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Comparison of the effects of green and sustainable extraction methods on the extraction yield and chemical composition of *Ruta chalepensis* roots

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ABSTRACT

Extraction of coumarin, furanocoumarin and alkaloids from *Ruta chalepensis* roots was carried out with subcritical water, subcritical ethyl alcohol, supercritical CO_2, and ultrasound assisted extraction. The effect of operating conditions for sub and supercritical extractions was determined by varying the extraction temperature (100–160 °C) and extraction pressure (60 and 80 atm). Maximum extraction efficiency was observed with subcritical water extraction at 125 °C and 60 atm (40.6%). Extracted compounds were identified by gas chromatography-mass spectrometry (GC-MS). Five main compound classes were identified in these extractions methods extracts. Furanocoumarins were the most abundant components of all extracts and maximum yields (31.0%) obtained with subcritical water extraction at 160 °C and 80 atm.

1. Introduction

Extraction of natural products from various traditional herbal plants is one of most attractive research is due to using these products in the treatment of health issues. In addition, the tremendous biodiversity of plants creates very important chemical and biochemical diversity. The *Rutaceae* family is seen as herbs, shrubs and small trees and grow in almost all parts of the world (Rahman and Gulshana, 2014). Furanocoumarins, furanoalkaloids, acridone alkaloids, phenolic-structured compounds and terpenes are the main components of this plant family. These compound classes are important in pharmacy (Riyanto et al., 2001) hence, extraction of these molecules required efficient and adequate strategy. Although, conventional extraction methods such as Soxhlet and maceration are still widely used, they have several drawbacks such as using hazardous solvents such as dichloromethane and methanol, time consuming and tedious labouring. Moreover, extraction rate may be affected by either the solubilisation or the diffusional processes of the plant matrices. On the other side, extraction type, extraction temperature, solvent-to-sample ratio and nature of solvent have significant effects on the extraction rate and the chemical composition of the extract.

In recent decades, more efficient green and sustainable extraction methods, such as supercritical fluid extraction (SCFE) and ultrasound assisted extraction (UAE) have been used for the extraction of bioactive compounds from natural products (Kubatova et al., 2001; Moldao-Martins et al., 2000; Giray et al., 2008). SCFE has a very important advantage that changing temperature and pressure has great effects on the physicochemical (such as viscosity, density, and diffusivity) properties of supercritical fluids (SCFs). These modifiable properties make SCFs promising alternative solvent for the extraction of several matrices.

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Subcritical water extraction (sbcW-E) uses water as an extractant at temperatures between 100 and 374 °C and at a pressure high enough to maintain the water at liquid state. sbcW-E can be realized in static and/or dynamic mode. In static mode, the plant material is loaded into the extraction cell with a certain amount of water. Extraction is carried out under certain pressure, temperature and time conditions. On the other hand, dynamic mode is a continuous process, and during extraction, pressurized hot water passes through the matrix in the cell. Optimization of extraction time and extraction cycles is also important in dynamic mode. In static mode, equilibrium quickly achieved (5–30 min) and allows relatively short extraction times. In dynamic extraction mode, the flow rate of water (usually in the range of 1–2 mL/min) is another important parameter for optimization of sbcW-E. Although mass transfer control is possible in dynamic extraction, it produces more diluted extracts (Mukhopadhyay and Panja, 2010).

Supercritical carbon dioxide extraction ($scCO_2$- E) technology is one of SFE technology and it has been widely used as a green strategy for the efficient extraction and recovery of valuable chemicals from a variety of plant materials. Critical temperature and pressure of CO_2 are 31.1 °C and 73 atm and over critical point CO_2 behaves as a gas and a liquid. $ScCO_2$ has the density of a liquid, the diffusivity of a gas, and the viscosity of a gas-liquid. Hence, $scCO_2$ has attracted a lot of interest as a solvent for extracting important bioactive chemicals from plant biomass matrices due to its unique properties. When determining the solvating power of solvents, density is also usually considered. For the separation of bioactive chemicals from plant materials without losing their useful qualities requires moderate temperature and pressure. $scCO_2$-E technique can fulfil these conditions. Also, after the extraction, a simple depressurizing setup allows the final product to be solvent-free. As a result, these properties of $scCO_2$-E technique put it in the centre of to extract bioactive chemicals from plant materials (Arumugham et al., 2021; Zhang et al., 2009; Jawade and Chavan, 2013; Mejri et al., 2012a; Ivanovic et al., 2011; Machmudah et al., 2008; Casas-Cardoso et al., 2021). Baldino et al. combined supercritical CO_2 ($scCO_2$) extraction proceses and cooling separator to concentrate the bioactive components of *Ruta graveolens*. They used 250 bar, 40 °C for the extraction process and −10 °C, for cooling (Baldino et al., 2018). In another study, Sovovà and co-workers decided that solubility of furanocoumarins of *R. graveolens* in $scCO_2$ becomes relevant at 40 °C and pressures above 200 bar (Sovov á, 2012). Mejri and co-workers carried out the extraction of essential oil of *Ruta chalepensis* L by using supercritical CO_2 extraction. While the yield of extraction by supercritical CO_2 was about 7.8%, the extract was including almost only undecan-2-one (100%) (Mejri et al., 2012b).

Ultrasound assisted extraction (UAE) has been reported as unique and environmentally friendly non-thermal techniques in the literature and it is an important approach for extracting valuable compounds from plant materials. It can be used on a small or large scale in lab or industry (Zhang et al., 2009). Several works on the UAE to extract bioactive chemicals from herbs, plant-derived metabolites have been published in the past (Londoño-Londoño et al., 2010; Santos et al., 2010; Pan et al., 2011; Khan et al., 2010; Sivakumar et al., 2011). Microcavitations can be created in the liquid around the plant material using an ultrasonic field. Mechanical breakage of the cell wall releases the contents of the cell, and local heating of the liquid increases diffusion. Following the bursting of cavitation bubbles at solid-liquid interfaces, kinetic energy and ultrasound induce in the entire volume, enhancing mass transfer across it (Jawade and Chavan, 2013).

In this study, the components of *Ruta chalepensis* were extracted by using sbcW-E, sbcEtOH-E, $scCO_2$-E, and UAE with three different solvents (*tert*-butyl methyl ether (tBME), methanol (MeOH) and ethanol (EtOH). The separated components are identified by using EIMS data's of the GC-MS results and literature (Wu et al., 2003; Cast ı llo et al., 1986; Yang et al., 2007; Orlita et al., 2008a; Orlita et al., 2008b). In addition according to the best knowledge, subcritical extraction of *Ruta chalepensis* was carried out in this study, as a first time.

2. Experimental

2.1. Plant material and chemicals

Ruta chalepensis, was collected from Kozan, South of Turkey, and by Prof. Saliha Kırıcı, from Agricultural Faculty of Cukurova University. Roots of the plant were separated and are dried at room temperature. All chemicals were purchased from Merck and used as received. All equipment used for sub and supercritical extraction was built in the laboratory.

2.2. Extraction

2.2.1. Sub-critical water extraction (sbcW-E)

Subcritical water extraction was done in an apparatus built in the laboratory. The water was used in a programmable HPLC pump (Alltech 426) for 1 mL/min constant flow. A 14 mL extraction cell equipped with 10 μm frit at the inlet and outlet, was connected to a 1 m cooling loop outside of the oven. Extraction cell contained sea sand at both ends. sbcW-E was carried out using 1 g of *Ruta chalepensis* at temperatures 100, 125, 140 and 160 °C, pressure of 60 and 80 atm and 30 min static extraction time with water and then the process completed 20 min dynamic extraction with ethyl alcohol at a flow rate of 2 mL per minute. The extractions were done in triplicate. The extraction yield, expressed in mass ratio of the extract to the starting dried material and calculated with the equation below.

$$[Y\ \% = \text{g of extract/g of dried material x } 100] \quad \text{(Eq 1)}$$

2.2.2. Sub-critical ethanol extraction (sbcEtOH-E)

It was studied with the device used in sub-critical water extraction experiments. sbcEtOH-E was carried out using 1 g of *Ruta chalepensis* roots at temperatures 100, 125, 140 and 160 °C, a pressure of 60 and 80 atm and 30 min static extraction time and then 20 min dynamic extraction with ethyl alcohol at a flow rate of 2 mL per minute. The extraction yield, expressed in mass ratio of the extract to the starting dried material and calculated with Eq (1). The extractions were done in triplicate.

