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ORIGINAL ARTICLE

HPLC-Q-Orbitrap-MS/MS phenolic profiles and biological activities of extracts from roxburgh rose (*Rosa roxburghii* Tratt.) leaves

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Abstract *Rosa roxburghii* Tratt. leaves (RRLs) have been exploited as a alternative tea product in China owing to its various biological properties. A comparative study was performed for the first time on high-performance liquid chromatography quadrupole orbitrap tandem mass spectrometry (HPLC-Q-Orbitrap-MS/MS) phenolic profiles, antioxidant, α-glucosidase inhibitory (α-GIA) and anti-bacterial activities of the RRLs extracts extracted by traditional and eco-friendly solvents. The RRLs extracts extracted with four screened deep eutectic solvents (DES) showed higher total phenolic content (TPC, 167.48–190.14 mg GAE/g DW) and moderate total flavonoid content (TFC, 3.78–4.11 mg RE/g DW), while the RRLs extracts extracted with choline chloride-1,2-propanediol, choline chloride-levulinic acid, and 50% methanol/ethanol extracts had the highest TFC. Ethyl acetate extracts had the lowest TPC and TFC. Additionally, the phenolic constituents of the RRLs extracts were identified and quantified via HPLC-Q-Orbitrap-MS/MS and HPLC-DAD methods. A total of 30 phenolic compounds were identified in RRLs extracts. Among them, arbutin, gallic acid, (+)-catechin, 3-hydroxybenzoic acid, quercetin-3-*O*-galactoside and myricetin were the representative ones. The selected DES (especially for choline chloride-lactic acid and choline chloride-levulinic acid) extracts exhibited higher 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{+\bullet}$) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) scavenging activities, cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP) except for reducing power, α-GIA and anti-bacterial activity as compared with the extracts extracted with other

Abbreviations: RRLs, *Rosa roxburghii* Tratt. leaves; DW, dried weight; DES, deep eutectic solvents; TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalent; RE, rutin equivalent; CUPRAC, cupric reducing antioxidant capacity; α-GIA, α-glucosidase inhibitory activity; MIC, minimum inhibitory concentration; HCA, hierarchical cluster analysis; PCA, principal component analysis; HPLC-Q-Orbitrap-MS/MS, high-performance liquid chromatography quadrupole orbitrap tandem mass spectrometry.

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solvents. Multivariate analysis results revealed that the extraction solvents significantly affected the phenolic constituents and biological activities of the RRLs extracts. The present study presented eco-friendly solvents for high-efficient extraction of the phenolic metabolites from RRLs. RRLs as a potential source enriched in phenolic constituents can be applied in the health, cosmetic and pharmaceutical industries.

1. Introduction

Roxburgh rose (*Rosa roxburghii* Tratt.), belonging to the *Rosaceae* family, has been widely cultivated in the plateau regions of China. Researchers have confirmed that various parts of roxburgh rose can be used as traditional herbs or functional foods. Owing to high ascorbic acid content and distinctive flavor, roxburgh rose fruits are usually used to produce various kinds of drinks, fruit wine, herbal tea and jams (Xu et al., 2019; Hou et al., 2020). *Rosa roxburghii* leaves (RRLs) have been exploited as leaf-tea product with healthcare functions. Many studies have also verified that the RRLs extracts had many pharmacological activities including scavenging ability of reactive oxygen species (ROS), anti-diabetic, anti-hypotensive, and anti-inflammatory characteristics (Akhtar et al., 2015; Wu et al., 2020a, 2020b). These healthcare functions are associated with its high phenolic/flavonoid contents (Schmitzer et al., 2012; Savic and Gajic, 2020a). At present, phenolic compounds, as a class of natural antioxidants, have attracted increasingly attentions due for their health-promoting properties (Souza et al., 2018; Savic and Gajic, 2020b). Many nutritionist advocated discovering natural antioxidants to take the place of synthetic compounds with potential risks to human health (Sahreen et al., 2010; Savic et al., 2019). At far as we know, there have been very few reports on the research of the phenolic compositions and biological activities of the RRLs.

In view of the complex composition of plant matrix, it is difficult to quantify each component of RRLs separately. The extraction solvents selected will greatly affect the amounts of the compounds extracted (Dong et al., 2015). Currently, various extraction solvents have been used to extract the phenolic compounds from plant matrix. These extraction solvents can be divided into two categories: traditional solvents (water and organic solvents) and green solvents (deep eutectic solvents and ionic liquids) (Wang et al., 2020; Lee and Row, 2016). However, organic solvents have many inherent drawbacks, such as strong volatility, high toxicity, poor bio-degradability, flammability, and high cost (Ma et al., 2020). Deep eutectic solvents (DES), as a new type of ionic liquids analogues, are also widely applied in extraction of phenolics, pigments, terpenoids and saponins from natural products (Leite et al., 2021; Pontes et al., 2021). Compared to traditional solvents, DES are not only eco-friendly, stable, less volatile but also have the advantages of easy synthesis and wide range of polarity (Bubalo et al., 2016; Cunha and Fernandes, 2018). It is no doubt that the properties of solvents will significantly affect the extraction efficiency of active compounds. The types and amounts of phenolic compounds extracted vary depending on the polarity, pH and viscosity of the extraction solvents. To the best of our knowledge, there have been no comparative research on the phenolic constituents and bioactivities of the RRLs extracts with respect to the influence of the extraction solvents.

The aim of the present study was to comprehensively investigate the phenolic profiles and multi-biological activities (antioxidant, α-glucosidase inhibitory and anti-bacterial) of RRLs extracts extracted with eco-friendly DES and traditional solvents, respectively. Additionally, phenolic compounds of RRLs extracts were identified and quantified by HPLC-Q-Orbitrap-MS/MS and HPLC-DAD for the first time. More importantly, the multivariate analysis was performed to investigate the effects of solvents on the extraction of active compounds from RRLs. Studies on RRLs will help local companies further develop the agricultural and sideline products.

2. Materials and methods

2.1. Materials and reagents

Folin-Ciocalteu reagent and chemicals used for antioxidant activity assays were acquired from Aladdin Biotechnology Co. Ltd. (Shanghai, China). Analytical-grade chemicals used in this study were purchased from Damou Chemical Reagent Co., Ltd. (Tianjin, China). Formic acid and acetonitrile used for HPLC analysis (HPLC-grade) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). RRLs were purchased from Puding Lyupin Agricultural Development Co., Ltd., (Anshun, Guizhou, China). The freshly leaf was vacuum freeze-dried for 48 h in a LGJ-10 type vacuum freeze-dryer (Beijing, China). The RRLs were ground and sieved into particles with size of less than 0.425 μm. Five tested strains including three $Gram^+$ bacteria (*Listeria monocytogenes* ATCC 51772, *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 14579) and two $Gram^-$ bacteria (*Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 14028) were purchased from Guangdong Microbial Culture Collection Center (Guangdong, China).

2.2. Preparation of deep eutectic solvents (DES)

Preparation of DES was conducted according to the method reported by Abbott et al. (2004). The DES included a mixture of two or three compounds at the suitable molar ratio. The mixture was placed in a water bath at 80 °C under continuous magnetic stirring, resulting in the formation of a homogeneous and transparent liquid (remained in liquid state after 18 h at 25 °C). The components for DES preparation are summarized in Table 1.

2.3. Extraction of phenolic compounds

In order to reduce the viscosity, the prepared DES were diluted with 30% deionized water (*v*/*v*) based on our previous assays (Wu et al., 2020a, 2020b). To evaluate the effects of extraction solvents on TPC and TFC, 1.0 g of the ground RRLs were mixed with 10 mL of the DES or traditional solvents (water, 50% ethanol, 50% methanol, and EtAc) in 15 mL tubes, respectively, and then the mixtures were sonicated at 320 W, under 40 °C for 30 min in an ultrasonic. Then, the supernatant was collected by centrifugation at 12,000*g* for 10 min. The resulting supernatant was used for the experiment.

