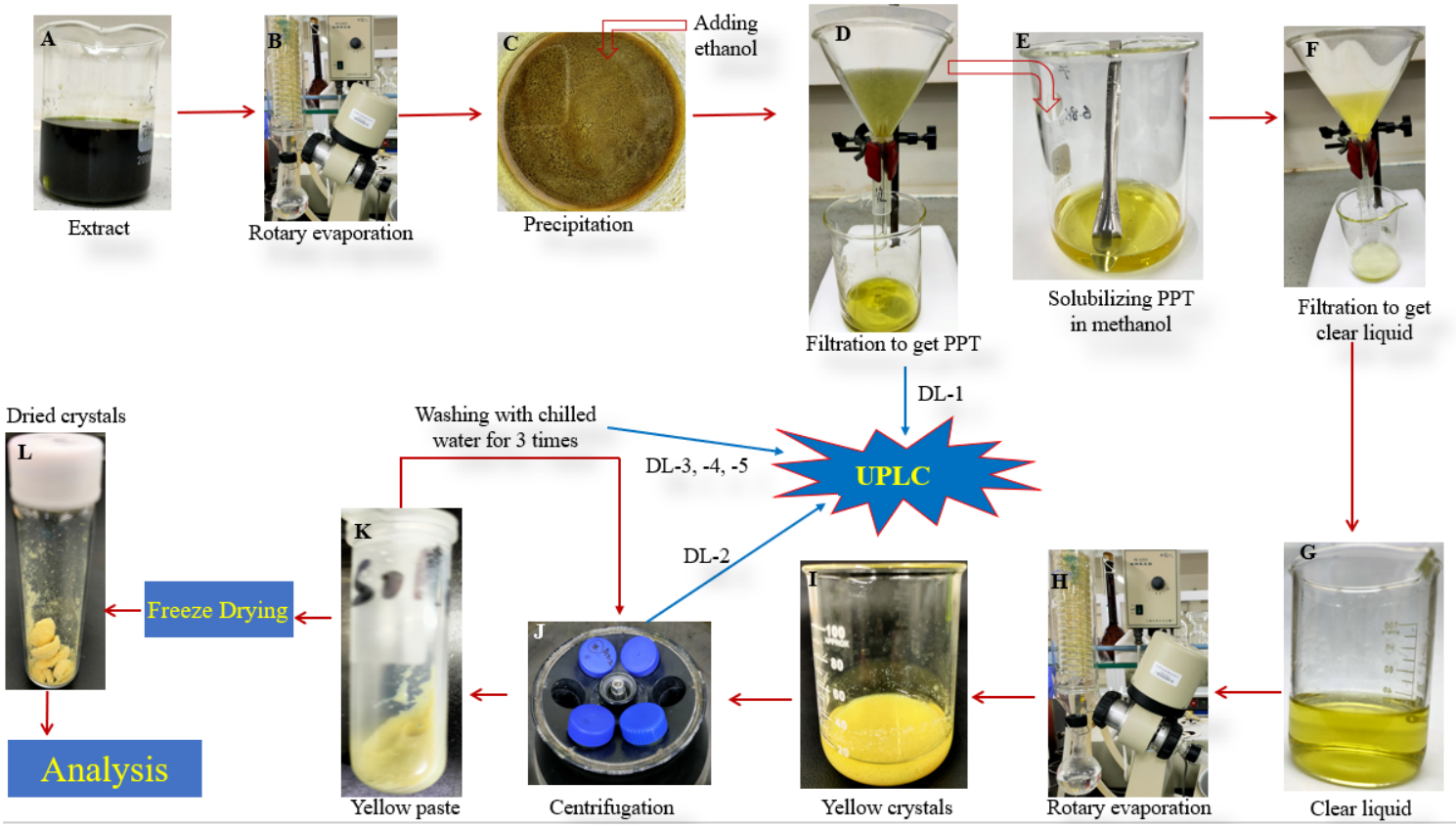


Figure 2

Step-by-step purification process of rutin, with multiple liquid-liquid back extraction using either ethanol, methanol or extra-pure water as solvents. Note: DL = discarded liquid; PPT = precipitates.

