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HPLC-ESI-MS AND GC-EI-MS IDENTIFICATION AND QUANTITATION OF POLYPHENOLICS AND ALKALOIDS IN MOROCCAN JUJUBE HONEYS

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Abstract

The aim of this study was to determine the phytochemical content of Moroccan Jujube Honey and to establish the principal components of this staple Moroccan food, which contributes health benefit for the local population. Total phytochemical compounds as determined by analytical HPLC-ESI-MS in the honey extracts were 29.39 ± 5.21 mg/kg (range 16.64-42.16) wet weight. The individual phytochemical compounds definitively identified by HPLC-DAD-ESI-MS in the Jujube honeys were the alkaloids: (I) 4-hydroxyquinoline glucoside, (II) 4-hydroxyquinoline and (V) kynurenic acid (17.66 ± 0.87 mg/kg), and the phenolic compounds: (III) p-hydroxybenzoic acid, (IV) caffeic acid and (VI) methyl syringate (11.73 ± 0.50 mg/kg). The structures were confirmed by GC-EI-MS apart from 4-hydroxyquinoline-glucoside. The major components were methyl syringate (8.34 ± 2.49 mg/kg), 4-hydroxyquinoline (6.61 ± 3.23 mg/kg) and kynurenic acid (6.56 ± 0.97 mg/kg). The mean content of polyphenolic compounds (11.73 ± 0.50 mg/kg) of Moroccan Jujube honeys compared favorably with those described for six Chinese Jujube honeys (2.49 mg/kg), five commercial Jujube honeys from different countries (0.92 mg/kg) and twelve Jujube honeys from Yemen (25.71 mg/kg). Reference studies did not identify alkaloids as components of Jujube honey, and therefore the phytochemical profile of Moroccan Jujube honey appears to be unique.

Keywords: 4-(1'-Hydroxy-1'-dimethyl) cyclohexa-1,3-diene-1 carboxylic acid-1-gentobioside, 4-hydroxyquinoline glucoside, 4-hydroxyquinoline, Jujube honey, kynurenic acid, methyl syringate, phenolic acid

INTRODUCTION

Honey refers to the fluid, pasty or crystalized functional food collected by bees (*Apis mellifera*) from the flowers and blossom nectar as well as from sweet deposits (honeydew) of living plants that bees forage on, transform and store to ripen in their hive. There are many varieties of honey with different or-

ganoleptic properties including color, texture, aroma and flavor (Cernak et al., 2012), which are dependent on flora type, geography and preparation techniques. According to Bogdanov, Ruoff, & Persano (2004), the color of honey is also associated with its flavor. Honey mainly comprises mixture of glucose and fructose, which represents around 70% of the total mass, and contains other such substances as volatile

organic and phenolic compounds, salts including potassium, enzymes (either of animal or plant origin) including diastases, invertase, catalase and glucose oxidase. Additionally, honey contains nitrogen compounds (e.g. Maillard reaction products, bioactive peptides and some alkaloids) as well as organic acids and vitamins (such as vitamins C, B1, B6, B9, riboflavin, nicotinic acid and pantothenic acid,) and other metabolites (Aliferis et al., 2010; Wang et al., 2014; Zha et al., 2016).

Historically, honey has been ascribed many properties, including wound healing and the amelioration of liver, cardiovascular and gastrointestinal problems. Ancient Egyptians, Greeks and Romans reported its effectiveness against bacterial infections, wounds and burns (Zulma & Lulat, 1989). Honey has also been recommended for the treatment of tuberculosis (Asadi-Pooya, Pnjehshahin, & Beheshti, 2003) and as a fortifying and invigorating agent (Descottes, 2009).

The wild Jujube *Ziziphus lotus* (L.) Lam. belongs to the Rhamnaceae family and is especially encountered in arid and semi-arid regions of the Mediterranean area, throughout Libya to Morocco and southern European countries (Benammar et al., 2010). Its fruits contain appreciable amounts of cyclopeptides alkaloids termed lotusines (Ghedira et al., 1993; 1995).

