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

Aicha BOUHAFSOUN[1*], Mustafa Abdullah YILMAZ[2,3], Ahmed BOUKELOUA[4], Hamdi TEMEL[3], Meriem KAID HARCHE[1]

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%),

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[1] *Laboratoire de Production et Valorisation Végétale et Microbienne, Département de Biotechnologie, Faculté des Sciences de la Nature et de la Vie, Université des Sciences et de la Technologie d'Oran Mohamed Boudiaf – USTO-MB, Oran, Algérie*
[2] *Dicle University Science and Technology Research and Application Center – DUBTAM, Dicle University, Diyarbakir, Turkey*
[3] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dicle University, Diyarbakir, Turkey*
[4] *Département de Biologie, Faculté des Sciences Exactes et Sciences de la Nature et de la Vie, Université d'Oum El Bouaghi, Oum Bouaghi, Algérie*
*Corresponding author: abouhafsoun@gmail.com

p-coumaric acid (98%), caffeic acid (98%), chlorogenic acid (95%), hyperoside (≥97%), myricetin (≥96%), coumarin (≥99%), kaempferol (≥97%) were obtained from Sigma (Germany); quinic acid (98%), *tr*-aconitic acid (98%), *4*-hydroxybenzoic acid (≥99%), fisetin (≥98%) were from Aldrich (Germany); gallic acid (≥99%), tannic acid (puris), salicylic acid (≥99%) were from Sigma-Aldrich (Germany); hesperidin (≥97%), luteolin (≥97%), apigenin (≥99%), rhamnetin (≥99%) were from Fluka (Germany). HPLC grade methanol was purchesed from Merck, USA.

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 filtrated 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,

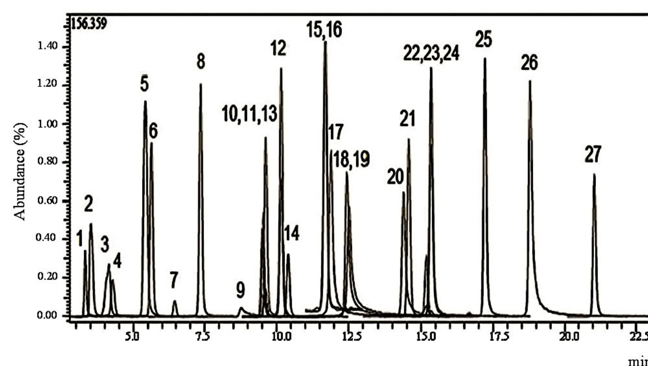

Figure 1. Total Ion Chromatogram (TIC) of 250 ppb standard mix. 1: Quinic acid, 2: Malic acid, 3: *tr*-Aconitic acid, 4: Gallic acid, 5: Chlorogenic acid, 6: Protocatechuic acid, 7: Tannic acid, 8: *tr*-caffeic acid, 9: Vanillin, 10: *p*-Coumaric acid, 11: Rosmarinic acid, 12: Rutin, 13: Hesperidin, 14: Hyperoside, 15: *4*-OH Benzoic acid, 16: Salicylic acid, 17: Myricetin, 18: Fisetin, 19: Coumarin, 20: Quercetin, 21: Naringenin, 22: Hesperetin, 23: Luteolin, 24: Kaempferol, 25: Apigenin, 26: Rhamnetin, 27: Chrysin.

Table 1. Analytical parameters of LC-MS/MS method.