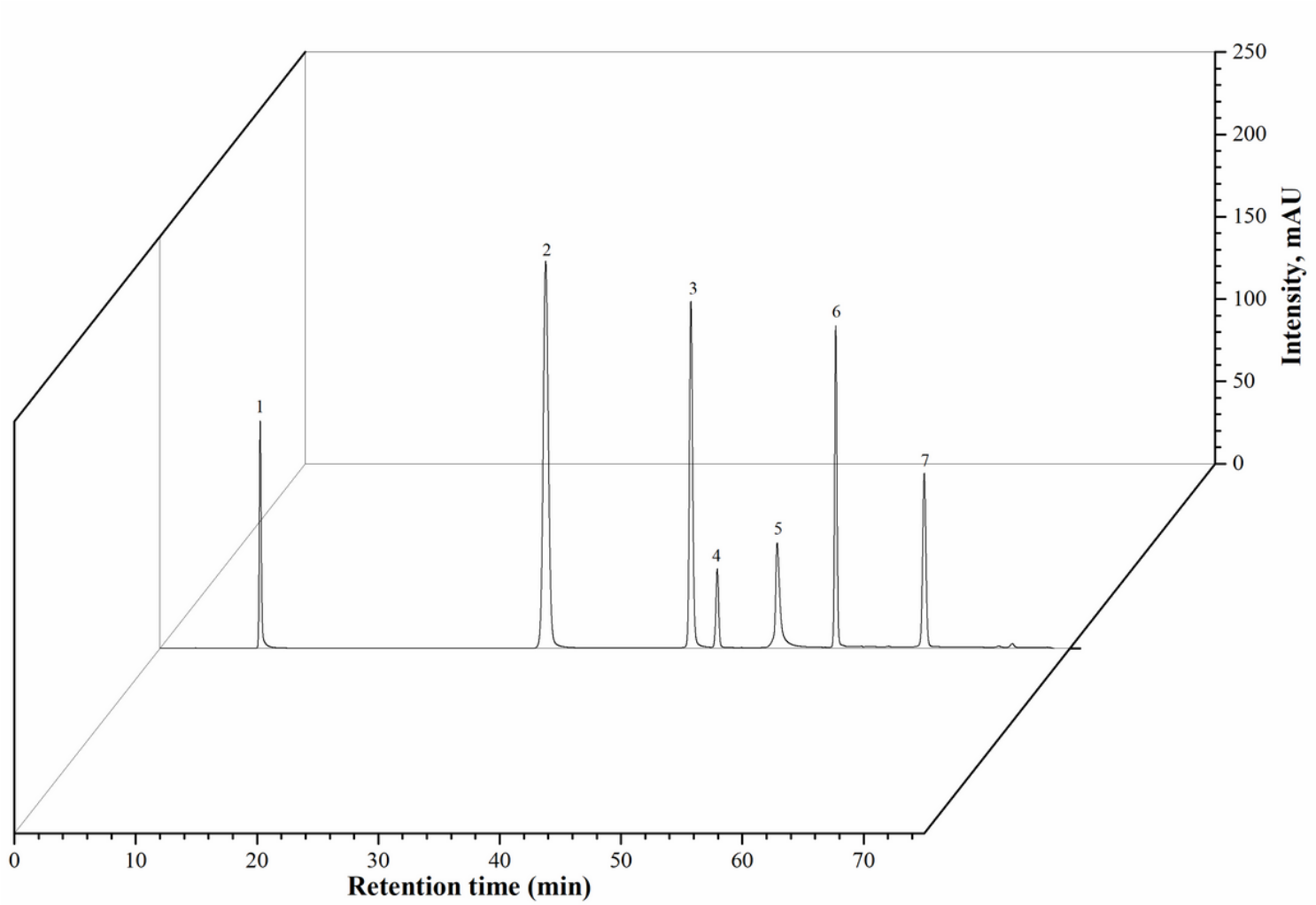


Figure 3

A chromatogram of mixed standards. 1) Gallic acid (8.21 - 8.63 min); 2) *p*-hydroxybenzoic acid (32.19 - 33.84 min); 3) Caffeic acid (43.27 - 45.49 min); 4) Vanillic acid (45.19 - 47.51 min); 5) Chlorogenic acid (49.94 - 52.50 min); 6) Ferulic acid (54.69 - 57.50 min); 7) Rutin (62.02 - 65.20 min).