A large body of literature associates honey metabolites with numerous biological functions. In particular Jujube honey treatment for twelve weeks significantly protects against hepatic disorders which are linked to chronic alcoholism. It also inhibits serum lipoprotein oxidation and reduces the impact of alcoholism on aspartate aminotransferase (AST) and alanine aminotransferase (ALT), two enzyme levels valuable primarily in the diagnosis of liver diseases. Moreover, Cheng et al. (2014) observed an inhibition of the generation of 8-hydroxy-2-deoxyguanosine (8-OHdG), a decreased malondialdehyde (MDA) concentrations and increased hepatic glutathione peroxidase (GSH-Px) activity. In other recent reports, natural Jujube honeys from Buraidah and Najran of northcentral and southwestern Saudi Arabia, respectively, were

screened and showed significant antibacterial effects particularly against gram positive bacterial strains. According to this report, the antibacterial characteristics of honey may be influenced by the area from which it was collected (Emad & Arafa, 2019). Furthermore, anticancer effects which entail apoptotic mechanism via DNA damage, p53 expression, and caspase activation by Jujube honey in HepG2 cells have been reported (Cheng et al., 2019).

From a phytochemical point of view, Algerian Jujube honeys were characterized by their organoleptic and physicochemical properties including high values of electrical conductivity, potassium, calcium, pH, phenol content, amber color and medium protein content. From a palynological point of view, the presence of some pollen types in the pollen spectra of such honeys as *Peganum harmala*, *Lotus*, *Eucalyptus*, *Taraxacum*, *Cistus*, *Trifolium*, *Carduus* and *Matricaria* was proposed as a geographical indicator of this honey type (Zerouk et al., 2018). Yemeni Jujube honey has a total of twenty-five compounds (ten phenolic acids, nine flavonoids and six monophenols) (Badjah Hadj Ahmed et al., 2014) and polyphenolics including 4-hydroxybenzoic and cinnamic acid as well as chrysin at concentrations of 1.41 mg/100 g, 1.34 mg/100 g and 0.85 mg/100 g, respectively. In Jujube honey varieties from other arid Asian regions including Yemen, the content of the phenolic compounds identified clearly differed from those of non-arid regions with higher polyphenolic levels and their bioactivities (Habib et al., 2014).

To date, the scientific literature on the chemical composition of Moroccan honeys is very sparse despite total Moroccan honey production being estimated at 5,815 tons in 2015. As part of the dynamics of the Morocco Green Plan (Plan Maroc Vert), a development program contract between the beekeeping associations and the Moroccan Government was established for the period of 2011-2020.

The region of Errachidia (Tafilalt, Southeast of Morocco), is a well-known source of Jujube honey, which is in high demand due to its medicinal properties, taste, flavor and fragrance. Because

there is a paucity of data, the aim of this pilot study was to evaluate the polyphenolic content of Moroccan Jujube honeys. The Jujube honeys studied here also contain appreciable levels of alkaloids and so were compared with a chestnut (*Castanea sativa* Mill.) honey produced in Italy, which is also renowned for its high alkaloid content.

MATERIAL AND METHODS

Chemicals and reagents

Acetic acid, acetonitrile and methanol were obtained from E. Merck (Darmstadt, Germany). 4-hydroxyquinoline and kynurenic acid and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (BSTFA) were obtained from Sigma-Aldrich Chemie (Deisenhofen, Germany). Caffeic acid, chrysin, *p*-coumaric acid, *p*-hydroxybenzoic acid, ferulic acid, methyl syringate, naringenin, syringic acid and pinocembrin were obtained from Extrasynthese (Lyon Nord, Genay, France) and Sep-Pak C18 cartridges for solid-phase extraction (Supelclean™ LC-18 SPE in 6-ml tubes, 500 and 5000 mg; Supelco, Bellefonte, Pennsylvania, USA). All solutions were made up in either double-distilled water, or methanol, unless otherwise stated.