Table 1 List of DESs prepared in this study.

No.	Abbreviation	Component A	Component B	Component C	Molar ratio (mol/mol)
1	ChCl-MA	Choline chloride	Malic acid	–	1:1
2	ChCl-Prop	Choline chloride	1,2-Propanediol	–	1:2
3	ChCl-Glu	Choline chloride	Glucose	–	5:2
4	ChCl-Xyl	Choline chloride	Xylitol	–	1:1
5	ChCl-LA	Choline chloride	Lactic acid	–	1:2
6	ChCl-Urea	Choline chloride	Urea	–	1:2
7	ChCl-Gly	Choline chloride	Glycerol	–	1:2
8	ChCl-ButG	Choline chloride	1,4-Butylene glycol	–	1:4
9	ChCl-EthG	Choline chloride	Ethylene glycol	–	1:2
10	ChCl-LevA	Choline chloride	Levulinic acid	–	1:2
11	ChCl-MA-Xyl	Choline chloride	Malic acid	Xylitol	1:1:1
12	ChCl-MA-Pro	Choline chloride	Malic acid	L-Proline	1:1:1
13	Bet-Gly	Betaine	Glycerol	–	1:1
14	Bet-CA	Betaine	Citric acid	–	1:1
15	Bet-LA	Betaine	Lactic acid	–	1:2
16	Bet-MA-LA	Betaine	Malic acid	Lactic acid	1:1:1
17	Pro-Gly	L-Proline	Glycerol	–	1:2
18	Pro-EthG	L-Proline	Ethylene glycol	–	1:2
19	Pro-LA	L-Proline	Lactic acid	–	1:2
20	Pro-LevA	L-Proline	Levulinic acid	–	1:2

2.4. Determination of total phenolic content (TPC) and total flavonoids content (TFC)

The TPC was determined by the spectrophotometric method as reported by Wang et al. (2019). Briefly, water, Folin Ciocalteu's reagent and 20% Na_2CO_3 solution were added to an aliquot of the diluted RRLs extracts in sequence. The mixture was incubated in the dark at 30 °C for 30 min followed by measurement of absorbance at 763 nm. The results were expressed as mg gallic acid equivalent per g DW (mg GAE/g DW), using a gallic acid calibration curve.

The TFC was analyzed by the protocols described by Wu et al. (2021). Briefly, ethanol, 5% $NaNO_2$ solution, 10% $AlCl_3$, and 1 M NaOH solution were added to an aliquot of the diluted RRL extracts successively. The mixture was incubated in the dark at 30 °C for 30 min, followed by measurement of absorbance at 517 nm. The results were expressed as mg rutin equivalent per g DW (mg RE/g DW), using a rutin calibration curve

2.5. HPLC-Q-Orbitrap-MS/MS analysis

One grams of the ground RRLs were mixed with 10 mL of different solvents in 15 mL tubes, and then the mixtures were sonicated at 320 W, under 40 °C for 30 min in an ultrasonic. Then, the supernatant was collected by centrifugation at 12,000*g* for 10 min. Phenolic compositions of the supernatant were identified and quantified by using HPLC-Q-Orbitrap-MS/MS (Zhu et al., 2020). The HPLC-MS/MS system comprised of an Agilent 1200 HPLC system equipped with a diode array detector and an Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). The chromatographic separation was carried out with an Zorbax Eclipse C18 plus column (250 mm × 4.6 mm, 3.5 μm, Aligent, USA). The binary mobile phase included phase A (0.1% formic acid in water) and phase B (acetonitrile), in a constant flow rate of 0.8 mL/min, with injection volume of 10 μL, under the following gradient elution conditions: 15% B, 0–5 min; 25–35% B, 5–25 min; 25–50% B, 25–40 min; 85% B, 40–45 min; and 15% B, 45–50 min. The column temperature was set to 30 °C. The Q Exactive HFX mass spectrometer was used because of its ability of acquiring MS/MS spectra on information-dependent acquisition mode in the control of the acquisition software (Xcalibur, Thermo). In this mode, the acquisition software continuously evaluated the full scan MS spectrum. The ESI source conditions were set as follow: sheath gas flow rate of 30 Arb, Aux gas flow rate of 10 Arb, capillary temperature of 350 °C, full MS resolution of 60000, MS/MS resolution of 7500, collision energy of 10/30/60 in NCE mode, spray voltage of 4.0 kV (positive) or −3.8 kV (negative). Phenolic compounds were detected at the wavelengths of 280 and 350 nm respectively. The individual compounds were quantified by using external standard calibration curves. All results were expressed as μg/g DW of RRLs.

2.6. Anti-oxidant activity assay

$ABTS^{+\bullet}$ and $DPPH^{\bullet}$ assays were performed based on our previous report (Zhu et al., 2020). The assay of reducing capacity was carried out according to the method proposed by Qin et al. (2018). Cupric reducing antioxidant capacity (CUPRAC) assays were conducted using the method reported by Saravanakumar et al. (2019). The $ABTS^{\bullet+}$/$DPPH^{\bullet}$ scavenging capacities, reducing capacity and CUPRAC values were expressed as μmol TE/g DW by using calibration curve established for Trolox. Ferric reducing antioxidant power (FRAP) was evaluated according to the method by Benzie and Strain (1996) with slight modifications. Briefly, the freshly FRAP working solution (3 M acetate buffer, 20 mM $FeCl_3{\cdot}6H_2O$ solution and 10 mM TPTZ solution at the volume radio of 10:1:1) was prepared. Afterwards, 25 μL of the extracts solution and 250 μL of the FRAP working solution were mixed and then incubated for 30 min at 20 °C, followed by measurement of absorbance at 593 nm. The FRAP value was

expressed as mM $FeSO_4$ equivalent/g DW (mM Fe(II)SE/g DW).

2.7. α-Glucosidase inhibitory activity assay

α-Glucosidase inhibitory activity (α-GIA) of the RRLs extracts was determined according to our previously reported method (Wu et al., 2020a, 2020b), where acarbose and the corresponding solvents were used as positive and negative controls, respectively. α-GIA was reported as half inhibit concentration value (IC_{50}), which was calculated by plotting inhibition-concentration curves via non-linear regression analysis.

2.8. Anti-bacterial activity assay

The anti-bacterial activity of the RRLs extracts extracted with different solvents was evaluated using the standard broth micro-dilution method reported by Boulekbache-Makhlouf et al. (2013). Minimum inhibitory concentration (MIC) refers to the lowest concentration of the extracts with no turbidity