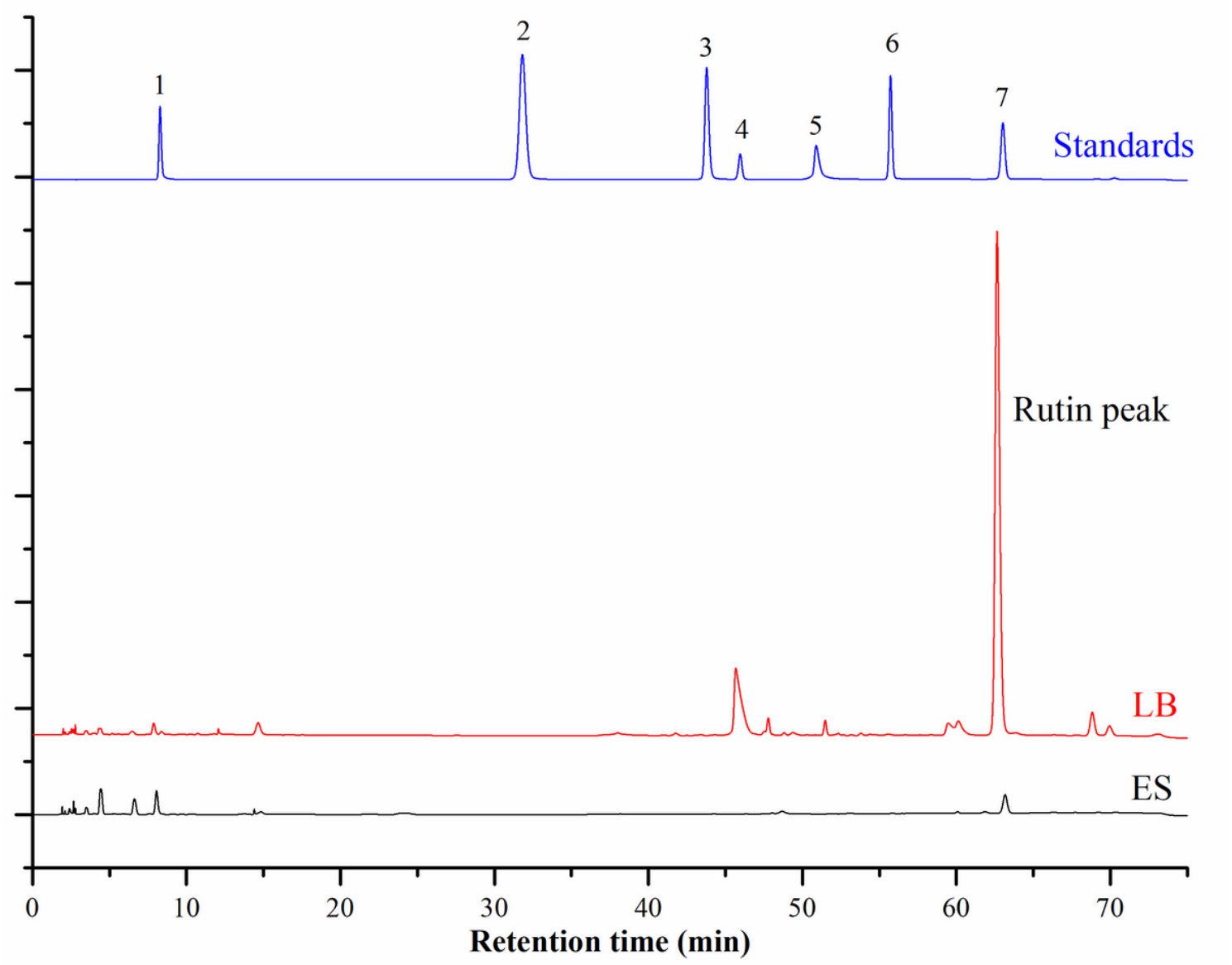


Figure 4

Chromatograms of edible spear (black chromatogram) and leafy by-products (red chromatogram) along with that of mixed standards (blue chromatogram). Standard numbers 1 to 7 correspond to those listed in Fig. 3. Note: ES = Edible spears; LB = leafy by-products.