Honey samples

Three commercial monofloral Jujube honeys (H1, H2 and H3) (*Ziziphus lotus* L.) harvested in 2018 were evaluated. The H1, H2 and H3 samples were collected from the El Khayr association of beekeepers at Kasbat Rahba Lkdima, Errachidia, Morocco (Latitude 31°55'31": Longitude 4°25'41": Altitude 1241 metres). They harvested the honey by a manual extractor, after the foraging of the bees during the Jujube flowering periods. The El khayr association, the leader in honey commercialization in the Errachidia province, has commercialized different honey types through the practice of transhumance to other areas of Morocco.

A fourth Jujube honey sample (H4) was collected in 2018 from beekeepers of the Manahil Ikarama association who harvest honey by a manual extractor, after the foraging of the bees in

the region of Er-Rich city, Morocco (Latitude 31°15'28": Longitude 4°29'42": Altitude 1409 metres) during the Jujube flowering periods. Commercial Chestnut (*Castanea sativa* Mill.) honey (H5) was purchased from a local supermarket in Heidelberg, Germany (Der Klassische Breitsamer honig; Kastania-Edelherb; produced in Italy (L6484151). Sell by date 10/2020).

Preparation of extracts

Duplicate Jujube and Chestnut honeys (2 x 10 g) were completely dissolved in double-distilled water, made up to exactly 50 mL in volumetric flasks, and initially analysed directly by HPLC-ESI-MS. Clean-up of the extracts was achieved through fractionation on SPE, C18 Sep-Pak cartridges.

Column chromatography on C18 Sep-Pak cartridges

Diluted honey samples 10 g in double-distilled water (50 mL), were applied to C18 Sep-Pak cartridges (5000 mg) for fractionation. The columns were preconditioned with methanol (50.0 mL), and distilled water (50.0 mL) and were not allowed to dry. Elution was performed with solvent mixtures (50.0 mL) containing increasing concentrations of methanol (5-50%) in 2% acetic acid followed by 100% methanol. The solvent was removed by lyophilization prior to spectroscopic analyses.

HPLC-DAD-ESI-MS

HPLC-ESI-MS was conducted on an Agilent 1100 HPLC coupled to an Agilent single-quadrupole mass-selective detector (HP 1101; Agilent Technologies, Waldbronn, Germany). Honeys dissolved in double distilled water, or SPE fractions dissolved in methanol, were chromatographically separated using a column of the same type and dimensions as for analytical HPLC (Phenomenex, Aschaffenburg, Germany). Mobile phase-1 consisted of 2% acetic acid in double-distilled water (solvent A) and acetonitrile (solvent B), with the gradient profile of initially 95% A for 10 min., to 90% A over 1 min., to 80% A over 9 min., to 60% A over 10 min., to 40% A over 10 min., to 0% A over 5 min and

continuing at 0% A until completion of the run. Mobile phase-2 consisted of 2% acetic acid in doubly distilled water (solvent A) and methanol (solvent B) with the gradient profile of initially 95% A for 2 min., to 75% A in 8 min., to 60% A in 10 min., to 50% A in 10 min., to 0% A in 5 min. and continuing at 0% A until completion of the run.

Phenolic compounds were detected by their UV absorbance (*A*) at 257, 278, 320 and 340 nm at 30°C. Negative-ion mass spectra were generated under the conditions of fragmentor voltage, 100; capillary voltage, 2500 V; nebulizer pressure, 30 psi; drying gas temperature, 350°C; *m/z* scan range, 100-1500 D. Positive-ion spectra were generated under the conditions of fragmentor voltage, 200; capillary voltage, 1500 V; nebulizer pressure of 30 psi, drying gas temperature of 350°C and *m/z* scan range of 100-1500 D. For HPLC-ESI-MS-MS experiments in negative-ion mode, the fragmentor voltage was increased to 300. Instrument control and data handling were performed on a personal computer with the Chemstation software.

