Simultaneous quantification of phenolic acids and flavonoids in Chamaerops humilis L. using LC–ESI-MS/MS

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Abstract

In this study, heated reflux extraction method has been used to identify the phenolic compounds from C. humilis var. argentea leaflets, rachis and fruits. Extractions were performed in both ultrapure water and 80% methanol solvents. The efficiency of procedures was determined in terms of the quality and quantity of phenolic acids and flavonoids identified. Chamaerops extracts have been characterized by high concentrations of phenolic compounds, which play a crucial role in protection against various diseases. LC-MS/MS was used to determine the chemical profile of various extracts obtained from Chamaerops. The results showed that the major components in leaflets and fruits extracts were quinic, malic and chlorogenic acids. In addition, nine minor acidic components were identified. On the other hand, rutin and hesperidin were found to be the major flavonoids. The methanol extract was shown as being the most efficient to identify phenolic compounds in C. humilis.

Keywords: Chamaerops humilis L.; LC-ESI-MS/MS; phenolic compounds; chemical composition; extracts.

Practical Application: Chamaerops humilis L. rich in flavonoids and phenolic acids was a great promising source of different bioactive components.

1 Introduction

Flavonoids and phenolic acids, a class of polyphenol compounds, are widely distributed in plants kingdom. Flavonoids include over 6000 identified family members. They play an important role in protection of plants from microbial and insect attack. Many studies reported that flavonoids exhibit various effects as antioxidant (Siahpoosh et al., 2016; Balci & Özdemir, 2016), anti-cancer (Androutsopoulos et al., 2010; Ma et al., 2015), anti-allergic (Park et al., 2006), anti-thrombotic and vasodilatory (Rahimi et al., 2010), anticholinesterase (Ertas et al., 2016) actions. Finally, because of their UV-absorbing properties, flavonoids protect plants from the UV radiation of the sun and scavenge UV-generated reactive oxygen species (Shirley, 1996).

Chamaerops humilis L., var. argentea, belonging to the Arecaceae family, is a palm widely distributed in the Mediterranean basin especially in Algeria which has been located mainly in Tlemcen and Oranian coast. In folk medicine, this plant has been widely applied by decoction in Algerian populations for a variety of illnesses as stomachache, toning (Hasnaoui et al., 2011), diabetes (Bnouham et al., 2002). Moreover, previous studies reported Chamaerops to contain phenolic compounds, such as tannins, flavonoids, saponins, quinons, coumarines (Benahmed-Bouhafsoun et al., 2013), sterols and terpenoids (Hasnaoui et al., 2013).

Additionally, antioxidant (Benahmed-Bouhafsoun et al., 2013; Khoudali et al., 2014), hypoglycemic and hypolipidemic (Gaamoussi et al., 2010), antilithic (Beghalia et al., 2008), anti-inflammatory and urinary antiseptic (Bellakhdar et al., 1991) activities of C. humilis were reported.

The flavonoids were previously reported as constituents of the Arecaceae family plants, but literature lacks detailed information on the phytochemical composition of C. humilis. This is the first study for the identification and quantification of phenolic acids and flavonoids of C. humilis. Therefore, the objective of the present study was to characterize the chemical composition of water and 80% methanol extracts of C. humilis leaflets, rachis and fruits by using liquid chromatography coupled with mass spectrometry (LC-ESI-MS/MS) as a potent analytical technique.

2 Materials and methods

2.1 Chemicals and instruments

The phenolic identification and quantification of C. humilis were determined by using LC-ESI-MS/MS (Shimadzu, Kyoto, Japan). (L)-Malic acid (purity: 95-100%), quercetin (95%), protocatechuic acid (97%), chrysin (97%), rutin (94%), hesperetin (95%), naringenin (95%), rosmarinic acid (96%), vanillin (99%),...
Table 1

L. var. argentea was collected by Dr. A. Bouhafsoun from western Algeria (Oran city) in June of 2014.

2.2 Plant material

Chamaerops humilis L. Var. argentea was collected by Dr. A. Bouhafsoun from western Algeria (Oran city) in June of 2014.