2.2.3. Supercritical carbon dioxide extraction ($scCO_2$-E)

After the extraction cell was filled with 2.0 g of sample, it was placed in the oven. Supercritical CO_2 at the desired pressure was sent to the cell by means of an ISCO pump. After 20 min of static extraction, 30 min of dynamic extraction was applied and samples were collected in a tube containing dichloromethane at a flow rate of 2 ml/min. Extraction temperature was 45 °C and pressure were varied as 90 atm and 200 atm. Extraction yield was calculated by Eq (1) and the extractions were done in triplicate.

2.2.4. Ultrasound assisted extraction (UAE)

Ultrasound assisted extraction experiments were carried out in Lab-Line Instrument Inc. 9314, Transsonic 890 model device (310 × 380 × 200 mm internal dimensions). 2 g *Ruta chalepensis* and 30 ml solvent were added into a 50 ml volume capped sample tube. Experiments were carried out at room temperature with three different solvents [t-butylmethylether (tBME), methanol (MeOH) and ethanol (EtOH)] at two different extraction times (2 h and 4 h). The extractions were done in triplicate. Extraction yield was calculated by Eq (1).

2.3. GC–MS analysis

GC-MS analysis was realized by using a Finnigan-Trace GC–MS. 1 μl sample volume injected with auto sampler. Chromatographic separations were accomplished with a Zebron ZB-5 capillary column (5% phenyl–95% dimethylpolysiloxane, 0.25 mm i.d. × 60 m, film thickness 0.25 m) with injections in the split mode with 10 split ratio. Analysis was carried out using helium as the carrier gas, flow rate 1.0 mL/min. GC conditions were: programmed heating from 50 to 180 °C at 7 °C/min and subsequent holding at 180 °C for 5 min, after heating from 180 to 280 at 10 °C/min and subsequent holding at 280 °C for 10 min. The injection port temperature was 280 °C. The ionization voltage applied was 70 eV, mass range *m/z* 50–650 a.m.u. The separated components identified using the literature and tentatively by matching with GC-MS results of National Institute of Standards and Technology (NIST) mass spectral library data because their reference reagents were not available (Wu et al., 2003; Cast ı llo et al., 1986; Yang et al., 2007; Orlita et al., 2008a; Orlita et al., 2008b).

3. Results and discussion

The hairy roots of *Ruta chalepensis* were dried and ground before the extractions and then were extracted by using sbcW-E, sbcEtOH-E, $scCO_2$-E, and UAE with three different solvents tBME, MeOH and EtOH.

During sub and supercritical solvent extraction of natural products, temperature and pressure are the main factor contributing to extraction efficiency. Especially, at higher temperatures viscosity and the surface tension of solvents decreases and then the solvent power increases with resulting the dissolution of target components in the solvent (Giergielewicz-Mozajska et al., 2001). One of the aim of this study was to define optimal conditions for sub and supercritical fluid extraction and suitable solvent for UAE to extract the bioactive components of *Ruta chalepensis* roots. To realize optimum conditions, temperature, pressure, and solvents were tested and the results are given below.

3.1. Optimization studies of extraction methods

In subcritical water extraction (sbcW-E), pressure and temperature have theoretical and practical implications on the extraction process. During sbcW-E, the effect of the extraction pressure and temperature on the extraction yield and chemical composition of *Ruta chalepensis* roots was tested at four different temperatures (100, 125, 140 and 160 °C) and pressures (60 and 80 atm) with static extraction (30 min) and dynamic extraction (20 min with EtOH, flow rate 2 ml/min). To preserve the quality of the extracts, temperatures kept relatively low. Fig. 1 shows extraction yields obtained by sbcW-E at different temperatures and pressures. The extraction yield was calculated on the base of dry extract (g) per g of dry plant material. The highest extraction yield (40.6 ± 0.2%, d.b) was obtained at 125 °C and 60 atm while the lowest extraction yield (20.4 ± 0.2%, d.b.) at 160 °C and 60 atm. It's obviously seen in Fig. 1 that the extraction yield was significantly dependent on the pressure and temperature. During the extractions at 60 atm, at first, an increase was observed at 125 °C and then decreased above 125 °C (Fig. 1). However, the processes at 80 atm, the extraction yield of *Ruta chalepensis* was smoothly decreased at temperatures above 100 °C.

Efficiency of sbcEtOH-E method on the extraction yield and chemical composition of *Ruta chalepensis* roots was investigated with

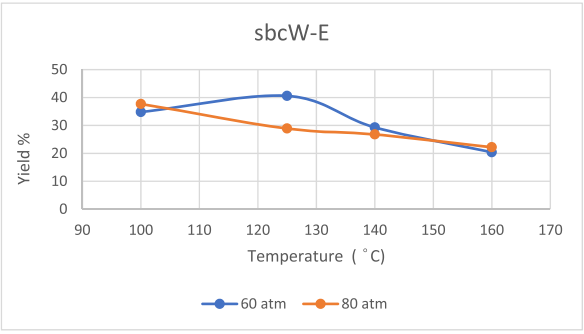


Fig. 1. The extraction yields of sbcW-E of *Ruta chalepensis* roots.

the same temperature, pressure and extraction times used in sbcW-E conditions. EtOH was used as solvent for both static and dynamic extraction. The results were presented in Fig. 2. During the extraction of *Ruta chalepensis* with sbcEtOH pressure and temperature have an obvious effect on the extraction yield. Overall, the extraction rate of *Ruta chalepensis* components was higher at 60 atm for all extraction temperatures. When temperature rises from 100 to 160 °C, extraction yields drop steadily. The highest extraction yield was obtained at 100 °C and 60 atm (18.9 ± 0.5%, d.b.), while the lowest yield at 160 °C and 80 atm (11.0 ± 0.1%, d.b.). These results show that sbcEtOH-E is less efficient method on the extraction yield of *Ruta chalepensis* roots than those of sbcW-E method. For water and ethanol, the solvatochromic indices, indicating the polarity/polarizability (Kamlet-Taft π *), hydrogen bond donation ability (Kamlet-Taft α) and hydrogen bond acceptance ability (Kamlet-Taft β) decrease with an increase in temperature, especially the critical point is approached. For example at 150 °C, for water, $\rho_{H2O} = 917$; $\varepsilon_{water} = 43{,}94$; Kamlet-Taft π * 0,98 while for EtOH $\rho_{EtOH} = 664{,}4$; $\varepsilon_{EtOH} = 9{,}88$; Kamlet-Taft π * = 0,27 which means subcritical water is a better solvent for the extraction of natural plants than those of subcritical EtOH (Marcus, 2018).

$scCO_2$-E of *Ruta chalepensis* roots was performed at constant temperature (45 °C) with two different pressures (90 and 200 atm). The extraction times were 20 min for static extraction and 30 min for dynamic extraction. The extraction yield higher at 200 atm (9.1 ± 0.7%) than those of extraction at 90 atm (6.5 ± 0.4%). Kamlet-Taft π * value increases with an increase in the density of the system. Hence, at higher pressures, $scCO_2$ becomes denser and causes an increase of the extraction yield. Our results are in a good agreement with literature data (Mejri et al., 2012b; Yutaka et al., 1991).

To determine optimal conditions for the extraction of chemical components from *Ruta chalepensis* roots, extracts were obtained at two different extraction times (2 h and 4 h) and using three different solvents tBME, MeOH and EtOH which have different polarity. During UAE, the choice of the most appropriate solvent for extracting of the valuable components of plants is a fundamental step (Li et al., 2004). Since cavitation is enhanced at low extraction temperature, all experiments were carried out at room temperature. The variations in extraction yields depending on the solvents used and given in Fig. 3. The highest extraction yield was obtained with EtOH (at 4 h: 10.3 ± 0.1%, d.b). Generally, 4 h extraction time is better than 2 h for all solvents used in UAE extraction of *Ruta chalepensis* roots.