Semi-preparative HPLC

Semi-preparative HPLC was conducted on a HP 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a Zorbax Phenyl-Hexyl reverse-phase (9.4 x 250 mm) C18 column (Agilent Technologies, Waldbronn, Germany). For the separation of individual compounds in the diluted *Castanea sativa* Mill. honey, the mobile phase (3 mL/min) consisted of 2% acetic acid in water (solvent A) and acetonitrile (solvent B), utilizing the following solvent gradient profile over a total run time of 50 min.: initially 95% A for 10 min.; reduced to 90% A over 1 min.; to 80% A over 9 min.; to 60% A over 10 min.; to 40% A over 10 min. and continuing at 0% A until completion of the run. Phytochemicals were detected by their UV absorbance (*A*) at 257, 278 305 and 340 nm at 30°C. Peaks eluting from the column were collected on a HP 220 Microplate Sampler and subsequently lyophilized.

Gas-chromatography mass spectrometry (GC-MS)

Analyses were performed on a HP 5973 mass spectrometer coupled to a HP 6890 gas chromatograph in the EI scan mode. Prior to GC-MS, TMS ether derivatives were prepared by reaction with BSTFA (100 µL) at 60°C for 30 minutes. Sample volumes of 1 µL were injected into the GC-MS. Separation of the analytes was achieved using a HP 5MS capillary column, (30 m x 0.25 mm I.D., 0.25 µm film thickness). Helium was used as carrier gas with a linear velocity of 0.9 mL/minute. The oven temperature program was an initial temperature of 160°C, 160°C to 270°C at 4°C/minute and 270°C for 20 minutes. The GC injector temperature was 250°C; and the transfer line temperature was held at 280°C. The mass spectrometer parameters for EI mode were ion source temperature at 230°C, electron energy at 70 eV, filament current at 34.6 µA and electron multiplier voltage at 1200 V.

Standard curves

The amounts of 4-hydroxyquinoline (II), *p*-hydroxybenzoic acid (III), caffeic acid (IV), kynurenic acid (V) and methyl syringate (VI) in the Jujube honeys were calculated from the HPLC-DAD-ESI-MS standard curves of authentic standards in the range of 50-1000 µM at 278, 257, 320 and 340 nm respectively. In the case of the 4-hydroxyquinoline glucoside (I), the concentration was calculated against the standard curve of 4-hydroxyquinoline with a relevant molecular weight correction.

The amounts of caffeic acid (IX) and *p*-coumaric acid (X) in the diluted *Castanea sativa* Mill. honey were calculated from the HPLC-DAD-ESI-MS standard curves of authentic standards in the range 50-1000 µM at 278, 257, 320 and 340 nm respectively. Likewise the kynurenic acid derivatives in the diluted *Castanea sativa* Mill. honey namely 3-pyrrolinyl-kynurenic acid (3-PKA) (VIII), (XI), kynurenic acid tautomer (XII), gamma-lact-3-PKA (XIII), and kynurenic acid (XIV) were calculated from the HPLC-DAD-ESI-MS standard curve of kynurenic acid with relevant molecular weight corrections. The amounts of 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-di-

ene-1 carboxylic acid-1-gentobioside (VII) and 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1 (XI) were calculated from a standard curve of 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1-gentobioside (VII) purified by semi-preparative HPLC with a molecular weight correction for the evaluation of 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1 (XI). Data are expressed in mg/kg wet weight. All the standard curves showed $R^2 > 0.99$.

RESULTS

Six major compounds were detected through HPLC-ESI-MS in the raw dilutions of Jujube honey of Moroccan origin (H1-H4) comprising alkaloids, and phenolic acids (Fig.1). These were the alkaloids, 4-hydroxyquinoline glycoside (I) 4-hydroxyquinoline (II) and kynurenic acid (V), the phenolic acids *p*-hydroxybenzoic acid (III) and caffeic acid (IV) and methyl syringate (VI). The compounds were identified by HPLC-ESI-MS (Figs. 2-4) in an negative ion mode (Tab. 1) following fractionation on Sep-Pak C18 cartridges and suspension of the lyophilised fractions in methanol (2.0 mL) and confirmed

by GC-El-MS (Tab. 2) as their trimethylsilyl ether derivatives. The levels of individual compounds in the Jujube honeys are shown in Tab. 3. On average a total of 29.39 ± 5.21 (range 16.64-42.16) mg/kg were quantitated in the Jujube honeys. The proportions of the polyphenolic species were alkaloids (58%) and phenolic acids (42%).