2.3 Extraction under continuous reflux

Three grams of dried samples were soaked separately in 50 ml of 80% aqueous methanol and ultrapure water at 60 °C for 30 min. The extracts were filtered through nylon filter. The extraction was repeated twice. The collected filtrates were dried under vacuum using a rotary evaporator at 30 °C until dry extracts were obtained. Dry filtrates were diluted to 1000 mg/L and filtered with 0.2 μm microfiber filter prior to LC–MS/MS analysis.

2.4 LC–MS/MS instrumentation and chromatographic conditions

LC–MS/MS analysis of the phenolic compounds was performed by using a Nexera model Shimadzu UHPLC coupled to a tandem MS instrument. The liquid chromatography was equipped with LC–30AD binary pumps, DGU-20A3R degasser, CTO-10ASvp column oven and SIL-30AC autosampler. The chromatographic separation was performed on a C18 reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3 μm) analytical column. The column temperature was fixed at 40 °C. The elution gradient consisted of mobile phase A (water, 5 mM ammonium formate and 0.1% formic acid) and mobile phase B (methanol, 5 mM ammonium formate and 0.1% formic acid). The gradient program with the following proportions of solvent B was applied t (min), B%: (0, 40), (20, 90), (23.99, 90), (24, 40), (29, 40). The solvent flow rate was maintained at 0.5 mL/min and injection volume was settled as 4 μL.

2.5 MS instrumentation

MS detection was performed using Shimadzu LCMS 8040 model triple quadrupole mass spectrometer equipped with an ESI source operating in both positive and negative ionization modes. LC–MS/MS data were collected and processed by LabSolutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analytes: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation. The optimum ESI conditions were determined as interface temperature; 350 °C, DL temperature; 250 °C, heat block temperature; 400 °C, nebulizing gas flow (nitrogen); 3 L/min and drying gas flow (nitrogen); 15 L/min.

2.6 Method validation parameters

In this study, twenty-four phenolic compounds (flavonoids, flavonoid glycosides, phenolic acids, phenolic aldehyde, coumarin) and three non-phenolic organic acids that are widespread in edible plant materials were qualified and quantified in two edible plants. Rectilinear regression equations and the linearity ranges of the studied standard compounds were given in Table 1. Correlation coefficients were found to be higher than 0.99. The limit of detection (LOD) and limit of quantitation (LOQ) of the reported analytical method were shown in Table 1. For the studied compounds, LOD ranged from 0.05 to 25.8 μg/L and LOQ ranged from 0.17 to 85.9 μg/L (Table 1) (Ertas et al., 2015). Moreover, the recoveries of the phenolic compounds ranged from 96.9% to 106.2%.

2.7 Statistical analysis

All experiments were conducted in triplicate and the data was presented as the mean value ± standard deviation (SD).

3 Results and discussion

In this study, twenty seven compounds (authentic markers) were studied for their dominant fragmentation pathways (Figure 1).

Most of the compounds in MS exhibited abundant [M - H]− in negative ion mode and [M + H]+ in the positive ion mode were subjected to MS/MS analysis, retention time (RT) and mass spectral characteristics of all marker compounds were given in Table 1.

The variables considered during reflux extraction process including 80% methanol and ultrapure water were tested for the extraction of polyphenols from rachis, leaflets and fruits of Chamaerops, in order to achieve high extraction efficiency of phenolic acids and flavonoids. After LC-MS/MS analysis,
the results indicated that phenolic acids were among the most abundant polyphenols detected in all plant parts including quinic, malic, chlorogenic, protocatechuic, p-hydroxybenzoic, p-coumaric, tr-aconitic, gallic and tannic acids, with quinic acid being equally predominant in both methanol and water leaflets extracts.

As shown in Table 2, a high content of quinic acid identified in leaflets was almost equal to 2.5 times of that found in rachis (37690 ± 1809 against 13198 ± 634 µg g⁻¹ extract) respectively. In this context, it could be said that Chamaerops humilis is a good source of quinic acid. Many studies in the literature showed that quinic acid has a potent broad spectrum antioxidant (Pero et al., 2009), hepatoprotective (Xiang et al., 2001) and can be used to combat prostate cancer (Onbathamizh & Padmini, 2013). Furthermore, protocatechuic acid was quantified in all Chamaerops extracts. However, in water solvents approximately equal amounts of protocatechuic acid was obtained (with 33 ± 2 µg g⁻¹). The other reflux extracts contained lower concentrations of that molecule. This aldehyde, contributes to the original natural flavour of vanilla and is a very popular flavouring agent used in large range of foods and as fragrance ingredients (Mitra et al., 2002).