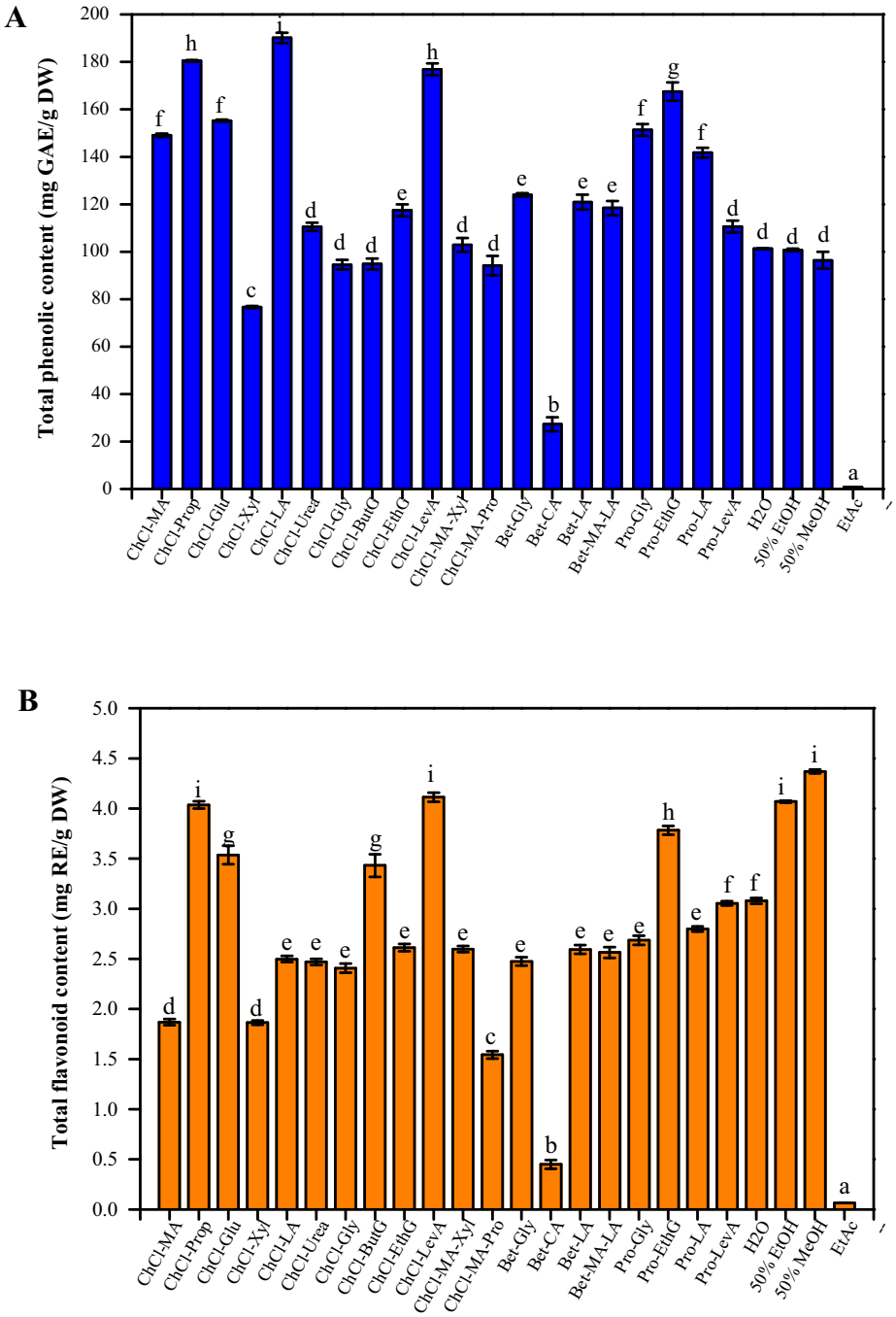

Fig. 1 TPC (A) and TFC (B) of the RRLs extracts extracted by traditional and eco-friendly deep eutectic solvents (DESs). Different lowercase letters (a-i) indicate statistically significant differences in TPC and TFC. TPC, total phenolic content; TFC, total flavonoid content.

observed in Luria-Bertani (LB) media. The bacteria were grown in LB media at 35 °C for 24 h, and then adjusted to the concentration of about 1×10^6 CFU/mL. The diluted RRLs extracts of various concentrations were used for MIC assays. Briefly, 100 μL of the diluted bacteria suspension was mixed with 50 μL of the diluted RRL extracts and 50 μL of LB culture in a 96-well plate, followed by incubation overnight (20 h) at 35 °C. Tetracycline hydrochloride and the extraction solvents were used as positive and negative control, respectively.

2.9. Statistical analysis

All results were expressed as the mean ± standard deviation based on three replicates. One way analysis of variance (ANOVA) and Duncan's multiple range test were carried out for comparison of difference. The differences were considered statistically significant at $p < 0.05$. Statistical analysis and multivariate analysis were performed by using IBM SPSS Statistics (Version 20.0, IBM Corp., NY, USA).

3. Results and discussion

3.1. TPC and TFC

Many researches confirmed that physico-chemical characteristics (viscosity, polarity, and pH, etc) of solvents greatly affected their extraction efficiency for phenolic compounds (Ma et al., 2020; Viell et al., 2020). In this study, different types of DES and traditional solvents were used and their extraction yields of phenolic compounds from RRLs were evaluated. As expected, TPC and TFC of the RRLs extracts were influenced by the extraction solvents used (Fig. 1A and B). Among these DESs, ChCl-Prop, ChCl-LA, ChCl-LevA and Pro-EthG all exhibited higher extraction efficiency and brought higher TPC (ranging from 167.48 to 190.14 mg GAE/g), indicating RRLs were a good source of polyphenols. ChCl-LA extracts had the highest contents of total phenolics (190.14 ± 2.15 mg GAE/g DW), but only have moderate extraction efficiency in total flavonoids (2.50 ± 0.03 mg RE/g DW). Bet-CA extracts exhibited the lowest TPC (27.33 mg GAE/g DW) and TFC (0.45 mg RE/g DW) compared to extracts extracted with other DES. For traditional solvents, extracts extracted with 50% MeOH and 50% EtOH exhibited high TFC (4.07–4.37 mg RE/g DW), but only had moderate TPC (96.40–100.73 mg GAE/g DW). Of those extracts extracted with traditional solvents, EtAc extracts indicated the lowest TPC (0.79 mg GAE/g DW) and TFC (0.06 mg GAE/g DW).

Due to the complex composition of plant matrix, it is difficult to quantify each component separately. Normally, the amounts of total phenolic/flavonoids extracted from plant matrix are related to the polarity of the extraction solvents and the solubility of those components in the solvent (Lim et al., 2019). The polarity of these traditional solvents can be ranked as water > MeOH > EtOH > acetone ≥ EtAc (Benzie et al., 1996; Wang et al., 2020). However, it should be noted that water having good polarity did not show high efficiency in extraction of phenolics/flavonoids. In addition, some compounds (i.e. flavonoid aglycones) with poor water solubility were very difficult to be extracted by using water. Sarikurkcu et al. (2020) investigated the effects of solvents (EtAc, MeOH and water) on the extraction of phenolic compounds from *Onosma pulchra* Riedl, and verified that MeOH was the best solvent. They also found that the EtAc had the worst extraction efficiency for phenolic compounds, which was consistent with the result of our study. However, Pintać et al. (2018) found that EtAc indicated the excellent extraction efficiency for phenolic compounds from grape pomace, which may be due to the difference in solubility of the components in the solvents. According to previous reports, DES with broad polarity and good solubility exhibited excellent efficiency in extraction of active compounds from various natural products, which was consistent with the results of our study (Wu et al., 2021). Additionally, low viscous or acidic-based DES (ChCl-LA and ChCl-LevA) had higher efficiency in extracting the active compounds from RRLs than other sugar- or alkaline-based DES. Viscosity of DES greatly affected the mass- and energy transfer in the phases, and thereby affecting the extraction efficiency of phenolic compounds (Wu et al., 2020a, 2020b, 2021). In conclusion, the extraction efficiency and amounts of phenolic compounds are not only related to the type and viscosity of solvents, but also associated with the solubility of those components in these solvents (Suchinina et al., 2011).

3.2. Phenolic compositions

The results of HPLC-Q-Orbitrap-MS/MS analysis of the RRLs extracts are shown in Fig. 2 and Table 2. Compound 1 (t_R 2.683 min, m/z 271.10 $[C_{12}H_{16}O_7+H]^+$) was easily identified as arbutin by comparing with the retention time of standard and MS/MS fragment ions. Compound 4 (t_R 3.253 min, m/z 127.01 $[C_6H_6O_3\text{-}H]^-$) was tentatively assigned as 1,2,3-trihydroxybenzene by analyzing its MS/MS fragment ions. Compound 5 (t_R 3.843 min, m/z 169.15 $[C_7H_6O_5\text{-}H]^-$) was easily identified as gallic acid by comparing with mass spectrometry data of the published study (Wu et al., 2019). Compound 6 (t_R 3.983 min) was identified as 3-chlorogenic acid because of its fragment ion at m/z of 353.09 $[C_{10}H_{10}O_4\text{-}H]^-$.