No	Analytes	RT[a]	r^2[b]	Ion. Mode[c]	Equation	RSD%[d]	Linearity (mg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)	U[f]
1	Quinic acid	3.36	0.9927	Neg	f(x) = 25133 + 33.6x	0.0388	250-10000	22.3 / 74.5	103.3	4.8
2	Malic acid	3.60	0.9975	Neg	f(x) = – 5674 + 93.6x	0.1214	250-10000	19.2 / 64.1	101.4	5.3
3	tr-Aconitic acid	4.13	0.9933	Neg	f(x) = – 28416 + 79.3x	0.3908	250-10000	15.6 / 51.9	102.8	4.9
4	Gallic acid	4.25	0.9901	Neg	f(x) = 26417 + 358.1x	0.4734	25-1000	4.8 / 15.9	102.3	5.1
5	Chlorogenic acid	5.29	0.9932	Neg	f(x) = 26780 + 49.0x	0.1882	250-10000	7.3 / 24.3	99.7	4.9
6	Protocatechuic acid	5.51	0.9991	Neg	f(x) = 6197 + 36.9x	0.5958	100-4000	25.8 / 85.9	100.2	5.1
7	Tannic acid	6.30	0.9955	Neg	f(x) = 30233 + 90.3x	0.9075	100-4000	10.2 / 34.2	97.8	5.1
8	tr- caffeic acid	7.11	0.9942	Neg	f(x) = 83958 + 1585.2x	1.0080	25-1000	4.4 / 14.7	98.6	5.2
9	Vanillin	8.57	0.9995	Neg	f(x) = – 575 + 44.5x	0.4094	250-10000	10.1 / 33.7	99.2	4.9
10	p-Coumaric acid	9.17	0.9909	Neg	f(x)= 27064 + 73.5x	1.1358	100-4000	15.2 / 50.8	98.4	5.1
11	Rosmarinic acid	9.19	0.9992	Neg	f(x) = – 1150 + 18.0x	0.5220	250-10000	10.4 / 34.8	101.7	4.9
12	Rutin	9.67	0.9971	Neg	f(x) = 3842 + 51.9x	0.8146	250-10000	17.0 / 56.6	102.2	5.0
13	Hesperidin	9.69	0.9973	Poz	f(x) = 105641 + 195.8x	0.1363	250-10000	21.6 / 71.9	100.2	4.9
14	Hyperoside	9.96	0.9549	Neg	f(x) = 827 + 1.0x	0.2135	100-4000	12.4 / 41.4	98.5	4.9
15	4-OH Benzoic acid	11.38	0.9925	Neg	f(x) = 5428 + 635.0x	1.4013	25-1000	3.0 / 10.0	106.2	5.2
16	Salicylic acid	11.39	0.9904	Neg	f(x) = 72571 + 915.2x	0.6619	25-1000	4 / 13.3	106.2	5.0
17	Myricetin	11.42	0.9991	Neg	f(x) = 5415 + 54.3x	2.8247	100-4000	9.9 / 32.9	106.0	5.9
18	Fisetin	12.10	0.9988	Neg	f(x) = 34409 + 331.9x	2.4262	100-4000	10.7 / 35.6	96.9	5.5
19	Coumarin	12.18	0.9924	Poz	f(x) = 34370 + 236.6x	0.4203	100-4000	9.1 / 30.4	104.4	4.9
20	Quercetin	13.93	0.9995	Neg	f(x) = 1693 + 206.1x	4.3149	25-1000	2.0 / 6.8	98.9	7.1
21	Naringenin	14.15	0.9956	Neg	f(x) = 39056 + 1100.6x	2.0200	25-1000	2.6 / 8.8	97.0	5.5
22	Hesperetin	14.80	0.9961	Neg	f(x) = 6545 + 160.3x	1.0164	25-1000	3.3/ 11.0	102.4	5.3
23	Luteolin	14.48	0.9992	Neg	f(x) = 3057 + 111.5x	3.9487	25-1000	5.8 / 19.4	105.4	6.9
24	Kaempferol	14.85	0.9917	Neg	f(x) = 571 + 21.0x	0.5885	25-1000	2.0 / 6.6	99.1	5.2
25	Apigenin	16.73	0.9954	Neg	(x) = 18526 + 543.8x	0.6782	25-1000	0.1 / 0.3	98.9	5.3
26	Rhamnetin	18.41	0.9994	Neg	f(x) = 632 + 110.1x	2.5678	25-1000	0.2 / 0.7	100.8	6.1
27	Chrysin	20.60	0.9965	Neg	f(x) = 23532 + 698.8x	1.5530	25-1000	0.05 / 0.17	102.2	5.3

[a]RT: retention time; [b]r^2: coefficient of determination; [c]Ion. Mode: Ionization mode of the analytes; [d]RSD: relative standard deviation; [e]LOD/LOQ (µg/L): limit of detection/limit of quantification; [f]U (%): percent relative uncertainty at 95% confidence level (k = 2). Neg: negative mode, Poz: positive mode.

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 *C. 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 (Inbathamizh & Padmini, 2013).

Furthermore, malic acid was the second most prevalent compound after quinic acid in leaflets and fruits. However, in rachis, it was the major organic acid with higher concentration in methanol extract (RM and RW contained 21747 ± 1153 against 17614 ± 933 µg g^{-1} of malic acid, respectively).