The comparison of the extraction yields of *Ruta chalepensis* roots after optimization studies are given in Table 1. The highest extraction yield was achieved with sbcW-E at 125 °C and 60 atm. Subcritical water has some unique properties as an extraction solvent. For example, ionization products of water increase, surface tension and viscosity decrease at high temperatures. Hence, penetration of subcritical water into the plant matrix and speeding up the dissolution of the components. In addition, breaking of van der Waals interactions, hydrogen bonds and dipole–dipole molecular forces between plant matrix and extractable compounds, thus activation energy of the extraction decreases, the mass transfer rate increases. Therefore, resulting in high extraction yields. Supercritical carbon dioxide has some important advantages as an extraction solvent for natural materials. They can be listed as chemically inert, low toxicity, no pollution problem, and shorter extraction time. On the other hand, $scCO_2$ as a nonpolar solvent, preferably dissolves non-polar and low polar substances from plants. Hence, during the extraction of polar compounds with $scCO_2$, some polar organic solvents have been added as modifiers to increase the polarity of extraction media (Keiichi et al., 1992; Lin et al., 1995, 1999).

Our first results with sbcW-E showed that *Ruta* roots are rich in polar substances such as furanocoumarin, alkaloids and phenols. It's well known that the solvent polarity parameter [E_T (30)] of $scCO_2$ increases with an increase in the pressure (Yutaka et al., 1991). Hence, we can explain the lower extraction yield of *Ruta* roots with lower polarity of $scCO_2$ at 90 atm. As seen from Table 1, we observed the highest $scCO_2$-E yield of *Ruta* roots at 200 atm due to increased polarity of $scCO_2$. These results are compatible with the previously reported results of Sovovà and co-workers where decided that solubility of furanocoumarins of *R. graveolens* in $scCO_2$ becomes relevant at 40 °C and pressures above 200 bar (Sovova et al., 2017).

3.2. Chemical composition and identification of components

Chemical composition of all extracts of *Ruta chalepensis* roots were analyzed by GC–MS. All detected compounds were identified through their mass spectral data which were found to be consistent with published data (Wu et al., 2003; Cast ı llo et al., 1986; Yang et al., 2007; Orlita et al., 2008a; Orlita et al., 2008b). These components of *Ruta chalepensis* roots are like those previously identified by Kostova et. all (Kostova et al., 1999). Tables of chemical components observed in the extracts of sbcW-E, sbcEtOH-E, $scCO_2$-E and UAE;

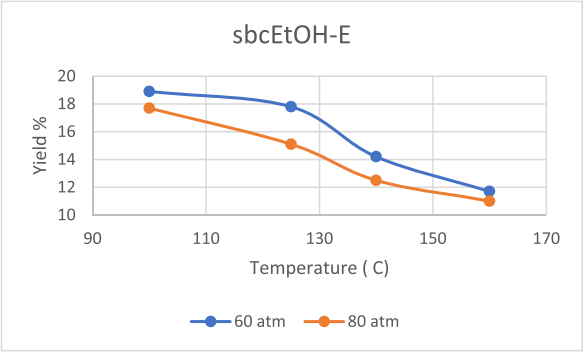


Fig. 2. The extraction yields of sbcEtOH-E of *Ruta chalepensis* roots.

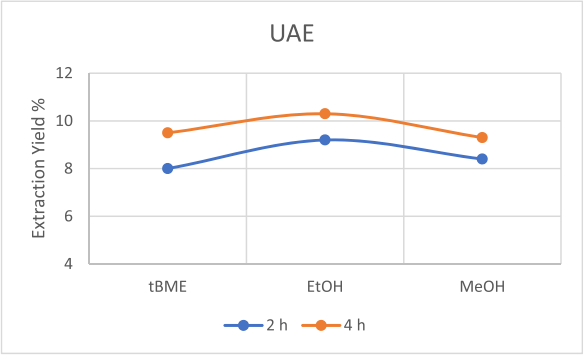


Fig. 3. The extraction yields of UAE of *Ruta chalepensis* roots.

Table 1
Optimized conditions for the extraction methods.

	Extraction methods			
	sbcW-E	sbcEtOH-E	$scCO_2$-E	UAE
Optimized parameters				
Temperature (° C)	125	100	45	Ambient
Pressure (atm)	60	60	200	Ambient
Time (static, min)	30	30	30	240
Solvent				EtOH
Yield%	40.6 ± 0.2	18.9 ± 0.5	9.1 ± 0.7	10.3 ± 0.1

GC-MS chromatograms with detected compounds and a structure list of all these compounds are given in the supplementary.

According to the GC-MS analysis, all extracts were rich in bioactive molecules. Two coumarins [3-(1,1′-dimethylallyl) scopoletin] and 5,7-dimethoxy-8-(3-methylbut-2-enyl)chromen-2-one, furanocoumarins and alkaloids (furano, acridinone quinoline and hydroquinolone) were the main components of all extraction methods. However, their distributions in the extracts varied depended on the extraction method. In addition, tashinones (diterpenes), shikimic acid derivatives (β-lapachone, and moskachan D), some phenols, several oxygenated hydrocarbons and hydrocarbons (Oxy-HC & HC) were detected as the main components of *Ruta chalepensis* roots. In addition, levoglucosenone (LGO), 5-hydroxymethyl-2-furfural (HMF), 5-methyl-2-furfural were detected as hydrolysis product in sbcW-E extracts and sbcEtOH-E extracts. The presence of all compounds was confirmed by mass spectral analysis and their relative amount were presented as peak are. A brief analysis of mass spectra of main components was given below.

3.2.1. *Furanocoumarins*

The mass spectrums of chalepensin and isochalepensin are quite similar and the only differences are in the intensities of fragment ion *m/z* 225 ($M^+ - C{=}O$). It's reported that the intensity of ion at *m/z* 225 is higher for chalepensin than those of isochalepensin (Sidwa-Gorycka et al., 2009). The molecular ion further eliminates a methyl radical to form an ion at *m/z* 239 (90%). Pinnarin was identified with an intensive molecular ion at *m/z* 274 $[M]^{+\bullet}$ (75%), and elimination of several groups such as 259 $[M–Me]^+$ (60%), 231 $[M–CH_3CO]^+$ (100%) and 219 $[M - 55\ u]^+$ (65%). The molecular ions of bergapten fragment are in the manner characteristic of coumarins; *m/z*: 216 $[M]^{+\bullet}$ (100%). Elimination of the methyl radical and the C═O moiety leads to the formation of *m/z* 201 $[M–Me]^+$ (29%), and *m/z* 188 $[M - CO]^+$ (14%). Other fragments were *m/z* 173 $[M–Me–CO]^+$ (54%), *m/z* 145 $[M–Me–CO–CO]^+$ (21%) and *m/z* 89 (26%).

Fragmentation ions of isopimpinelin were *m/z*: 246 $[M]^{+\bullet}$ (79%), 231[M–Me] + (100%), 203 [M–Me–CO] + (19%), 188 (28%), 175 (26%), 147 (11%) and 89 (25%).

In the mass spectra of rutamarin, fragmentation ions were *m/z*: 356 $[M]^{+\bullet}$ (11%), 341$[M–Me]^+$, (7%), 313 (9%) $[M - 43\ u]^+$, 281 [M–aryl] + (100%). Chalepin was determined with the ions *m/z*: 314 $[M]^{+\bullet}$(94%), 299 [M–Me] + (100%), 255 $[M–OHC\bullet(CH_3)_2]$ + (50%), 281 (36%) 206 (70%). Angecine was identified by means of the ions at *m/z*: 186 $[M]^{+\bullet}$ (100%), 158 [M − CO] + (65%), 130 [M–CO–CO] + (19%) and 102 [M–CO–CO–CO] + (32%). A very rare coumarin, 3-(1,1-dimethyl-2-propenyl) scopoletin was identified by means of the following ions at *m/z* 260 $[M]^{+\bullet}$ (80%), 245 [M − Me] + (95%), 230 [M–2H–CO] + (25%), 217 (50%), 205 (100%). The characteristic mass fragment ions of 5,7-dimethoxy-8-(3-methylbut-2-enyl)chromen-2-one were *m/z* 274 $[M]^{+\bullet}$ (80%), 245 [M − Me] + (100%), 231 $[M–Me–CO]^+$ (25%), 219 $[M - 55\ u]^+$ (90%).