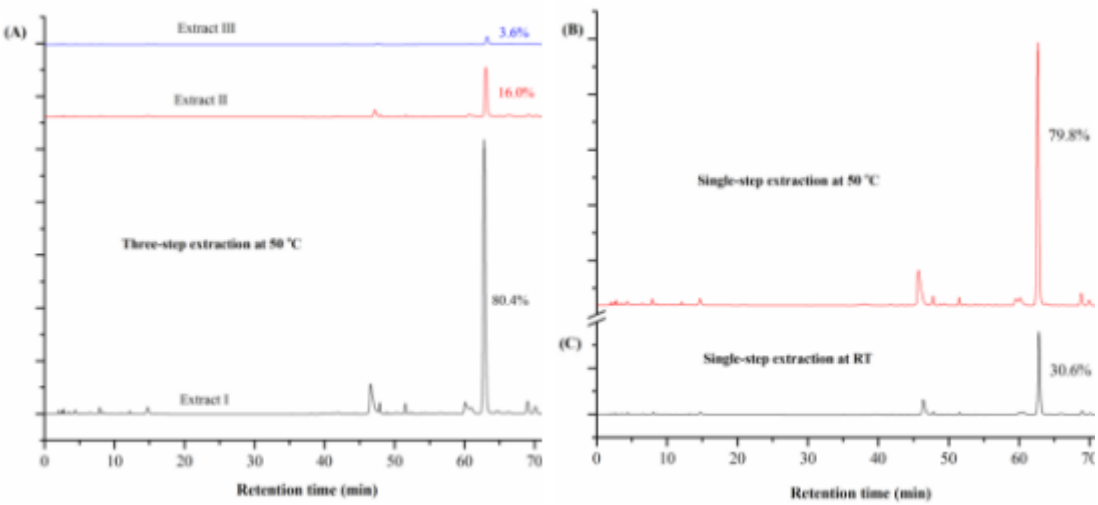


Figure 5

Chromatograms of extracts by different methods of extraction: (A) Three-step extraction at 50 ^{o}C (1 h each step); (B) Single-step extraction at 50 ^{o}C for 3 h; (C) Single-step extraction at RT for 3 h. Note: RT = room temperature.

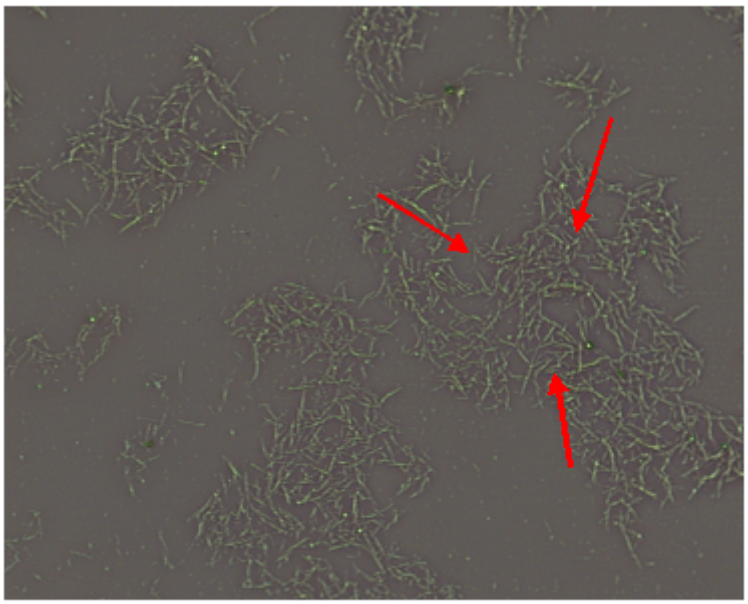


Figure 6

Microscopic view of purified crystals as seen through fluorescence microscopy.