Previous work has already demonstrated in other Arecaceae that malic acid was identified as the major compound in *Phœnix canariensis* (Al-Farsi et al., 2005), this organic acid was thought to play an important role in cardioprotective properties (Khazanov et al., 2008).

Methanol showed the highest extraction capacity for chlorogenic acid which took the third place after quinic and malic acid in all extracts. Its presence was dominant in leaflets and rachis. Previous studies have shown that chlorogenic acid blocked chemically induced carcinogens in the large intestine (Mori et al., 1986).

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*-caffeic, *tr*-aconitic and salycilic acids. However, traces of rosmarinic acid were found only in few Chamaerops extracts.

Table 2. Identification and quantification of compounds of water and methanol reflux extracts of *C. humilis* by LC–MS/MS.

Analytes	Parent ion (m/z)[a]	MS² (Collision energy)[b]	Quantification (µg analyte/g extract)[c] RW	LW	FW	RM	LM	FM
Quinic acid	191.0	85 (22), 93 (22)	13198 ± 634	37690 ± 1809	1842 ± 88	15092 ± 724	38920 ± 1868	1507 ± 72
Malic acid	133.1	115 (14), 71 (17)	17614 ± 933	12215 ± 647	67 ± 4	21747 ± 1153	12590 ± 667	54 ± 3
***tr*-Aconitic acid**	172.9	85 (12). 129 (9)	1.7 ± 0.08	ND	3.8 ± 0.2	2.97 ± 0.14	2.8 ± 0.1	1.30 ± 0.06
Gallic acid	169.1	125 (14), 79 (25)	0.70 ± 0.03	1.40 ± 0.07	0.20 ± 0.01	0.40 ± 0.02	1.50 ± 0.07	0.1 ± 0.0
Chlorogenic acid	353.0	191 (17)	205 ± 10	415 ± 20	51 ± 3	605 ± 30	1490 ± 73	53 ± 3
Protocatechuic acid	153.0	109 (16), 108 (26)	10.3 ± 0.5	33 ± 2	17.3 ± 0.9	12.4 ± 0.6	27 ± 1	10.9 ± 0.6
Tannic acid	183.0	124 (22), 78 (34)	6.5 ± 0.3	1.70 ± 0.08	1.50 ± 0.07	7.6 ± 0.4	12.9 ± 0.7	3.5 ± 0.2
***tr*- caffeic acid**	179.0	135 (15), 134 (24), 89 (31)	1.20 ± 0.06	2.0 ± 0.1	2.1 ± 0.1	1.20 ± 0.06	6.8 ± 0.4	2.4 ± 0.2
Vanillin	151.1	136 (17), 92 (21)	5.1 ± 0.24	33 ± 2	3.5 ± 0.2	5.3 ± 0.3	22 ± 1	4.6 ± 0.2
***p*-Coumaric acid**	163.0	119 (15), 93 (31)	26 ± 1	70 ± 4	1.60 ± 0.08	21 ± 1	79 ± 4	1.00 ± 0.05
Rosmarinic acid	358.9	161 (7), 133 (42)	0.20 ± 0.01	ND	ND	0.30 ± 0.01	0.30 ± 0.01	ND
Rutin	609.0	300 (37), 271 (51), 301(38)	0.60 ± 0.02	10.9 ± 0.54	11.5 ± 0.6	3.9 ± 0.2	33 ± 2	12.9 ± 0.64
Hesperidin	611.0	303 (24), 465 (12)	0.2 ± 0.0	6.1 ± 0.3	13.0 ± 0.6	4.1 ± 0.2	35 ± 2	14 ± 0.7
Hyperoside	463.1	300 (27), 301 (26)	0.60 ± 0.02	0.50 ± 0.02	0.90 ± 0.04	1.80 ± 0.08	2.00 ± 0.09	1.10 ± 0.05
***4*-OH Benzoic acid**	137.0	93 (17), 65 (27)	0.40 ± 0.02	0.40 ± 0.02	0.20 ± 0.01	0.80 ± 0.04	0.40 ± 0.02	0.20 ± 0.01
Salicylic acid	137.0	93 (16), 65 (31), 75 (30)	0.40 ± 0.02	0.80 ± 0.04	0.60 ± 0.03	0.1 ± 0.0	0.70 ± 0.03	0.50 ± 0.02
Myricetin	317.0	179 (19), 151(23), 137 (26)	ND	0.09 ± 0.00	ND	0.1 ± 0.0	ND	ND
Fisetin	285.0	135 (22), 121 (27)	0.1 ± 0.0	0.30 ± 0.01	ND	ND	ND	0.50 ± 0.02
Coumarin	147.0	103 (17), 91 (26), 77 (27)	1.60 ± 0.07	1.80 ± 0.08	1.60 ± 0.07	1.30 ± 0.06	1.90 ± 0.09	0.80 ± 0.03
Quercetin	300.9	179 (19), 151 (21), 121 (28)	ND	0.1 ± 0.0	ND	ND	0.1 ± 0.0	0.40 ± 0.02
Naringenin	271.0	151 (18), 119 (24), 107 (26)	0.04 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	1.30 ± 0.07	0.60 ± 0.03
Hesperetin	301.0	164 (25), 136 (33), 108 (42)	ND	ND	ND	ND	ND	0.07 ± 0.00
Luteolin	285.0	217 (25), 199 (28), 175 (29)	ND	4.6 ± 0.3	ND	ND	6.1 ± 0.4	0.40 ± 0.02
Kaempferol	285.0	217 (29), 133 (32), 151 (23)	0.09 ± 0.00	5.60 ± 0.02	0.20 ± 0.01	0.1 ± 0.0	4.0 ± 0.2	0.1 ± 0.0
Apigenin	269.0	151 (25), 117 (35)	ND	0.1 ± 0.0	0.04 ± 0.00	0.08 ± 0.04	0.3 ± 0.01	0.009 ± 0.0
Rhamnetin	315.0	165 (23), 121 (28), 300 (22)	ND	ND	ND	ND	ND	0.1 ± 0.0
Chrysin	253.0	143 (29), 119 (32), 107 (26)	ND	ND	ND	ND	ND	ND