3.2.2. *Tanshinones (Diterpens)*

The mass spectrums of cryptotanshinone and isocryptotanshinone were almost same and it was difficult to identify each other. Molecular ion fragments of cryptotanshinone were *m/z* 296 $[M]^{+\bullet}$ (60%), 281 $[M - Me]^+$(100%); 253 [M–Me–CO] + (70%); 241 [M − 55 u] + (80%) and of isocryptotanshinone were *m/z* 296 $[M]^{+\bullet}$ (90%), 281 [M − Me] + (100%); 253 [M–Me–CO] + (50%); 241 [M − 55 u] + (70%).

Furoquinoline alkaloids: Four alkaloids were identified with EI-MS data agreed with published data (Sidwa-Gorycka et al., 2009).
Dictamine: *m/z*: 199 [M]+• (100%), 184 [M–Me]+ (65%), 156 (45%), 128 (45%)
Skimmianine: *m/z* 259 [M]+• (50%), 244 [M–Me]+ (100%), 230 (55%)
Kokusagine: *m/z*: 259 [M]+• (100%), 244 [M–Me]+ (60%), 230 [M − CHO•]+ (10%), 216 (25%), 191 (35%), 178 (38%).

3.2.3. Acridone alkaloids

1-hydroxy-10-methyl-9-acridanone: *m/z* (%), 225 ([M]+ •, 100%), 197 ([M − CO]+, 15%), 182 ([M–CO–CH_3]+, 35%).
1-hydroxy-3-methoxy-10-methylacridon: *m/z* (%), 255 ([M]+ •, 100%), 226 ([M-H-CO]+, 90%), 203 (85%).

3.2.4. Quinolone alkaloids

One N-methyl-2-alkyl-4(1H)-quinolone alkaloid was determined by characteristic fragment ion, such as *m/z* 173(base peak) due to a McLafferty rearrangement, a rearrangement fragment ion at *m/z* 186, and *m/z* 144.

Graveoline belongs to hydroquinolones which are contain a hydrogenated quinoline with a ketone group. Characteristic ions: *m/z* (%), 279 ([M]+ •, 100%), 251 ([M − CO]+, 55%), 191 (35%), 178 (30%)

β-lapachone.: *m/z* 242 (M^+, 80%) and *m/z* 227 (M − CH_3, 100%).

Moskachan D: *m/z* (%), 248 ($[M]^+$, 25%), 148 ([M–CH_2–CH_2-CH_2-CH_2-$COCH_3$] $^+$, 15%), 135 ([M − CH_2–CH_2–CH_2–CH_2–$COCH_3$–CH]$^+$,100%). An unidentified compound with *m/z* 290 ($[M]^{+\bullet}$, 25%), 207 (15%), 148 (25%); *m/z* 135 (100%), *m/z* 105 (15%) were detected which is characterized with *m/z* 135 ($C_8H_7O_2$) peak. The base peak at *m/z* 135 ($C_8H_7O_2$) is characteristic for the Moskachan-type compounds (Ulubelen and G ü ner, 1988).

3.2.5. Phenols and the others

3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one: *m/z* (%), 144 ($[M]^+$, 25%), 115 ([M-H-CO, 20%] $^+$, 101 ([M − O–CH_2–CH_3]$^+$,100%).
Syringol: *m/z* (%), 154 ($[M]^+$, 100%), 139 ([M − CH_3] $^+$, 55%), 111 ([M −]$^+$, 50%).
4-vinyl syringol: *m/z* (%), 180 ($[M]^+$, 100%), 165 ([M − CH_3] $^+$, 65%), 137 ([M − C_2H_3O]$^+$,50%).
4-vinyl guaiacol (2-methoxy-4-vinyl phenol): *m/z* (%), 154 ($[M]^+$, 100%), 135 ([M − CH_3] $^+$, 90%), 107 ([M − CH_3O_2]$^+$,55%).
Coniferyl alcohol: *m/z* (%), 180 ($[M]^+$, 60%), 163 ([M − OH] $^+$, 10%), 137 ([M − C_2H_3O]$^+$,100%); 124 ([M-C_3H_4O],30%).
Eugenol: *m/z* (%), 164 ($[M]^+$, 100%), 149 ([M − CH_3] $^+$, 60%).
2.6-dimethyl-4-(oct-7-en-1-yl)phenol: *m/z* (%), 232 ($[M]^+$, 30%), 161 ([M − CH_3] $^+$, 65%), 137 ([M − C_2H_3O]$^+$,50%).
5-hydroxymethyl-2-furfural (HMF): *m/z* (%), 126 ($[M]^+$, 60%), 97 ([M-H-CO] $^+$, 100%), 69 ([M − H–CH_2O–CO]$^+$,40%)

Generally, the relative yields, molecular diversity, and selectivity of all these classes of compounds varied with temperature and pressure in sbcW-E, sbcEtOH-E and scCO_2-E extracts. Coumarin and furanocoumarins (C&FC) were most abundant components of the sbcW-E extracts and had an upward tendency with increasing temperature from 100 to 160 °C at 60 atm (14.8–31.0%, Fig. 4). It's known that during sbcW-E, although the extraction rate increases with temperature, some organic components of natural products

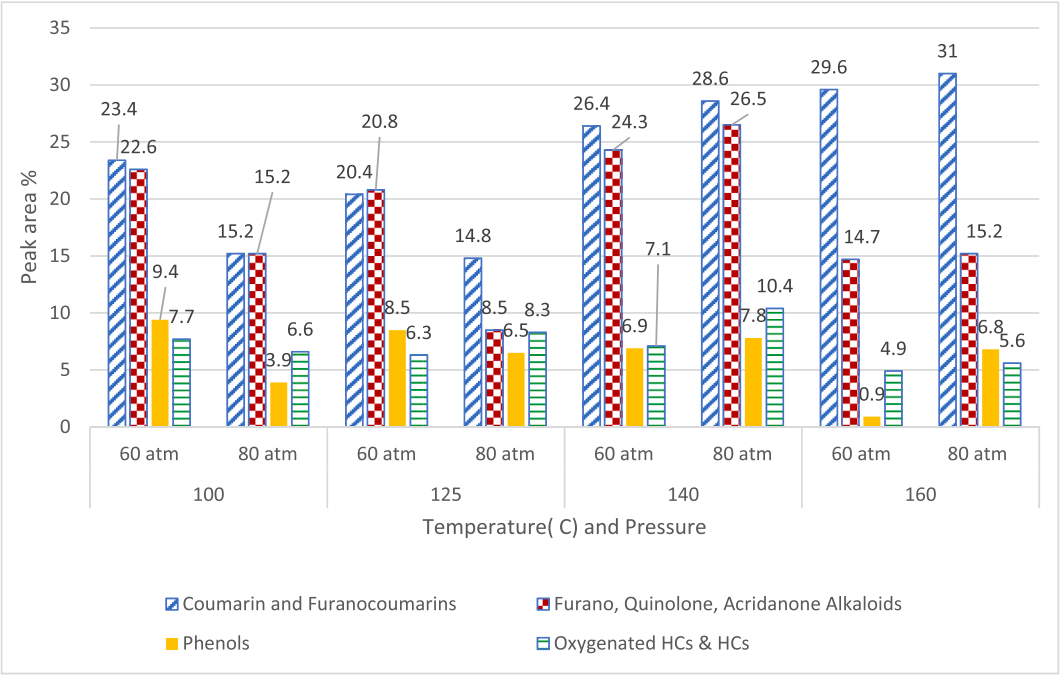


Fig. 4. Relative abundances of chemical components of sbcW-E of *Ruta chalepensis* roots.

show substantial degradation above temperatures 150 °C, because of instability of them at high temperatures (Moldao-Martins et al., 2000). However, the maximum yield of C&FC was observed at 160 °C and 80 atm (31,0%). It could be said that the efficiency of sbcW-E on the extraction of C&FC is greatly affected by temperature. As expected, among the C&FC, isochalepensin was detected as the most abundant component in all samples (max 17.0% at 160 °C and 80 atm). C&FC were the most abundant component classes in sbcEtOH-E extracts (21.5%–30.9%, Fig. 5), as well. Among the C&FC, isochalepensin was detected as the most abundant component of sbcEtOH-E extracts (12.5 at 140 °C, 60 atm). These results show that efficiency of sbcW-E and sbcETOH-E on the extraction yield of C&FC is almost similar.