In the raw dilution of the *Castanea sativa* Mill. honey (H5), eight compounds were detected (five major and three minor). These were again identified by HPLC-ESI-MS (Fig. 5) as 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1-gentobioside (VII), 3-pyrrolinyl-kynurenic acid (3-PKA) (VIII), caffeic acid (IX), *p*-coumaric acid (X), 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1 (XI), kynurenic acid tautomer (XII), gamma-lact-3-PKA (XIII), and kynurenic acid (XIV). The individual levels of compounds in the *Castanea sativa* Mill. honey are also shown in Tab. 3. The proportions of the polyphenolic species were alkaloids (77%) and terpenoids (23%). The structures of the secondary metabolites detected in the Jujube and *Castanea sativa* Mill. honey samples are given in Figs. 6 and 7 respectively.

Fig. 1. Analytical HPLC-DAD-ESI-MS chromatogram (at 278, 257 and 320 nm) of a Moroccan Jujube (*Ziziphus lotus*) honey (10g) dissolved in doubly distilled water (50.0 mL) I. 4-hydroxyquinoline glucoside, II. 4-hydroxyquinoline, III. *p*-hydroxybenzoic acid, IV. caffeic acid, V. kynurenic acid, VI. methyl syringate.

Fig. 2. Analytical HPLC-DAD-ESI-MS chromatogram (at 257 and 320 nm) of a SPE (10% methanol) fraction of Moroccan Jujube (*Ziziphus lotus*) honey (10g) I. 4-hydroxyquinoline glucoside, II. 4-hydroxyquinoline, III. p-hydroxybenzoic acid.

Fig. 3. Analytical HPLC-DAD-ESI-MS chromatogram (at 257 and 320 nm) of a SPE (25% methanol) fraction of Moroccan Jujube (*Ziziphus lotus*) honey (10 g) II. 4-hydroxyquinoline, III. p-hydroxybenzoic acid, IV. caffeic acid, V. kynurenic acid.

Fig. 4. Analytical HPLC-DAD-ESI-MS chromatogram (at 257 and 320 nm) of a SPE (100% methanol) fraction of Moroccan Jujube (*Ziziphus lotus*) honey (10 g) VI. methyl syringate.

Fig. 5. Analytical HPLC-DAD-ESI-MS chromatogram (at 320 nm) of an Italian Chestnut (*Castania sativa* Mill.) honey (10 g) dissolved in doubly distilled water (50.0 mL) VII. 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1-gentobioside, VIII. 3-pyrrolinyl-kynurenic acid (3-PKA), IX. caffeic acid, X. p-coumaric acid, XI. 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1, XII. kynurenic acid tautomer, XIII. gamma-lact-3-PKA, XIV. kynurenic acid.

Fig. 6. Structures of the phenolic compounds detected and identified in Moroccan Jujube (*Ziziphus lotus*) honeys I. 4-hydroxyquinoline glucoside, II. 4-hydroxyquinoline, III. p-hydroxybenzoic acid, IV. caffeic acid, V. kynurenic acid, VI. methyl syringate.

Fig. 7. Structures of the major polyphenolic compounds identified in Italian chestnut (*Castania sativa* Mill.) honey VII. 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1-gentobioside, VIII. 3-pyrrolinyl-kynurenic acid (3-PKA), IX. caffeic acid, X. p-coumaric acid, XI. 4-(1'-hydroxy-1'-dimethyl)cyclohexa-1,3-diene-1 carboxylic acid-1, XII. kynurenic acid tautomer, XIII. gamma-lact-3-PKA, XIV. kynurenic acid.