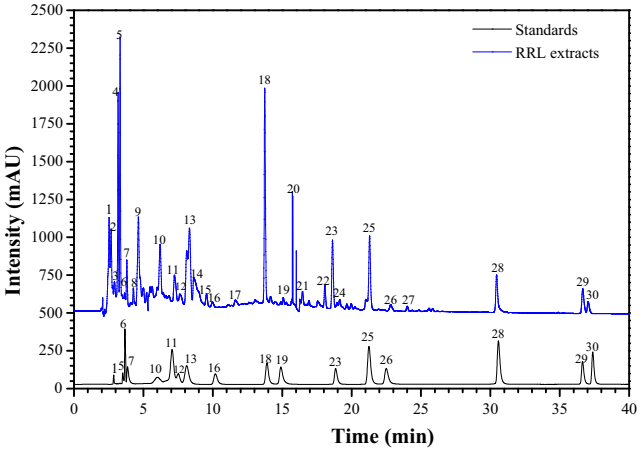

Fig. 2 HPLC-Q-Orbitrap-MS/MS phenolic profiles of the RRLs extracts and the standards. 1, Arbutin; 5, Gallic acid; 6, 3-Chlorogenic acid; 7, (+)-Catechin; 10, Protocatechuic acid; 11, Epicatechin; 12, Caffeic acid; 13, 3-Hydroxybenzoic acid; 16, Vanillin; 18, Quercetin-3-*O*-galactoside; 19, Ellagic acid; 23, Kaempferol-3-*O*-glucoside; 25, *m*-Coumaric acid; 26, Myricetin; 28, Quercetin; 29, Isorhamnetin; 30, Kaempferol.

Table 2 Identification of the main the main phenolic compounds from RRLs.

No.	Retention time (min)	λ_{max} (nm)	Molecular ion (m/z)	MS ion fraction (m/z)	Mw	Formula	Compounds	Error	Identification
1	2.683	245, 280	271.10 $[M-H]^-$	271.10, 125.10, 143.01	272	$C_{12}H_{16}O_7$	Arbutin	0.12	Standard, MS/MS
2	2.917	215	179.23 $[M-H]^-$	179.23, 115.12, 87.29	180	$C_8H_8O_6$	–	–	MS/MS
3	3.021	256	333.11 $[M-H]^-$	333.11, 171.20, 153.10	334	–	–	–	MS/MS
4	3.253	280	127.01 $[M+H]^+$	127.01, 109.21, 81.37	126	$C_6H_6O_3$	1,2,3-Trihydroxybenzene	1.38	MS/MS
5	3.843	245, 278	169.15 $[M-H]^-$	169.15, 125.03, 97.27, 81.32	170	$C_7H_6O_5$	Gallic acid	2.13	Standard, MS/MS
6	3.983	215, 268	353.09 $[M-H]^-$	353.09, 191.06, 185.05	354	$C_{10}H_{10}O_4$	3-Chlorogenic acid	0.57	Standard, MS/MS
7	4.119	260, 280	289.70[M-H]$^-$	289.07	290	$C_{15}H_{14}O_6$	(+)-catechin	−0.35	Standard, MS/MS
8	4.213	260, 280	369.10 $[M+H]^+$	369.10, 177.12, 145.23	368	$C_{17}H_{20}O_9$	3-*O*-Feruloylquinic acid	0.98	MS/MS
9	4.539	280	337.12 $[M+H]^+$	337.12, 201.03	336	$C_{16}H_{16}O_8$	–	–	MS/MS
10	6.018	260, 281	153.02 $[M-H]^-$	153.02, 108.02	154	$C_7H_6O_4$	Protocatechuic acid	1.54	Standard, MS/MS
11	7.085	256, 280	289.71 $[M-H]^-$	289.71, 245.08	290	$C_{15}H_{14}O_6$	L-Epicatechin	2.11	Standard, MS/MS
12	7.629	214, 280	179.04 $[M-H]^-$	179.04, 135.05	180	$C_9H_8O_4$	Caffeic acid	0.71	Standard, MS/MS
13	8.400	208, 279	137.0254 $[M-H]^-$	137.0254	138	$C_7H_6O_3$	3-Hydroxybenzoic acid	0.43	Standard, MS/MS
14	8.532	258, 360	617.10 $[M-H]^-$	617.10, 303.21, 153.20	618	–	–	–	MS/MS
15	9.219	254, 279	337.10 $[M-H]^-$	337.10, 191.13, 163.21	338	$C_{16}H_{18}O_8$	3-*O*-p-Coumaroylquinic acid	3.15	MS/MS
16	10.334	214, 260	147.15 $[M+H]^+$	147.15	146	$C_9H_6O_2$	*p*-Coumarin	1.07	Standard, MS/MS
17	11.879	254, 350	433.01 $[M+H]^+$	433.01, 301.10, 271.12, 163.05	434	$C_{20}H_{18}O_{10}$	Quercetin 3-arabinoside	−0.32	MS/MS
18	14.596	257, 352	463.37 $[M-H]^-$	463.37, 301.11, 159.03, 151.20	464	$C_{21}H_{20}O_{12}$	Quercetin-3-*O*-galactoside	−0.91	Standard, MS/MS
19	15.833	256, 350	303.05 $[M+H]^+$	303.05, 193.12	302	$C_{14}H_6O_8$	Ellagic acid	0.08	Standard, MS/MS
20	15.945	256, 350	505.12 $[M-H]^-$	505.42, 301.21, 271.03, 161.21	506	$C_{23}H_{22}O_{13}$	Quercetin 3-*O*-(6′′-acetyl-glucoside)	1.78	MS/MS
21	16.537	256, 350	478.20 $[M-H]^-$	478.20, 317.10, 302.10, 161.10, 153.61	479	$C_{22}H_{22}O_{12}$	Isorhamnetin 7-glucoside	1.25	MS/MS
22	18.209	258, 350	601.10 $[M-H]^-$	601.10, 315.10, 286.13, 153.21	602	–	Kaempferol 7-(6′′-galloylglucoside)	3.05	MS/MS
23	19.012	254, 350	449.11 $[M+H]^+$	449.11, 287.06, 161.05	448	$C_{21}H_{20}O_{11}$	Kaempferol-3-*O*-glucoside	0.46	Standard, MS/MS
24	19.213	260, 360	449.10 $[M+H]^+$	449.10, 287.10, 153.02	448	$C_{21}H_{20}O_{11}$	Luteolin-4′-glucoside	2.87	MS/MS
25	21.609	254, 280	165.50 $[M+H]^+$	165.05, 119.05	164	$C_9H_8O_3$	*m*-Coumaric acid	0.43	Standard, MS/MS
26	23.317	254, 350	319.60 $[M+H]^+$	320.60, 319.60, 273.10, 179.21	318	$C_{15}H_{10}O_8$	Myricetin	0.11	Standard, MS/MS
27	24.078	258, 360	271.11 $[M-H]^-$	271.11, 256.21, 151.32	272	$C_{15}H_{12}O_6$	–	–	MS/MS
28	31.311	256, 367	303.07 $[M+H]^+$	303.15, 137.62	303	$C_{15}H_{10}O_7$	Quercetin	0.08	Standard, MS/MS
29	37.233	257, 350	316.17 $[M-H]^-$	316.17, 229.13, 153.21	316	$C_{16}H_{12}O_7$	Isorhamnetin	0.12	Standard, MS/MS
30	37.843	254, 364	287.27 $[M+H]^+$	287.27	286	$C_{15}H_{10}O_6$	Kaempferol	0.17	Standard, MS/MS