C&FC were also recognized as most abundant molecule classes of $scCO_2$-E extracts and isochalepensin was detected as the most abundant components (13.1%). As mentioned before, $scCO_2$-E was more efficient at 200 atm for the extraction of C&FC components of *Ruta chalepensis* roots (Fig. 6) (Sovova et al., 2017). On the other side Khellin, a furanochrome molecule which was substituted with methoxy groups at C-4 and C-9 and a methyl at C-7 was detected only in $scCO_2$-E extract (1.1% at 90 atm).

C& FC were also detected as main molecule classes of UAE extracts (Fig. 7). As mentioned at experimental section, three different solvents tBME, MeOH and EtOH were used for the extraction of *Ruta chalepensis* roots and tBME was observed as the most efficient solvent for the extraction of C&FC (31.1% at 4 h). As discussed in sbcW-E, sbcEtOH-E and $scCO_2$-E results, isochalepensin draws attention as the most abundance compound among C&FC and tBME was decided as more efficient for extraction of isochalepensin (12.9%, in tBME-2h). In second order, relative amount of rutamarin is 5.2% in the extract of UAE with tBME for 2 h. Among C&FC components pinnarin (4.2% at 2 h) and, 5,7-dimethoxy-8-(3-methylbut-2-enyl) chromen-2-one (3.9% at 2 h) were also significant components of UAE with tBME extraction.

The *Rutaceae* family is also source of furoquinoline alkaloids, acridone alkaloids and quinoline alkaloids (Ek ı ert and K ı s ı el, 1997). In this study, several alkaloids were determined as second most found molecule classes in the extracts of *Ruta chalepensis* roots.

Graveoline which is a hydroquinolone molecule was the most abundant alkaloid component in all extracts of all extraction methods. In sbcW-E extract, maximum amount of graveoline (8.9%) was obtained at 140 °C and 80 atm while it was detected in $scCO_2$-E extract with relative amount of 9.0% at 200 atm and was also decided as the most abundant alkaloid compound of sbcEtOH-E extracts and reached its maximum (7.9%) at 160 °C, 80 atm). It was noticed that during sub and supercritical fluid extraction, pressure has an influence on the yield of Graveoline as obviously as the extraction temperature. Under UAE conditions, tBME was decided as most efficient solvent for the extraction of Graveoline (6.6% at 4 h). On the other side, Hallacridone which is a rare alkaloid was detected as the most abundant compound in the extract of UAE with methyl alcohol (8.8% in 2 h and 4 h). It was also observed in $scCO_2$-E extracts with a low amount (0.9% at 200 atm). Extracts of UAE of *Ruta chalepensis* roots with MeOH, EtOH and tBME were also rich in alkaloids and MeOH was observed as most efficient solvent for the extraction of alkaloids (20.1% at 2 h). A 2-alkylated N-methyl-quinolinone was obtained as second most abundant alkaloid in extracts of all extraction methods.

In addition, two acridone alkaloids, 1-hydroxy-10-methylacridin-9(10*H*)-one and 1-hydroxy-3-methoxy-10-methylacridin-9(10*H*)-one were detected in all extracts of all methods used. Acridone alkaloids are heterocyclic aromatic compounds, and their heterocyclic unit contains a carbonyl and secondary amino group at its 9th and 10th position, respectively. In sbcW-E extracts total yields of acridone alkaloids reached their maximum (5.4% at 160 °C, 80 atm). During the extraction of alkaloids with sbcW-E, efficiency of the method is greatly affected by the extraction temperature. While extraction rate increases with temperature, some desired components of the extract can be degraded at temperatures above 150 °C (Kubatova et al., 2001; Ko et al., 2011). However, for acridone alkaloids, we observed opposite of this determination.

Another molecular classes of the extracts of *Ruta chalepensis* roots were shikimic acid derivatives and phenols were detected in relatively lower amounts. In addition, hydrocarbons and oxygenated hydrocarbons, except for $scCO_2$-E at 90 atm, were also detected in

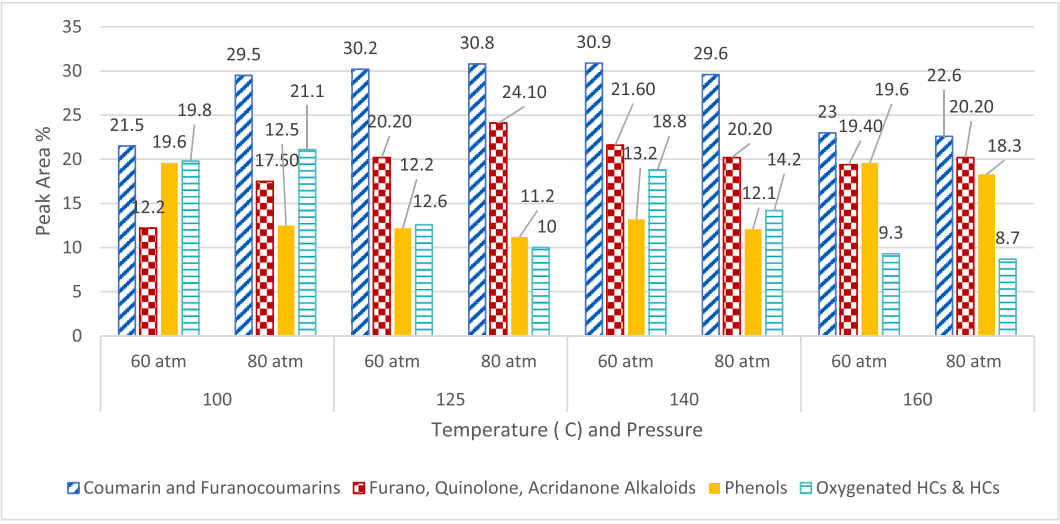


Fig. 5. Relative abundances of chemical components of sbcEtOH-E of *Ruta chalepensis* roots.

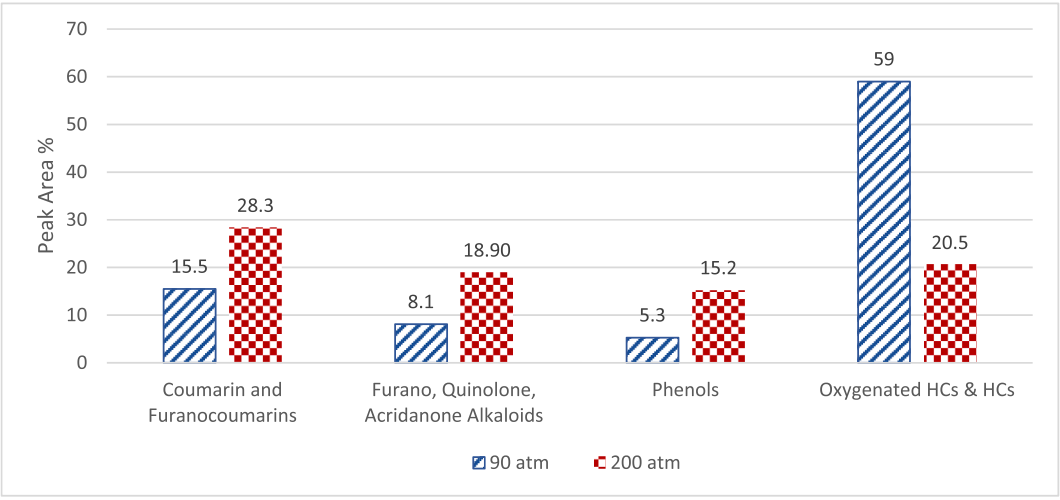


Fig. 6. Comparison of relative abundances of chemical components of $scCO_2$-E of *Ruta chalepensis* roots.