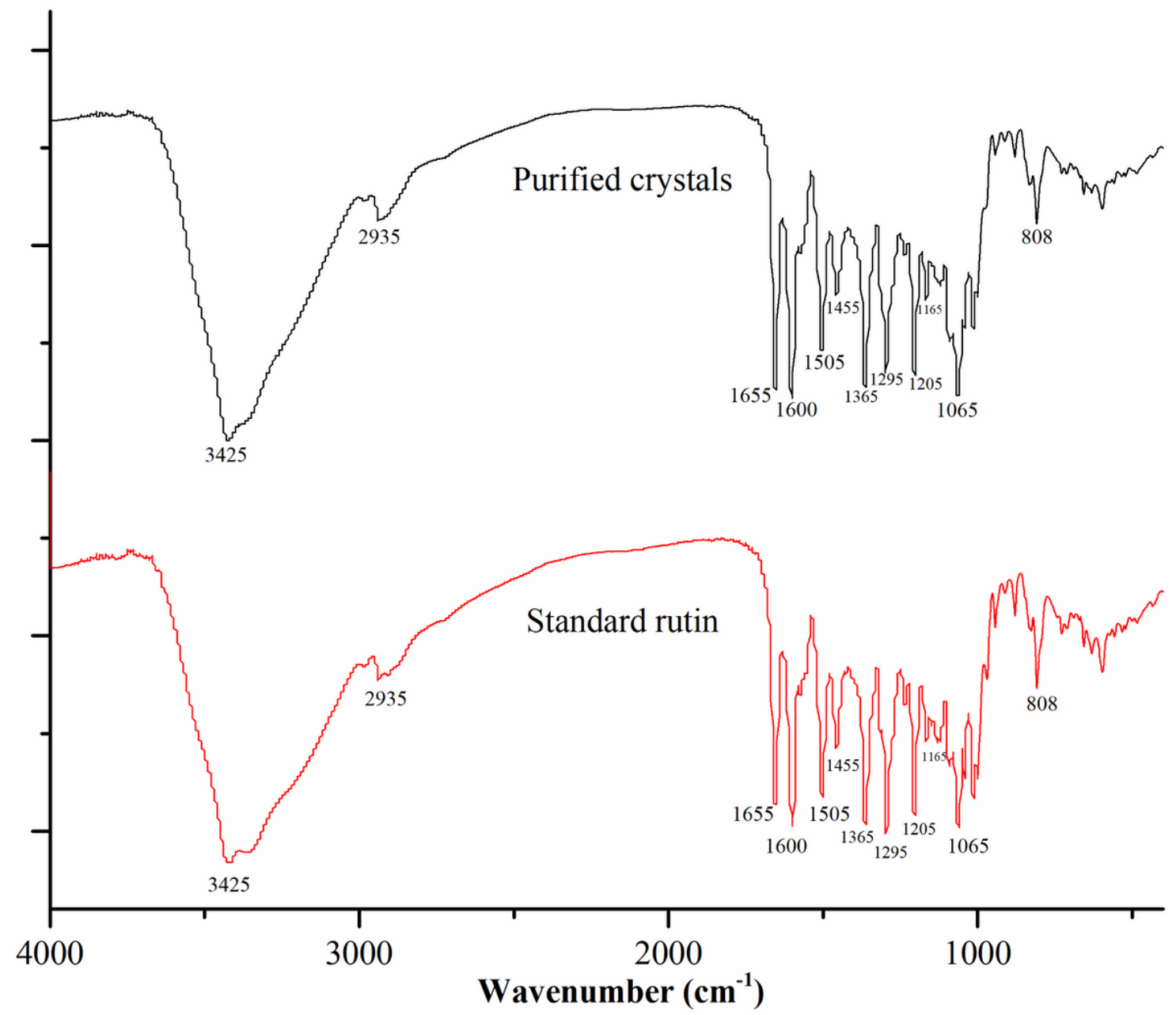


Figure 7

FTIR spectra of purified crystals (black line) and rutin standard (red line).