Compounds 7 and 11, two isomers of catechins (t_R 4.119 and 7.085 min, 289.70 [$C_{15}H_{14}O_6$-H]$^-$), were identified as (+)-catechin and L-epicatechin by comparing them with retention time of the standards. Compound 8 (t_R 4.213 min, *m/z* 369.10 [$C_{17}H_{20}O_9$ + H]$^+$) was tentatively assigned as 3-*O*-feruloylquinic acid by referring to related reference (Schwarz et al., 2021). Compound 10 (t_R 6.018 min, *m/z* 153.02 [$C_7H_6O_4$-H]$^-$) was easily verified as protocatechuic acid. Compounds 12 (t_R 7.629, *m/z* 179.0420 [$C_9H_8O_4$-H]$^-$) and 13 (t_R 8.400, *m/z* 137.02 [$C_7H_6O_3$-H]$^-$) were easily determined as caffeic acid and 3-hydroxybenzoic acid by comparing with authentic standards. Compound 15 (t_R 3.253 min, *m/z* 337.10 [$C_{16}H_{18}O_8$-H]$^-$) was temporarily assigned as 3-*O*-*p*-coumaroylquinic acid by analyzing its mass fragment ions. Compound 16 (t_R 10.334) indicating the molecular ion at *m/z* 147.15 [$C_9H_6O_2$ + H]$^+$ was identified to be *p*-coumarin. Compound 17 (t_R 11.879 min, *m/z* 433.01 [$C_{20}H_{18}O_{10}$ + H]$^+$) showed fragment ions at *m/z* of 301.11 [$C_{15}H_{10}O_7$-H]$^-$ and 151.20 [M−$C_{15}H_{10}O_7$−H]$^-$ corresponding to quercetin-3-arabinoside (Wang et al., 2017). Compound 18 (t_R 14.596 min, *m/z* 463.37 [$C_{21}H_{20}O_{12}$ + H]$^+$) indicated fragment ions at *m/z* of 271.12, 300.11 [$C_{15}H_{10}O_7$-H]$^-$ and 151.20 [gla-H]$^-$ corresponding to quercetin-3-*O*-galactoside. Compound 19 (t_R 15.833 min), showing the parent ion at 303.05 [M + H]$^+$, was easily verified as ellagic acid by comparing with authentic standard. Compound 20 (t_R 15.945 min, *m/z* of 505.12 [$C_{23}H_{22}O_{13}$-H]$^-$), was tentatively characterized as quercetin 3-*O*-(6″-acetyl-glucoside) because of its fragment ions at *m/z* of 301.11 [$C_{15}H_{10}O_7$-H]$^-$, corresponding to the quercetin moiety and at *m/z* of 161.21 (missing of glucose). Compound 21, indicating the parent ion at 478.20 [$C_{22}H_{22}O_{12}$-H]$^-$ plus the fragment ions at *m/z* 317.10 [$C_{16}H_{12}O_7$-H]$^-$ and 161.10 [glc + H]$^+$, was temporarily assigned as isorhamnetin-7-glucoside. Compound 22 (t_R 18.209 min, *m/z* of 601.10 [M−H]$^-$), which indicated fragment ions at *m/z* of 286.13 [$C_{15}H_{10}O_6$-H]$^-$ corresponding to the kaempferol moiety and at *m/z* of 315.10 [M−$C_{15}H_{10}O_6$−H]$^-$, was temporarily assigned as kaempferol-7-(6″-galloylglucoside). Compound 23 (t_R 19.012 min, *m/z* 449.11 [$C_{21}H_{20}O_{11}$ + H]$^+$) indicated fragment ions at *m/z* of 287.0611 [$C_{15}H_{10}O_6$ + H]$^+$ and *m/z* 161.05 [glc + H]$^+$ corresponding to kaempferol-3-*O*-glucoside. Compound 24 (t_R 19.213 min) indicating the parent ion at *m/z* 449.10 [M + H]$^+$) produced fragment ions at *m/z* of 287.10 [$C_{15}H_{10}O_6$ + H]$^+$ and *m/z* 161.02 [M−$C_{15}H_{10}O_6$ + H]$^+$, was temporarily assigned as luteolin-4′-glucoside. Compound 25 (t_R 21.609 min, 165.50 [$C_9H_8O_3$ + H]$^+$) was identified as *m*-coumaric acid by comparing with the retention time of the standard. Compound 26 (t_R 23.317 min) showed fragment ions at *m/z* of 319.60 [$C_{15}H_{10}O_8$ + H]$^+$) corresponding to myricetin by comparing with the standard. Compounds 28 (t_R 31.311 min, 303.07 [$C_{15}H_{10}O_7$ + H]$^+$), 29 (t_R 37.233 min, 316.17 [$C_{16}H_{12}O_7$-H]$^-$), and 30 (t_R 37.843 min, 287.27 [$C_{15}H_{10}O_6$ + H]$^+$) were easily verified as quercetin, isorhamnetin and kaempferol by comparing with authentic standards. Compounds 2, 3, 9, 14 and 27 could not be identified temporarily, but compounds 14 and 27 could be inferred as flavonoid compounds based on their UV–vis spectrum (λ_{max} = 258 nm and 360 nm).

As shown in Table 3 and Fig. S1, the extraction solvents greatly affected the compositions and contents of phenolic compounds extracted from the RRLs. Regarding organic extraction solvents used in this study, it can be seen that all of extraction solvents except for EtAc could help obtain much components. 50% MeOH/EtOH extracts showed certain similarities in the number of extracted compounds, but had the difference in the contents of compounds. 50% MeOH showed excellent extraction efficiency for gallic acid (1832.56 μg/g DW), 3-chlorogenic acid (1592.57 μg/g DW) and epicatechin (1505.15 μg/g DW). EtAc extracts exhibited the least number of compounds and the lowest contents of chemical components. Water exhibited the moderate extraction efficiency for phenolic compounds especially for 3-chlorogenic acid (1302.81 μg/g DW) and catechin (1629.63 μg/g DW). In additions, the contents of most compounds in traditional solvents extracts were lower than those in selected DES-based extracts. Compared with other solvents, ChCl-LA had good extraction efficiency for arbutin (19333.18 μg/g DW), caffeic acid (619.82 μg/g DW), 3-hydroxybenzoic acid (4860.16 μg/g DW), quercetin-3-*O*-galactoside (778.12 μg/g DW), quercetin (221.92 μg/g DW), isorhamnetin (161.51 μg/g DW) and kaempferol (90.98 μg/g DW). Especially, two DES (Pro-EthG and ChCl-LevA) extracts had the highest contents of (+)-catechin and *m*-coumaric acid. However, epicatechin was not almost detected in ChCl-LA and Pro-EthG extracts. ChCl-Prop also showed good extraction efficiency for arbutin (17110.87 μg/g DW), epicatechin (3537.51 μg/g DW) and 3-hydroxybenzoic acid (2958.36 μg/g DW).

In this study, it was found that four DES had significantly higher efficiency in extraction of most of phenolic compounds from RRLs compared with traditional solvents. The amounts of phenolic compounds extracted were affected by the polarity of the solvent and the solubility of those components in the solvent (Wu et al., 2020a, 2020b; Suchinina et al., 2011). Eco-friendly DES, which has a wide range of polarity and good solubility for water-insoluble compounds, extracted higher contents of active components from RRLs than other solvents. In additions, some water-insoluble flavonoids (such as quercetin-3-*O*-galactoside, quercetin, isorhamnetin and kaempferol) were almost not detected in water extracts, which was consistent with the results of the previous study (Ma et al., 2020). Our result was also similar to the research results of Zhu et al. (2020) who reported EtAc extracts contained the lowest quantities of phenolic components. In conclusion, the types and amounts of extracted compounds are related to the extraction solvents used (Suchinina et al., 2011; Llorent-Martíneza et al., 2020; Wu et al., 2020a, 2020b). Many studies have confirmed that using suitable DES instead of organic solvents can achieve efficient extraction of active components from medicinal plants.