Table 1.
HPLC-ESI-MS data in negative ion mode at a fragmentor voltage (100 V) of polyphenolic, alkaloid
and terpenoid compounds detected in Jujube and Chestnut honey extracts

Number	Phenolic compound	Molecular formula	R _t (min)	Molecular weight (exact)	[M-H] ⁻	[2M-H] ⁻
Jujube honeys (Morocco)*						
I	4-Hydroxyquinoline glucoside	C ₁₅ H ₁₇ NO ₆	11.97	307.106	306.2	613.1
II	4-Hydroxyquinoline	C ₉ H ₇ NO	17.45	145.053	144.2	289.1
III	<i>p</i> -Hydroxybenzoic acid	C ₇ H ₆ O ₃	19.82	138.032	137.2	275.2
IV	Caffeic acid	C ₉ H ₈ O ₄	22.11	180.042	179.1	359.1
V	Kynurenic acid	C ₁₀ H ₇ NO ₃	26.03	189.043	188.1	377.1
VI	Methyl syringate	C ₁₀ H ₁₂ O ₅	35.69	212.068	211.1	423.0
Chestnut honey (Italy)#						
VII	4-(1'-Hydroxy- 1'-dimethyl) cy- clohexa-1,3-diene-1 carboxylic acid- 1-gentobioside	C ₂₂ H ₃₄ O ₁₃	15.18	505.199	505.2	1011.4
VIII	3-PKA	C ₁₄ H ₁₄ N ₂ O ₃	16.28	258.100	257.1	n.d.
IX	Caffeic acid	C ₉ H ₈ O ₄	17.99	180.042	179.1	359.1
X	<i>p</i> -Coumaric acid	C ₉ H ₈ O ₃	21.48	164.047	163.1	327.1
XI	4-(1'-Hydroxy- 1'-dimethyl) cy- clohexa-1,3-diene-1 carboxylic acid	C ₁₀ H ₁₄ O ₃	22.31	182.094	181.2	363.1
XII	Kynurenic acid tautomer	C ₁₀ H ₇ NO ₃	22.54	189.042	188.1	377.1
XIII	Gamma-Lact-3-PKA	C ₁₄ H ₁₂ N ₂ O ₂	24.32	240.089	239.1	n.d.
XIV	Kynurenic acid	C ₁₀ H ₇ NO ₃	30.62	189.042	188.1	377.1

R_t = retention time: *HPLC mobile phase-1: #HPLC mobile phase-2

n.d. = not detected

Table 2.

GC-EI-MS of aglycone polyphenolic and alkaloid compound TMS-derivatives detected in Jujube (*Ziziphus lotus*) honey

No	Polyphenolic compound	R _t (min.)	M ⁺ calc.	Major neutral fragments (m/z %)
II	4-Hydroxyquinoline	16.38	217	73(29), 101(13), 172(18), 202(100) , 217(49)
III	<i>p</i> -Hydroxybenzoic acid	16.51	282	73(74), 193(56), 223(71), 267(100) , 282(28)
IV	<i>trans</i> -Caffeic acid	29.30	396	73(100) ,191(14), 219(7), 307(11), 381(24), 396(99)
V	Kynurenic acid	27.67	333	73(85), 231(81), 288(21), 304(34), 318(100) , 333(10)
VI	Methyl syringate	21.30	284	73(26), 223(24), 254(100) , 269(46), 284(31)

Base peak = bold

DISCUSSION

The polyphenol content (11.73±0.50 mg/kg) of Moroccan Jujube honeys compares favorably with those described for six Chinese (Cheng et al., 2014) Jujube honeys (2.49 mg/kg), which comprise six monophenolic acids including ferulic acid (1.50 mg/kg), chlorogenic acid and one diphenolic acid namely ellagic acid (0.37 mg/kg), and five wild Jujube commercial honey brands from UAE, Oman, Pakistan and Kashmir, evaluated within the framework of honeys from arid and non-arid regions (Habib et al., 2014). The mean value of total phenolic compounds in these five honeys was 0.92 mg/kg comprising gallic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, cinnamic acid, catechin, epi-catechin and rutin. Badjah Hadj Ahmed et al. (2014) reported on the polyphenolic content of twelve Jujube honeys from various regions of the Yemen. The following twenty-three phenolic compounds were identified and quantitated in these honeys: the phenolic acids gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, caffeic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid; the flavonoids naringin, myricetin, quercetin, naringenin, kaempferol, apigenin, chrysin, galangin,y phenol, benzoic acid, cinnamic