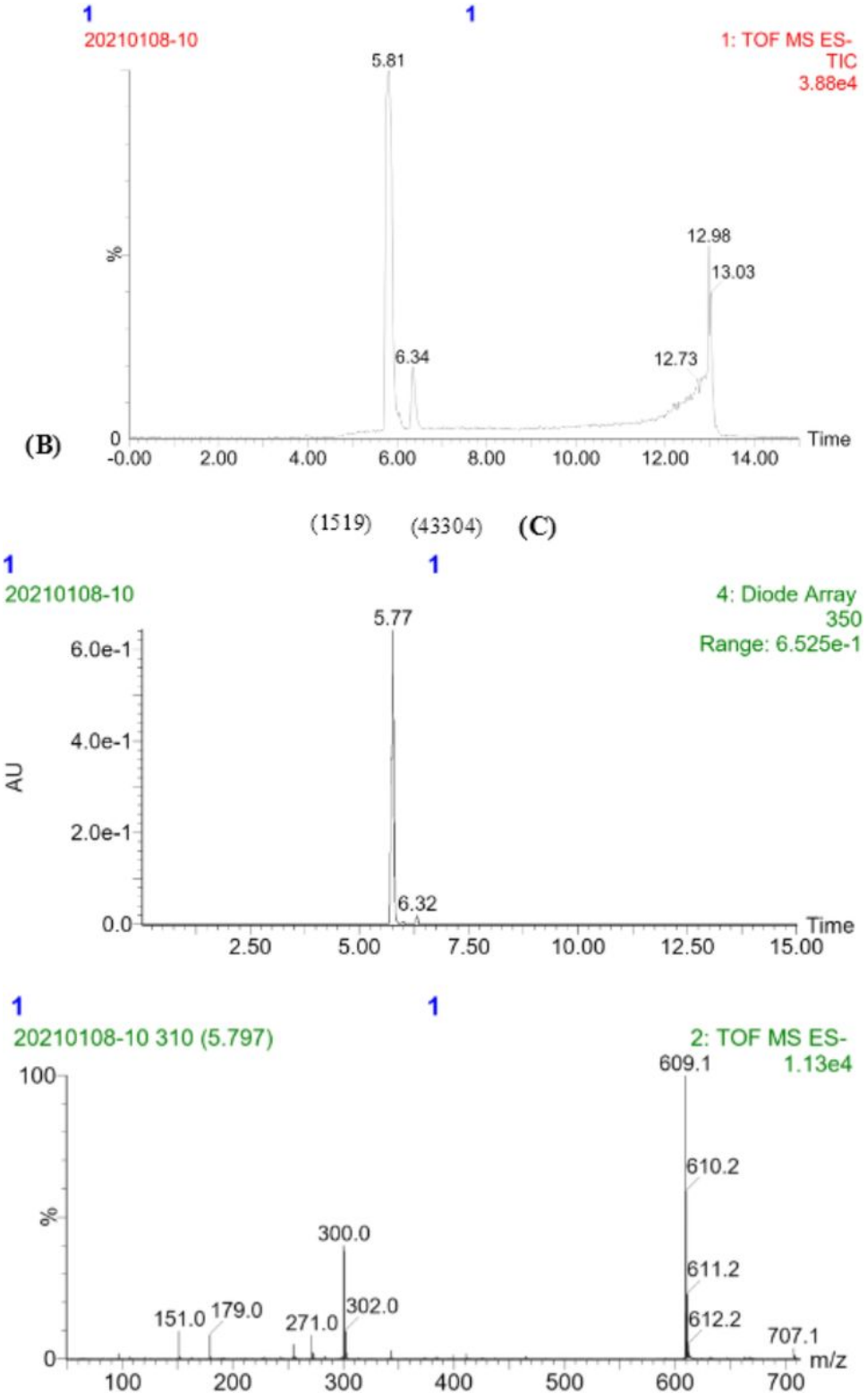


Figure 8

(A) TIC chromatogram of purified crystals; (B) Diode array chromatogram of purified crystals; (C) Mass fragmentation pattern of purified rutin peak at RT = 5.797 min.