3.3. Antioxidant activity

The antioxidant activities of the RRLs extracts extracted with different solvents are shown in Table 4. Three types of DES (ChCl-LA, ChCl-LevA and Pro-EthG) extracts showed stronger scavenging capacity for ABTS$^{+\bullet}$ (1800.34–2044.96 μmol TE/g DW), DPPH$^\bullet$ (1117.79–1322.00 μmol TE/g DW), and higher FRAP values (5386.76–5853.27 μM Fe(II)E/g DW) than extracts extracted with other solvents. In terms of reducing capacity, the highest reducing capacity was observed in 50% MeOH/EtOH extracts (26.82 mmol TE/g DW), followed by water extracts (18.52 mmol TE/g DW), and DES extracts (8.67–11.22 mmol TE/g DW). Additionally, high CUPRAC

Table 3 The contents of the main phenolic compounds of RRLs extracts with different solvents.

Contents	Extraction solvents							
	H_2O	50% MeOH	50% EtOH	EtAC	ChCl-Prop	ChCl-LA	ChCl-LevA	Pro-EthG
Arbutin	646.24 ± 26.28b	1541.81 ± 261.09d	1201.43 ± 32.95c	506.13 ± 46.30a	17110.87 ± 868.92f	19333.18 ± 760.84h	12958.62 ± 246.59e	18155.90 ± 808.22g
Gallic acid	706.52 ± 19.49cd	1002.97 ± 1.92f	1592.57 ± 12.00g	118.60 ± 9.19a	793.64 ± 5.28e	535.30 ± 21.72b	733.45 ± 25.61d	670.93 ± 59.07c
Chlorogenic acid	1302.81 ± 22.82g	568.86 ± 15.59d	1832.56 ± 123.91h	185.68 ± 15.16a	475.85 ± 30.68c	693.56 ± 31.82e	352.73 ± 14.06b	973.28 ± 18.01f
(+)-Catechin	1629.63 ± 29.81d	1150.19 ± 77.07c	1627.10 ± 43.87d	128.67 ± 11.50a	805.42 ± 34.23b	1272.61 ± 48.73c	6041.55 ± 369.68f	5132.04 ± 936.18e
Protocatechuic acid	67.73 ± 5.57c	333.80 ± 14.76f	0.32 ± 0.04a	0.00	5.70 ± 1.94b	254.18 ± 18.23e	138.44 ± 11.86d	869.08 ± 869.44g
Epicatechin	214.38 ± 52.56a	1746.70 ± 324.68d	1505.15 ± 211.74c	0.00	3537.51 ± 761.51e	0.00	1027.34 ± 103.94b	0.00
Caffeic acid	547.03 ± 15.03d	152.81 ± 24.54c	45.87 ± 1.35b	37.85 ± 1.45a	0.00	619.82 ± 4.07e	117.11 ± 11.68c	59.47 ± 3.64b
3-Hydroxybenzoic acid	986.21 ± 34.25a	877.19 ± 62.06a	1424.33 ± 13.97b	0.00	2958.36 ± 36.30c	4860.16 ± 411.93d	1393.11 ± 132.61b	4275.19 ± 64.49d
Vanillin	30.50 ± 5.00a	113.62 ± 12.6c	160.39 ± 27.9e	0.00	145.98 ± 21.98d	135.79 ± 8.01d	159.40 ± 10.20e	96.06 ± 21.23b
Quercetin-3-*O*-galactoside	89.04 ± 8.02a	205.91 ± 39.01b	544.98 ± 47.01c	0.00	489.87 ± 33.52c	778.12 ± 19.40d	0.00	2052.18 ± 107.44e
Ellagic acid	97.03 ± 5.53a	66.98 ± 4.36a	242.68 ± 6.09c	0.00	271.11 ± 36.94c	194.21 ± 6.60b	268.83 ± 17.87c	613.68 ± 39.63d
Kaempferol-3-*O*-glucoside	140.71 ± 16.53c	132.61 ± 27.55c	147.69 ± 1.16c	0.00	119.27 ± 22.39b	205.77 ± 26.29d	94.85 ± 12.68a	150.92 ± 18.45c
m-Coumaric acid	30.22 ± 2.62a	31.69 ± 8.42a	29.51 ± 2.52a	0.00	49.95 ± 7.04b	35.48 ± 2.12a	193.07 ± 20.63d	141.19 ± 12.63c
Myricetin	233.12 ± 3.70c	149.92 ± 23.67a	131.49 ± 1.13a	0.00	205.16 ± 9.42c	221.92 ± 14.84c	187.85 ± 9.68b	345.85 ± 25.74d
Quercetin	11.01 ± 1.34b	16.72 ± 2.26c	36.93 ± 2.29d	0.00	2.49 ± 0.01a	75.83 ± 4.79f	110.20 ± 3.36g	43.66 ± 3.57e
Isorhamnetin	12.40 ± 0.43a	20.86 ± 1.72b	42.61 ± 1.84c	0.00	0.00	161.51 ± 5.34e	89.62 ± 6.91d	0.00
Kaempferol	0.12 ± 0.01a	7.40 ± 1.23c	40.72 ± 4.83d	0.00	4.81 ± 0.22b	90.98 ± 9.04e	35.64 ± 1.34d	6.06 ± 0.74c

Each value was expressed as mean ± standard deviation (n = 3). Values with different lowercase letters (a-g) within rows are significantly different ($p < 0.05$).

Table 4 Antioxidant activities and α-glucosidase inhibitory activity (α-GIA) of the RRLs extracts obtained by various solvents. Vehicle experiments of the corresponding solvents have been carried out. Acarbose is used as the positive control of α-GIA assay.

Extracts/control	$ABTS^{+\bullet}$ (μmol TE/g DW)	$DPPH^{\bullet}$ (μmol TE/g DW)	FRAP reducing (μM Fe(Ⅱ)E/g DW)	Reducing power (mmol TE/g DW)	CUPRAC reducing (μmol TE/g DW)	α-GIA (IC_{50}, μg GAE/mL)
H_2O	1238.25 ± 27.03b	966.16 ± 13.33c	2904.70 ± 9.50b	18.52 ± 0.05e	985.52 ± 17.62b	200.33 ± 2.52f
50% MeOH	1652.72 ± 23.84c	923.96 ± 10.18c	4322.17 ± 36.54c	26.82 ± 0.02f	2110.89 ± 40.50c	81.65 ± 1.13d
50% EtOH	1289.31 ± 13.76b	1312.64 ± 6.66f	4553.43 ± 34.01c	23.84 ± 0.04f	2406.10 ± 10.17d	147.57 ± 2.11e
Ethyl acetate	7.13 ± 0.14a	0.40 ± 0.01a	65.99 ± 3.40a	0.14 ± 0.01a	43.06 ± 1.68a	3993.33 ± 104.71 g
ChCl-Prop	1547.47 ± 26.05c	715.76 ± 6.88b	4493.62 ± 22.64c	8.67 ± 0.03b	2037.64 ± 24.01c	4.92 ± 0.15b
ChCl-LA	1800.34 ± 12.99d	1246.17 ± 10.70e	5853.27 ± 45.29e	10.66 ± 0.03d	2561.47 ± 10.17e	2.70 ± 0.20a
ChCl-LevA	2044.96 ± 13.03e	1322.00 ± 11.26f	5386.76 ± 15.05d	11.22 ± 0.03d	2681.33 ± 39.02f	3.59 ± 0.26a
Pro-EthG	1905.70 ± 22.09d	1117.79 ± 10.62d	5448.56 ± 31.65d	9.29 ± 0.03c	2193.01 ± 25.21c	7.49 ± 0.24c
Acarbose (Positive drug)	–	–	–	–	–	189.21 ± 3.57 μg/mL

Each value was expressed as mean ± standard deviation (n = 3). Values with different lowercase letters (a–g) within columns are significantly different ($p < 0.05$).

value was found in ChCl-LevA and ChCl-LA extracts, followed by 50% EtOH/MeOH, Pro-EthG and ChCl-Prop extracts. Of all extracts studied, EtAc extracts exhibited the lowest antioxidant activity.