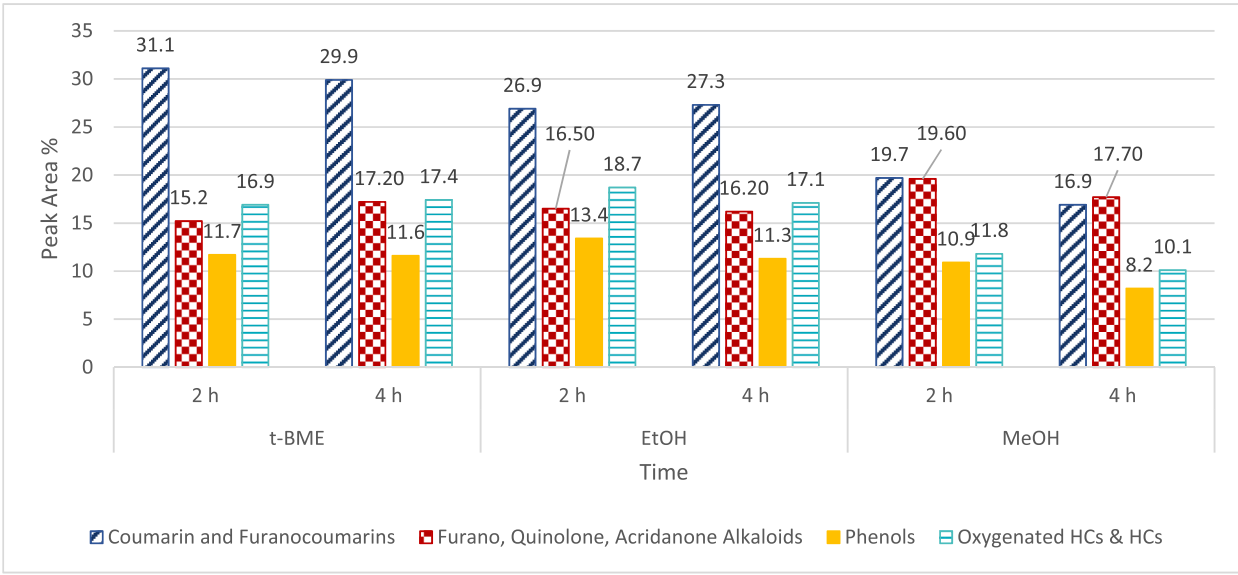
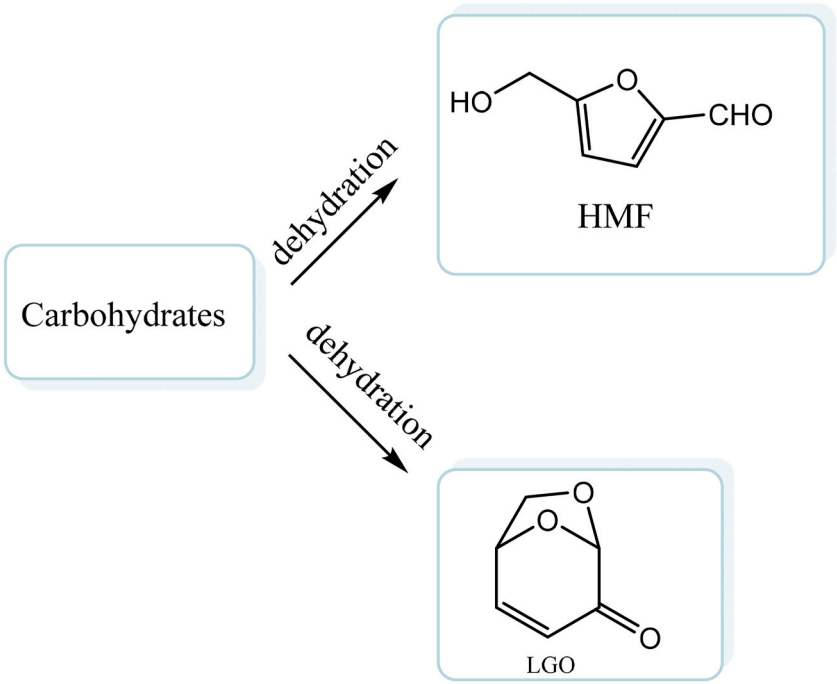


Fig. 7. Comparison of relative abundances of chemical components of UAE-E of *Ruta chalepensis* roots.

lower relative amount.

SbcW-E at 140 °C and 60 atm was the best condition for maximum extraction yield of β-lapachone (4.7%). UAE with tBME for 2 h is also an efficient method for β-lapachone extraction (3.6%). On the other side, two tanshinones, Cryptotanshinone and Isocryptotanshinone were observed only in sbcW-E extract (6.3% at 160 °C and 80 atm) and in UAE with tBME extract (1.1%). To the best of our knowledge, tashinones which are classified biogenetically as diterpenes did not reported before in *Ruta* species. Some phenols were also observed in extracts of *Ruta chalepensis* and (Z)-8-(3,5-dimethyl-4-hydroxyphenyl)-2-octene was the most abundant components of phenols. The highest yields of phenols (19.6%) were observed in sbcW-E at 100 °C and 60 atm.

Some hydrolysis products of carbohydrates were observed in sbcW-E and sbcEtOH-E extracts. It's well known that ion products of subcritical water higher than ambient water due to the destruction of hydrogen bonds between water molecular at high temperature. During subcritical water extraction, due to decomposition and hydrolysis of sugar molecules, HMF, 5-methyl-2-furfural, 5-etoxymethyl-2-furfural, LGO, levulinic acid and lactic acid form (Scheme 1) (Pourali et al., 2010; Wahyudiono Sasaki and Goto, 2008). HMF, LGO, 5-methyl-2-furfural, 5-etoxymethyl-2-furfural were observed as hydrolysis products in sbcW-E extracts. The highest amount of HMF was 1.8% at 160 °C at 60 atm. LGO was observed at 125 °C, 80 atm and 160 °C, 80 atm with same amount (1.6%). During sbcEtOH-E, only HMF was obtained as hydrolysis product and the relative highest amount was 1.3% (125 °C, 80 atm and 140 °C, 60 atm).



Scheme 1. The potential formation pathway of LGO and HMF carbohydrate.

3.3. Effect of static extraction time on the yield of sbcW-E

Since sbcW-E method was decided as most efficient method for the extraction of *Ruta chalepensis* roots, we wanted to examine the effect of extraction time on the yield of the chemical components. Hence, we could develop an efficient method to extract especially for C&FC and alkaloids which are the most abundant two molecule classes of sbcW-E extract. For this study, we choose extraction conditions such as 140 °C, 60 atm with extraction time between 5 and 120 min due to relative amount of C&FC and alkaloids was near each other (Fig. 8). C&FC's are the fastest-extracting class of molecules and their highest amount were extracted in the first 5 min of the process (31.2%). Isochalepensin which was the most abundant component of sbcW-E extract of *Ruta chalepensis* roots reached its maximum in only 5 min at 140 °C and 60 atm (13.6%). As mentioned before, the second most abundant group was alkaloids. Graveoline was the most abundant hydroquinolone alkaloid of sbcW-E extracts of *Ruta chalepensis* roots. Maximum extraction of graveoline was received in 30 min (7.7%). As well as furanoalkaloids, acridon alkaloids also reached their maximum amount (3.8%) in 30 min. The yield of phenols, moschakan D and shikimic acid derivatives reached their maximum in only 5 min. Hence, we can conclude that optimum extraction time of furanocoumarin and alkaloids from *Ruta chalepensis* root is 30 min at 140 °C and 60 atm.