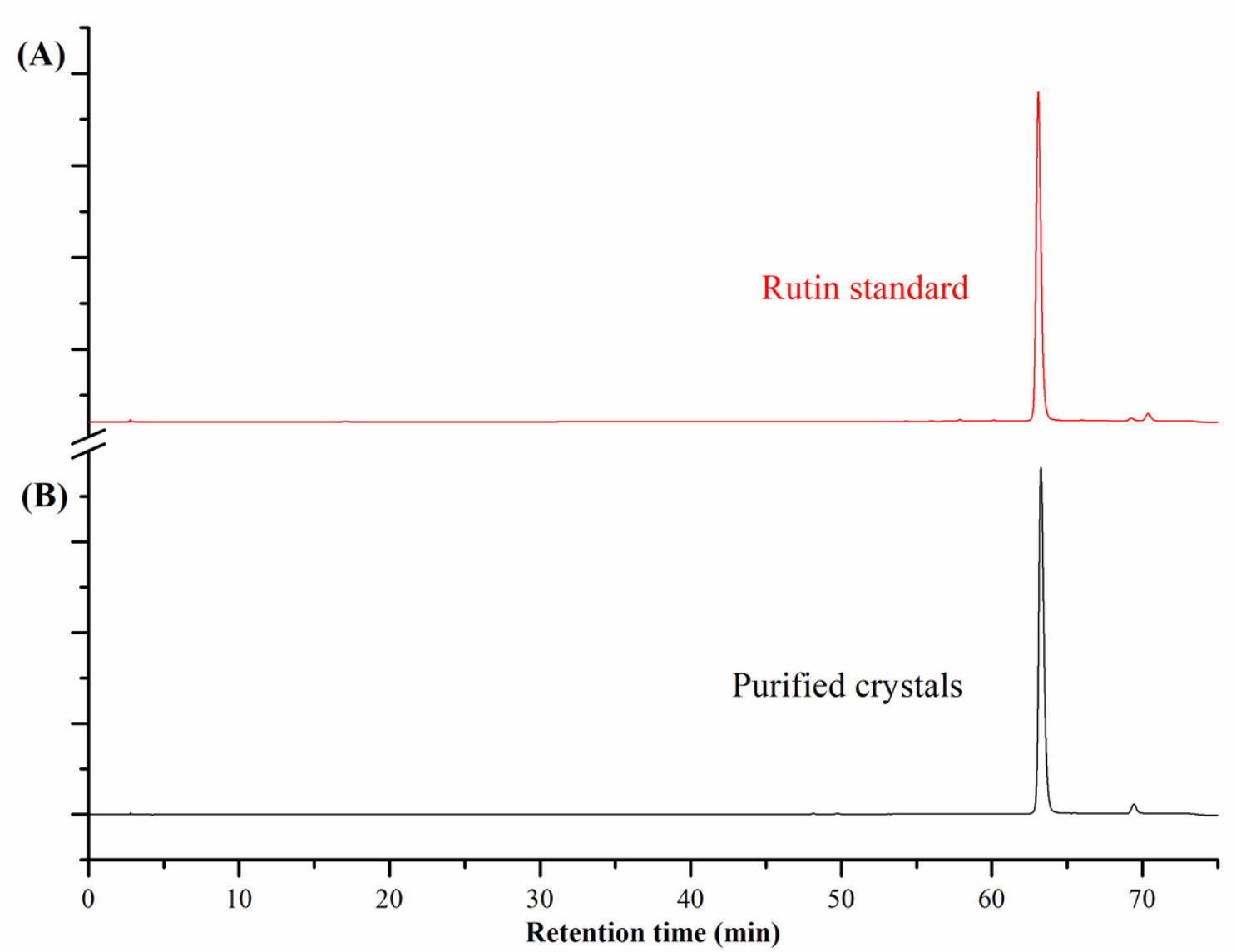


Figure 9

UPLC chromatograms of (A) rutin standard and (B) purified crystals.