The difference in the antioxidant activity of the RRLs extracts was due to the difference in the components and contents of the phenolic compounds existed in the solvents. It was found that four types of DES extracts with the higher TPC exhibited the stronger $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ scavenging capacities and higher FRAP. High reducing capacity of 50% MeOH/EtOH extracts was associated with its high TFC. The results confirmed that the antioxidant activities of the RRLs extracts had a strong positive correlations with their TPC ($0.710 < r < 0.909$, $p < 0.01$) or TFC ($0.662 < r < 0.812$, $p < 0.01$), which was consistent with the reports of Zhu et al. (2020). Importantly, eco-friendly DES were significantly more effective in extracting the natural antioxidants from RRLs than traditional solvents.

3.4. Alpha-glucosidase inhibitory activity (α-GIA)

Alpha-glucosidase is one of important enzymes involved in digestion of carbohydrates and glucose absorption. It was found that suppressing α-glucosidase activity significantly delayed the carbohydrate digestion and reduced the postprandial blood glucose levels (Zengin et al., 2020; Hao et al., 2020). In the α-GIA assays, it was observed that extraction solvents greatly affected the activity of the RRLs. The activity ranking of the different solvents extracts can be ranked as follows: ChCl-LA (IC_{50} = 2.70 μg GAE/mL) > ChCl-LevA (IC_{50} = 3.59 μg GAE/mL) > ChCl-Prop (IC_{50} = 4.92 μg GAE/mL) > Pro-EthG (IC_{50} = 7.49 μg GAE/mL) > 50% MeOH (IC_{50} = 81.65 μg GAE/mL) > 50% EtOH (IC_{50} = 147.57 μg GAE/mL) > water (IC_{50} = 200.33 μg GAE/mL) > EtAc (IC_{50} = 3993.33 μg GAE/mL). In comparison, DES extracts (especially for ChCl-LA and ChCl-LevA) with the higher TPC showed the stronger α-GIA, but EtAc extracts had the weakest α-glucosidase inhibitory activity. Our results were in agreement with the report of Zhu et al. (2020), which also verified that EtAc extracts extracted from noni leaf had the lowest amounts of active compounds, and thereby indicated the weakest inhibitory activity on α-glucosidase. Many researches have confirmed that contents and compositions of phenolics in plant extracts had great influences on their α-GIA (Suchinina et al., 2011; Hao et al., 2020). In this work, it was found that ChCl-LA and ChCl-LevA extracts evidently had higher TPC and TFC, especially for arbutin, isorhamnetin, quercetin and kaempferol. These compounds have been reported to be associated with stronger α-GIA (Wang et al., 2018; Cai et al., 2020).

3.5. Anti-bacterial activity

The data with regard to the anti-bacterial activities of the RRL extracts are shown in Table 5. As expected, the extraction solvent greatly affected the anti-bacterial activity of the RRLs extracts. Two DES (ChCl-LA and ChCl-LevA) extracts showed evidently incredible anti-bacterial activities against the five tested pathogen strains, with MIC values ranging from 0.012 to 0.049 mg/mL. Particularly, the lowest MIC value was observed in Pro-LA extract against *S. typhimurium* (0.012 mg/mL). A relative lower MIC value was found in the ChCl-Prop and Pro-EthG extracts, and these two extracts exhibited similar anti-bacterial activities except that the MIC of ChCl-Prop extract for *L. monocytogenes* (0.391 mg/mL) and *S. aureus* (0.781 mg/mL). 50% EtOH/MeOH extracts showed similar anti-bacterial activities except for *S. aureus* and *E. coli*. The EtAc extracts did not almost show anti-bacterial activities against all the tested strains. The water extract also exhibited moderate anti-bacterial activities against the tested strains with MIC value ranging from 0.781 to 3.125 mg/mL, especially for *B. subtilis* (0.781 mg/mL) and *S. typhimurium* (0.781 mg/mL).

Many researchers have reported that the anti-bacterial activities of plant extracts are related to the presence of secondary metabolites (i.e. phenolics, flavonoids and essential oil, etc.) (Sepahpour et al., 2018; Sim et al., 2019). In this study, the anti-bacterial activity of extracts was dependent on the contents and compositions of phenolics in the extracts. As previously reported, many phenolics have antimicrobial effects, such as flavonoids (e.g. myricetin, quercetin, kaempferol and phenolic acids) (Sepahpour et al., 2018). With higher TPC and TFC (especially for myricetin, quercetin, and kaempferol), the DES extracts indicated better anti-bacterial activities than the extracts extracted with other solvents. However,

Table 5 Anti-microbial activities of the RRLs extracts extracted by various solvents. Vehicle experiments of the corresponding solvents have been carried out.

Extracts/antibiotic	Minimum inhibitory concentration (MIC, mg/mL)				
	Gram$^+$ bacteria			Gram$^-$ bacteria	
	Listeria monocytogenes	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhimurium*
H_2O	3.125	3.125	0.781	1.563	0.781
50% MeOH	3.125	3.125	0.391	1.563	0.391
50% EtOH	3.125	1.563	0.391	3.125	0.391
EtAc	> 50.00	> 50.00	> 50.00	> 50.00	> 50.00
ChCl-Prop	0.391	0.781	0.098	1.563	0.781
ChCl-LA	0.024	0.049	0.049	0.024	0.012
ChCl-LevA	0.024	0.024	0.024	0.024	0.012
Pro-EthG	0.781	1.563	0.049	1.563	0.781
Tetracycline hydrochloride	0.002	0.004	0.004	0.004	0.002

the 50% EtOH/MeOH extracts were more effective than water extracts in inhibiting the growth of the tested strains. EtAc extracts had the lowest TPC and TFC, thereby indicating the lowest anti-bacterial activity. The result was consistent to that of Zhu et al. (2020) who reported that *Morinda citrifolia* L. leaves extracts extracted by DES exhibited higher anti-bacterial abilities than the extracts extracted with other solvents. Additionally, it was observed that the RRLs extracts had higher resistance against Gram$^+$ bacteria than against *E. coli*, which was probably due to the high complexity of *E. coli*'s cell-wall as a Gram$^-$ bacterium (Francisco et al., 2019). In conclusion, TPC and TFC in the RRLs extracts are of great importance to the anti-bacterial abilities against foodborne pathogens.