In addition, protocatechuic acid was quantified in all Chamaerops extracts. However, in water solvents approximately equal amounts of protocatechuic acid was obtained (with 33 ± 2 µg g⁻¹ in LW as high concentration). This value was equal to vanillin found also in LW (33 ± 2 µg g⁻¹), and identified slightly less in LM (22 ± 1 µg g⁻¹). The other reflux extracts contained lower concentrations of that molecule. This aldehyde, contributes to the original natural flavour of vanilla and is a very popular flavouring agent used in large range of foods and as fragrance ingredients (Mitra et al., 2002).

Chamaerops extracts contain also minor amounts of p-coumaric, tannic, p-hydroxybenzoic, gallic, tr-cafeic, tr-aconitic and salicylic acids. However, traces of rosmarinic acid were found only in few Chamaerops extracts.
Different types of flavonoids such as flavones, flavonols, and flavanones were found in Chamaeops extracts. Flavonoids identified included various flavone-C-glycosides of apigenin and luteolin.

Our results showed that heat reflux was a good attractive procedure for the extraction of luteolin in both methanol and water extracts of leaflets. However, it was not identified in rachis and fruits parts.

Flavone C-glycosides and tricin were previously identified in C. humilis leaves (Williams et al., 1973; Hirai et al., 1986). These results showed that the rutin and kaempferol were identified in all Chamaeops extracts, a higher content was obtained in methanol extracts of leaflets (33 ± 2 µg g⁻¹), water was extracted three times less rutin LW (10.9 ± 0.54 µg g⁻¹). In a previous study rutin was identified from the C. humilis leaves (Hirai et al., 1986) and Phoenix dactylifera L. fruits (Hamad et al., 2015).

According to the literature, kaempferol glycosides are widely distributed in the Arecaceae family (Williams et al., 1973).

LC-ESI-MS-MS detected traces of fisetin, rhamnatin and myricitin in only some Chamaeops extracts. The presence of quercetin traces has been present only in methanol extracts. Nonetheless, flavonoid C-glycosides were already identified from plants of the Arecaceae family. Flavone C-glycosides (84%), tricin (51%), luteolin (30%) and quercetin glycosides (24%)
Phenolic compounds of Chamaerops humilis L.

were found out in the leaves of the 125 species of the Palmae (Williams et al., 1973).

Regarding the flavanone group, naringenin was weakly identified in all Chamaerops extracts. It is important to note that hesperitin was not identified, while its glycoside hesperidin (which is conjugates with rhamnosyl-α-1,6-glucose) was found. However, its concentration has decreased about 6 times less in water extracts (35 ± 2 and 6.2 ± 0.3 µg g⁻¹ in LM and LW respectively), this reflects the solubility behaviour of hesperidin (Grandi et al., 1994).

Finally, methanol was more effective extraction solvent, which resulted in the coextraction of lots of compounds (Figure 2).

4 Conclusion

In the present study, phenolic composition of leaflets, rachis and fruits parts of C. humilis var. argentea were identified by using heated reflux extraction and liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis techniques. The LC-MS/MS results revealed that quinic, malic and chlorogenic acids and rutin and hesperidin were the major phenolic compounds in leaflets and fruits extracts. Besides, the methanol extract was detected to be the most efficient solvent to identify phenolic compounds in C. humilis. This approach showed that Chamaerops was a great promising source of different bioactive components, particularly phenolic acids and flavonoids. In vivo studies are required to determine its benefits as potential food ingredients and being agents for protection against various diseases. Therefore, the growing use of Chamaerops in foods was encouraged.

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Figure 2. Graph that shows the concentrations (µg analyte/g extract) of chlorogenic, malic and quinic acids in methanol and water extracts of different parts. RW: Rachis water extract, LW: Leaflets water extract, FW: Fruit water extract, RM: Rachis methanol extract, LM: Leaflets methanol extract, FM: Fruit methanol extract.

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