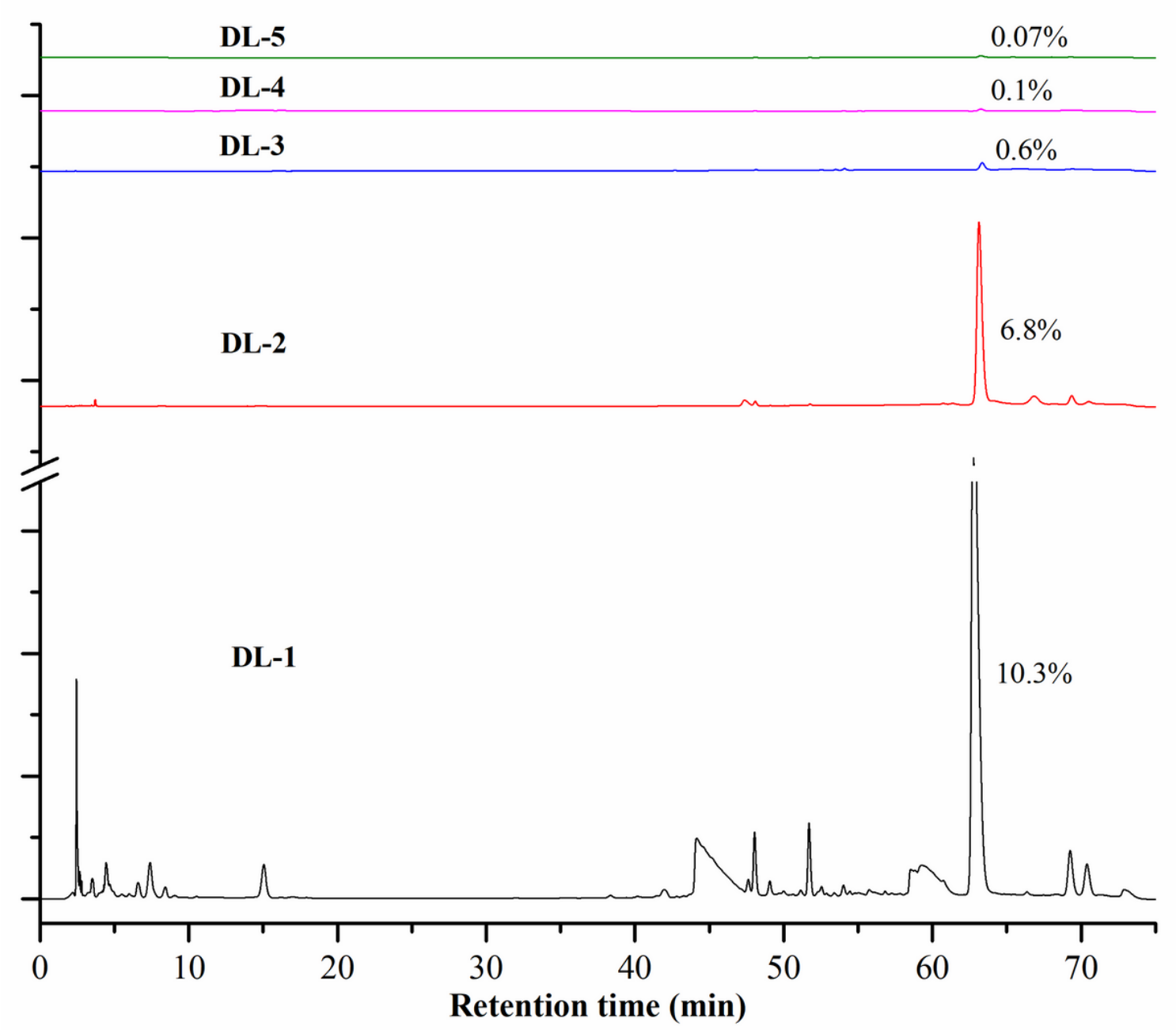


Figure 10

Chromatograms of discarded liquids during purification process with respective rutin contents (% of the amount in initial extract).