3.6. Multivariate analysis

Multivariate analysis was carried out to assess the effects of the extraction solvents on phenolic compounds and biological activities of the RRLs extracts (Suchinina et al., 2011; Zengin et al., 2020). With respect to HCA plot established

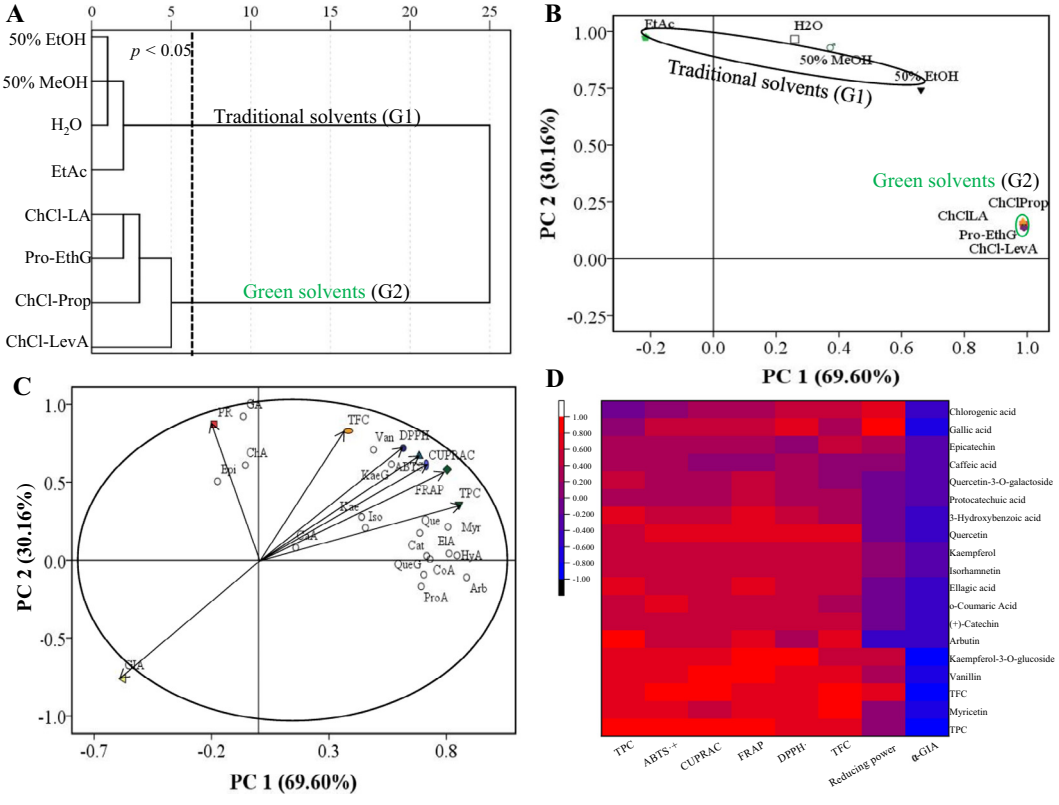

Fig. 3 Multi-variate analysis on the datasets of the RRLs extracts. A, HCA analysis; B, PCA loading plot; C. The PCA score plots; D, Heat-map analysis. Arb, Arbutin; GaA, Gallic acid; ChA, 3-Chlorogenic acid; Cat, (+)-Catechin; ProA, Protocatechuic acid; Epi, Epicatechin; CaA, Caffeic acid; HydA, 3-Hydroxybenzoic acid; Van, Vanillin; QueG, Quercetin-3-*O*-galactoside; ElA, Ellagic acid; KaeG, Kaempferol-3-*O*-glucoside; CA, *m*-Coumaric acid; Myr, Myricetin; Que, Quercetin; Iso, Isorhamnetin; Kae, Kaempferol.

by Ward's method, it was observed that all samples were clearly divided into two major groups (Fig. 3A). Group 1 (G1) included traditional solvents extracts (50% MeOH, 50% EtOH, EtAc, and water); Group 2 (G2) included DES extracts (ChCl-LA, ChCl-Prop, ChCl-LevA and Pro-EthG). PCA was carried to visualize the effects of solvents on active components and the bio-activities of the RRLs extracts. PC1 69.60% and PC2 30.16% accounted for 99.76% of the total variances, which indicated that these two principal components could load maximum information of the original data. For PCA loading plot, traditional solvents and DES were respectively divided into G1 and G2, which was in agreement with the result of HCA (Fig. 3B). With respect to PCA score plot, the relationship between samples can be represented by the distance between the points, and the relationship between the variables can be reflected by the cosine values (Fig. 3C). Among them, TPC, TFC, arbutin (Arb), vanillin (Van), quercetin (QUE), kaempferol-3-*O*-glucoside (KaeG), myricetin (Myr), isorhamnetin (Iso), ellagic acid (ElA) and 3-hydroxybenzoic acid (HyA) were positively correlated with $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ scavenging capacity, FRAP, and CUPRAC. GA, chlorogenic acid (ChA), kaempferol (Kae), KaeG and TFC were positively correlated with reducing power. In addition, TPC, TFC, GA, KaeG, Van, and Myr were correlated with the α-GIA. The results of HCA and PCA demonstrated the significant influences of extraction solvents on the biological activities of the RRLs extracts.

Heatmap analysis can better visualize the relationships between chemical constituents and the bio-activities of the RRLs extracts (Fig. 3D and Table S2). Significant positive correlations can be observed between TPC and $ABTS^{+\bullet}$ ($r = 0.895$; $p < 0.01$), $DPPH^{\bullet}$ ($r = 0.71$; $p < 0.05$), FRAP ($r = 0.909$; $p < 0.01$), and CUPRAC ($r = 0.823$; $p < 0.05$). In these components, Van, Que, KaeG, Myr, Iso, ElA and HyA had a significant positive correlation with antioxidant activities ($r > 0.570$; $p < 0.05$). However, the reducing capacity of extracts was positively correlated with GA ($r = 0.816$; $p < 0.01$), ChA ($r = 0.608$; $p < 0.05$), KaeG ($r = 0.505$; $p < 0.05$) and TFC ($r = 0.662$; $p < 0.05$). α-GIA was significantly correlated with TFC ($r = 0.899$; $p < 0.01$), TPC ($r = 0.817$; $p < 0.01$), KaeG ($r = 0.844$; $p < 0.01$), GA ($r = 0.606$; $p < 0.05$), Van ($r = 0.726$; $p < 0.05$), and Myr ($r = 0.765$; $p < 0.05$). Consequently, TPC and TFC (especially for Arb, Van, KaeG, and Que) increased antioxidant activities ($ABTS^{+\bullet}$, $DPPH^{\bullet}$, FRAP, and CUPRAC) and α-GIA of the RRLs extracts, and TFC (especially for GA and ChA) increased the reducing capacity. Many researchers alsoverified that TPC/TFC in food or plant extracts was evidently associated with the antioxidant activities and α-GIA (Figueiredo-González et al., 2018; Zengin et al., 2020).

4. Conclusions

Rosa roxburghii Tratt. leaves extracts extracted with traditional solvents and eco-friendly solvents showed significant differences in the TPC, TFC, phenolic components and anti-oxidant, anti-bacterial, α-glucosidase inhibitory activity. Four types of DES extracts had the highest TPC. 50% MeOH/EtOH extracts showed the highest TFC. EtAc extracts had the lowest TPC and TFC. Seventeen compounds were identified and quantified, including phenolic acids and flavonoids that had not been previously reported in RRLs. Among them, arbutin, gallic acid, (+)-catechin, 3-hydroxybenzoic acid, quercetin-3-*O*-galactoside and myricetin were the dominant compounds. In addition, four types of DES extracts exhibited stronger antioxidant activities, α-GIA and anti-bacterial activity than extracts extracted with other solvents. Multivariate analysis revealed that the TPC, TFC and individual phenolic compounds were key factors affecting the bio-activities of the RRLs extracts. In conclusions, RRLs as a good source rich in phenolic compounds, have significant bio-activities and can be used in the pharmaceutical industry. This work provides a reference for obtaining extracts rich in phenolic compounds from natural products using eco-friendly and high-efficient solvents.

CRediT authorship contribution statement

Ruimin Wang: Methodology, Investigation. **Ruiping He:** Methodology, Investigation. **Zhaohui Li:** Resources, Investigation. **Xue Lin:** Supervision, Data curation, Writing - review & editing. **Lu Wang:** Supervision, Data curation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103257.

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