[a]Parent ion (m/z): molecular ions of the standard compounds (mass to charge ratio); [b]MS² (CE): MRM fragments for the related molecular ions (CE refers to related collision energies of the fragment ions); [c]Values in µg/g (w/w) of plant methanol extract; N.D.: not detected. RW: Rachis water extract, LW: Leaflets water extract, FW: Fruit water extract, RM: Rachis methanol extract, LM: Leaflets methanol extract, FM: Fruit methanol extract.

Different types of flavonoids such as flavones, flavonols, and flavanones were found in Chamaerops 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 Chamaerops 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, rhamnetin and myricitin in only some Chamaerops 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%)

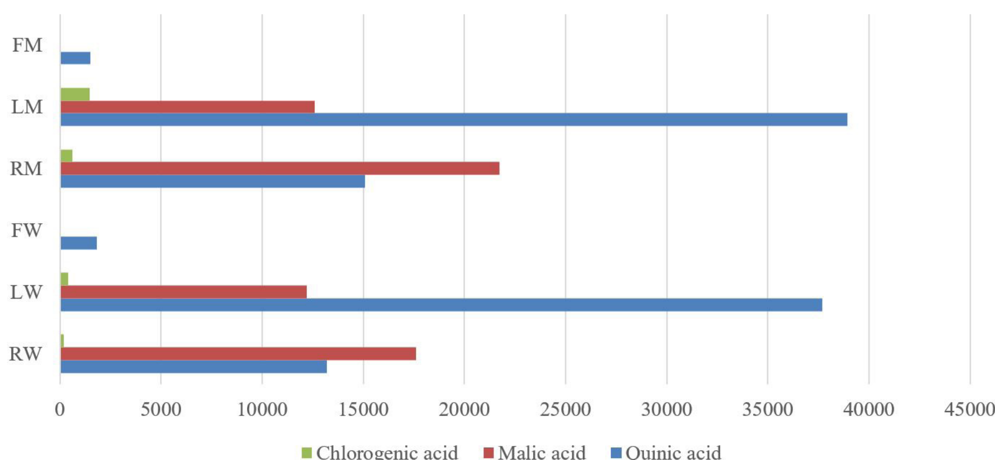

Figure 2. Graph that shows the concentrations (µg analyte/g extract) of chlorogenic, malic and quinic acids in methanol and water extracts of different parts. RW: Rachis water extract, LW: Leaflets water extract, FW: Fruit water extract, RM: Rachis methanol extract, LM: Leaflets methanol extract, FM: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

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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