As a result, we can conclude that optimum extraction time of C&FC and alkaloids from *Ruta chalepensis* root is 30 min at 140 °C and 60 atm. Due to relatively highest hydrolysis products distribution, 60 min extraction time was observed as less efficient extraction time for the extraction of bioactive components of *Ruta chalepensis* roots. In addition, with increasing extraction time to 60 min relative amount of hydrolysis products reached to the highest amount (11.8%). It means that relative amount of classes decreased.

4. Conclusion

The results obtained in this study demonstrate that sbcW-E is a highly efficient and rapid method for extracting bioactive compounds such as coumarins, furanocoumarins, alkaloids, phenolics, shikimic acid derivatives, oxygenated hydrocarbons and hydrocarbons from. Hence, we conclude that subcritical water could be an excellent environmentally friendly alternative to using organic solvents such as methanol and ethanol for extracting bioactive compounds. The highest extraction yield (40.6 ± 0.2%, d.b) of *Ruta chalepensis* roots was observed with sbcW-E at 125 °C and 60 atm. The efficiency of the methods used on the extraction yield was ranked as sbcW-E (40.6 ± 0.2%, 125 °C and 60 atm) > sbcEtOH-E (18.9% at 100 °C and 60 atm) > UAE (10.3%, EtOH, 2 h)> $scCO_2$-E (9.1%, 45 °C, 200 atm). Coumarin and furanocoumarins were the most abundant components of all extracts of extraction methods used, and maximum yields of coumarin and furanocoumarin were observed in sbcW-E extract (160 °C and 80 atm). Isochalepensin and graveolin were most abundant components of these two classes. The most appropriate extraction method for phenol derivatives was decided as sbcEtOH-E while $scCO_2$-E was efficient to extract oxygenated hydrocarbons and hydrocarbons.

Author statements

Onur Demirkol: Validation, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing. **Mehmet Erşatır:** Validation, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing. **Elife Sultan Giray:** Validation,

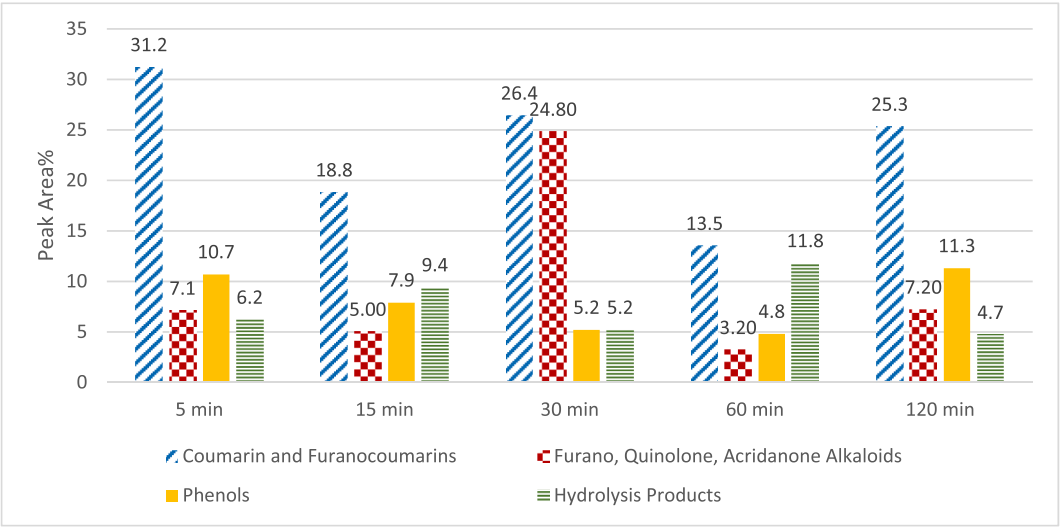


Fig. 8. Comparing the effect of static extraction time on the relative abundances of chemical classes of sbcW-E extract.

Investigation, Resources, Conceptualization, Methodology, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision. **Saliha Kırıcı:** Investigation, Resources, Conceptualization, Methodology, Visualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scp.2022.100750.

References

Arumugham, T., Rambabu, K., Hasan, S.W., Show, P.L., Rinklebe, J., Banat, F., 2021. Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications- A review. Chemosphere 271, 129525. https://doi.org/10.1016/j.chemosphere.2020.129525.

Baldino, L., Adami, R., Reverchon, E., 2018. Concentration of Ruta graveolens active compounds using SC-CO_2 extraction coupled with fractional separation. J. Supercrit. Fluids 131, 82–86. https://doi.org/10.1016/j.supflu.2017.09.008.

Casas-Cardoso, L., Mantell, C., Obregón, S., Cejudo-Bastante, C., Alonso-Moraga, Á., Martínez de la Ossa, E.J., Haro-Bailón, A., 2021. Health-promoting properties of borage seed oil fractionated by supercritical carbon dioxide extraction. Foods 10, 2471. https://doi.org/10.3390/foods10102471.

Castıllo, J.B.D., Secundıno, M., Luis, F.R., 1986. Four aromatic derivatives from *Ruta angustifolia*. Phytochemistry 25 (9), 2209–2210. https://doi.org/10.1016/0031-9422(86)80093-0.

Ekıert, H., Kısıel, W., 1997. Coumarins and alkaloids in shoot culture of *Ruta graveolens* L. Acta Soc. Bot. Pol. 66 (3–4), 329–332. https://doi.org/10.5586/asbp.1997.039.

Giergielewicz-Mozajska, H., Dąbrowski, Ł., Namieśnik, J., 2001. Accelerated solvent extraction (ASE) in the analysis of environmental solid samples-some aspects of theory and practice. Crit. Rev. Anal. Chem. 31 (3), 149–165. https://doi.org/10.1080/20014091076712.

Giray, E.S., Kırıcı, S., Kaya, D.A., Türk, M., Sönmez, Ö., İnan, M., 2008. Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical composition of *Lavandula stoechas*. Talanta 74, 930–935. https://doi.org/10.1016/j.talanta.2007.

Ivanovic, J., Ristic, M., Skala, D., 2011. Supercritical CO_2 extraction of *Helichrysum italicum*: influence of CO_2 density and moisture content of plant material. J. Supercrit. Fluids 57, 129–136. https://doi.org/10.1016/j.supflu.2011.02.013.

Jawade, N.R., Chavan, A.R., 2013. Ultrasonic-assisted extraction of aloin from aloe vera gel. Procedia Eng. 51, 487–493. https://doi.org/10.1016/j.proeng.2013.01.069.

Keiichi, S., Mitsuhiro, M., Tadao, M., Kazuhiko, S., Taku, M., 1992. Shoyakugaku 46, 9.

Khan, M.K., Abert-Vian, M., Fabiano-Tixier, A.-S., Dangles, O., Chemat, F., 2010. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) peel. Food Chem. 119, 851–858. https://doi.org/10.1016/j.foodchem.2009.08.046.

Ko, M.-J., Cheigh, C.-I., Cho, S.-W., Chung, M.-S., 2011. Subcritical water extraction of flavonol quercetin from onion skin. J. Food Eng. 102, 327–333. https://doi.org/10.1016/j.jfoodeng.2010.09.008.

Kostova, I., Ivanova, A., Mikhova, B., Klaiber, I., 1999. Alkaloids and coumarins from Ruta graveolens. Monatsh. Chem. 130 (5), 703–707. https://doi.org/10.1007/PL00010251.

Kubatova, A., Lagadec, A.J.M., Miller, D.J., Hawthorne, S.B., 2001. Selective extraction of oxygenates from savory and peppermint using subcritical water. Flavour Fragrance J. 16, 64–73. https://doi.org/10.1002/1099-1026(200101/02)16:1<64::AID-FFJ949>3.0.CO;2-D.

Li, H., Pordesimo, L., Weiss, J., 2004. High intensity ultrasound-assisted extraction of oil from soybeans. Food Res. Int. 37, 731–738. https://doi.org/10.1016/j.foodres.2004.02.0.

Lin, Y., Smart, N.G., Wai, C.M., 1995. Supercritical fluid extraction and chromatography of metal chelates and organometallic compounds. Trends Anal. Chem. 14, 123. https://doi.org/10.1016/0165-9936(95)94045-G.

Lin, M.-C., Tsai, M.-J., Wen, K.-C., 1999. Supercritical fluid extraction of flavonoids from Scutellariae Radix. J. Chromatogr. A 830, 387–395. https://doi.org/10.1016/S0021-9673(98)00906-6.

Londoño-Londoño, J., de Lima, V.R., Lara, O., Gil, A., Pasa, T.B.C., Arango, G.J., Pineda, J.R.R., 2010. Clean recovery of antioxidant flavonoids from citrus peel: optimizing an aqueous ultrasound-assisted extraction method. Food Chem. 119, 81–87. https://doi.org/10.1016/j.foodchem.2009.05.075.

Machmudah, S., Kondo, M., Sasaki, M., Goto, M., Munemasa, J., Yamagata, M., 2008. Pressure effect in supercritical CO_2 extraction of plant seeds. J. Supercrit. Fluids 44, 301–307. https://doi.org/10.1016/j.supflu.2007.09.024.

Marcus, Y., 2018. Extraction by subcritical and supercritical water, methanol, ethanol and their mixtures. Separations 5 (4), 1–18. https://doi.org/10.3390/separations5010004.

Mejri, J., Bouajila, J., Aydi, A., Barth, D., Abderrabba, M., Mejri, M., 2012a. Supercritical CO_2 extract and essential oil of *Ruta* chalepensis L. Growing in Tunisia: a natural source of undecan-2-one. Anal. Chem. Lett. 2 (5), 290–300. https://doi.org/10.1080/22297928.2012.1064280.

Mejri, J., Bouajila, J., Abdelkarim, A., Barth, D., Mejri, M.A.M., 2012b. Supercritical CO_2 extract and essential oil of Ruta chalepensis L. Growing in Tunisia: a natural source of undecan-2-one. Anal. Chem. Lett. 2 (5), 290–300. https://doi.org/10.1080/22297928.2012.10648280.

Moldao-Martins, M., Palavra, A., Beirao da Costa, M.L., Bernardo-Gil, M.G., 2000. Supercritical CO_2 extraction of Thymus zygis L. subsp. sylvestris aroma. J. Supercrit. Fluids 18, 25–34. https://doi.org/10.1016/S0896-8446(00)00047-4.

Mukhopadhyay, M., Panja, P., 2010. Hot water as a novel extractant of natural products: a review. Indian Chem. Eng. 51 (4), 311–324. https://doi.org/10.1080/00194500903430655.

Orlita, A., Sidwa-Gorycka, M., Kumirska, J., Malinski, E., Siedlecka, E.M., Gajdus, J., Lojkowska, E., Stepnowski, P., 2008a. Identification of *Ruta graveolens* L. metabolites accumulated in the presence of abiotic elicitors. Biotechnol. Prog. 24, 128–133. https://doi.org/10.1021/bp070261.

Orlita, A., Sidwa-Gorycka, M., Paszkiewicz, M., Malinski, E., Kumirska, J., Siedlecka, E.M., Lojkowska, E., Stepnowski, P., 2008b. Application of chitin and chitosan as elicitors of coumarins and furoquinolone alkaloids in *Ruta graveolens* L. (common rue). Biotechnol. Appl. Biochem. 51, 91–96. https://doi.org/10.1042/BA20070200.

Pan, Z., Qu, W., Ma, H., Atungulu, G.G., McHugh, T.H., 2011. Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. Ultrason. Sonochem. 19 (2), 365–372. https://doi.org/10.1016/j.ultsonch.2011.05.015.

Pourali, O., Asghari, F.S., Yoshida, H., 2010. Production of phenolic compounds from rice bran biomass under subcritical water conditions. Chem. Eng. J. 160 (1), 259–266. https://doi.org/10.1016/j.cej.2010.02.057.

Rahman, A.H.M.M., Gulshana, M.I.A., 2014. Taxonomy and medicinal uses on amaranthaceae family of rajshahi, Bangladesh. Appl. Ecol. Environ. Sci. 2 (2), 54–59. https://doi.org/10.12691/aees-2-2-3.

Riyanto, S., Sukari, M.A., Rahmani, M., Gwendoline, C. L. Ee, Taufiq-Yap, Y.H., Aimi, N., Kitajima, M., 2001. Alkoloids from aegle marmelos (*Rutaceae*), Malaysian. J. Anal. Sci. 7 (2), 463–465.

Santos, D.T., Veggi, P.C., Meireles, M.A.A., 2010. Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: yield, composition and economical evaluation. J. Food Eng. 101, 23–31. https://doi.org/10.1016/j.jfoodeng.2010.06.005.

Sidwa-Gorycka, M., Krolicka, A., Orlita, A., Malinski, E., Golebiowski, M., Kumurska, J., Chromik, A., Biskup, E., Stepnowski, P., Lojkowska, E., 2009. Genetic transformation of *Ruta graveolens* L. by *Agrobacterium rhizogenes*: hairy root cultures a promising approach for production of coumarins and furanocoumarins. Plant Cell Tissue Organ Cult. 97, 59–69. https://doi.org/10.1007/s11240-009-9498-x.

Sivakumar, V., Vijaeeswarri, J., Anna, J.L., 2011. Effective natural dye extraction from different plant materials using ultrasound. Ind. Crop. Prod. 33, 116–122. https://doi.org/10.1016/j.indcrop.2010.09.007.

Sovová, H., 2012. Steps of supercritical fluid extraction of natural products and their characteristic times. J. Supercrit. Fluids 66, 73–79. https://doi.org/10.1016/j.supflu.2011.11.004.

Sovova, H., Sajfrtova, M., Stateva, R.P., 2017. A novel model for multicomponent supercritical fluid extraction and its application to Ruta graveolens. J. Supercrit. Fluids 120 (1), 102–112. https://doi.org/10.1016/j.supflu.2016.10.008.

Ulubelen, A., Güner, H., 1988. İsolation of dehydromoskachan C from *Ruta chalepensis* var. Latifolia. J. Nat. Prod. 51 (5), 1012–1013.

Wahyudiono, M., Sasaki, Goto, M., 2008. Recovery of phenolic compounds through the decomposition of lignin in near and supercritical water. Chem. Eng. Process 47, 1609–1619. https://doi.org/10.1016/j.cep.2007.09.001.

Wu, T.-S., Shi, L.-S., Wang, J.-J., Iou, S.-C., Chang, H.-C., Chen, Y.-P., Kuo, Y.-H., Chang, Y.-L., Teng, C.-M., 2003. Cytotoxic and antiplatelet agregation principles of *Ruta graveolens*. J. Chin. Chem. Soc. 50, 171–178. https://doi.org/10.1002/jccs.200300024.

Yang, Q.-Y., Tıan, X.-Y., Fang, W.-S., 2007. Bioactive coumarins from BoenninghaUAEnia sessilicarpa. J. Asian Nat. Prod. Res. 9 (1), 59–65. https://doi.org/10.1080/10286020500382397.

Yutaka, I., Norio, S., Masahiko, A., Kunio, A., 1991. Solvent polarity parameters of supercritical carbon dioxide as measured by infrared spectroscopy. Bull. Chem. Soc. Jpn. 64 (7), 2224–2229. https://doi.org/10.1246/bcsj.64.2224.

Zhang, H.-F., Yang, X.-H., Zhao, Li-D., Wang, Y., 2009. Ultrasonic-assisted extraction of epimedin C from fresh leaves of Epimedium and extraction mechanism. Innovat. Food Sci. Emerg. Technol. 10, 54–60. https://doi.org/10.1016/j.ifset.2008.09.007.