acid, thymol and carvacrol. The mean concentration of polyphenolic compounds detected in these Jujube honeys from the Yemen was 25.71 mg/kg represented by phenolic acids (12.85 mg/kg), flavonoids (7.49 mg/kg) and other phenol compounds (5.37 mg/kg). The mean major polyphenol of each class in this report was *p*-hydroxybenzoic acid at 7.66mg/kg or phenolic acids, chrysin at 0.50 mg/kg for flavonoids and cinnamic acid at 0.52 mg/kg for other phenols. The detection of alkaloids especially kynurenic acid, 4-hydroxquinoline and its glycoside as a major phytochemical class (58%) in the Moroccan Jujube honeys was studied here. Kynurenic acid was reported (Truchado et al., 2009) to be a major alkaloid component of monofloral chestnut honey (over 70%). Given that the Moroccan Jujube honeys also contained appreciable levels of alkaloids, we compared our data with that of chestnut (*Castanea sativa* Mill.) honey produced in Italy. The phytochemical content of this honey sample (Tab. 3) was dominated by alkaloids, especially kynurenic acid (1246 ± 17 mg/kg). However the lackof evidence for the presence of 4-hydroxyquinoline (II) and its glycosylated derivative (I) in chestnut honey indicates that the polyphenolic profile of Jujube honey described in this paper appears to be unique and to differ from Jujube

Table 3.

Major polyphenolic compounds, alkaloids and terpenoids detected in Jujube honeys and Chestnut honey (Italy)

Number	Phenolic compound	Jujube honey samples (mg/kg)			
		H1	H2	H3	H4
Jujube honeys (Morocco)					
I	4-Hydroxyquinoline glucoside	3.58±0.28	3.67±0.19	2.61±0.10	8.11±0.32
II	4-Hydroxyquinoline	3.38±0.37	3.38±0.09	3.38±0.18	16.29±0.86
III	<i>p</i> -Hydroxybenzoic acid	1.55±0.06	1.60±0.28	1.16±0.11	2.43±0
IV	Caffeic acid	1.41±0.13	1.18±0.04	0.77±0.07	3.44±0
V	Kynurenic acid	7.08±0.30	6.69±0.30	3.89±0.30	8.58±0.20
VI	Methyl syringate	12.24±0.18	12.98±0.92	4.83±0.12	3.31±0.09
Total (mg/kg)		29.24±1.42	29.50±1.27	16.64±0.67	42.16±1.07
Chestnut honey (Italy)					
Number	Phenolic compound	H5 mg/kg			
VII	4-(1'-Hydroxy-1'-dimethyl) cyclohexa-1,3-diene-1 carboxylic acid-1-gentobioside	305±4			
VIII	3-PKA	187±3			
IX	Caffeic acid	13±0.1			
X	<i>p</i> -Coumaric acid	7±0.1			
XI	4-(1'-Hydroxy-1'-dimethyl) cyclohexa-1,3-diene-1 carboxylic acid	179±11			
XII	Kynurenic acid tautomer	94±2			
XIII	Gamma-Lact-3-PKA	43±2			
XIV	Kynurenic acid	1246±17			
Total terpenoids (mg/kg)		484			
Total alkaloids (mg/kg)		1570			
Total polyphenols (mg/kg)		20			
Total (mg/kg)		2074			

honeys studied of China, UAE, Oman, Pakistan, Kashmir and Yemen.

The levels of kynurenic acid (V) at 1246 ± 17 mg/kg detected in the Italian chestnut honey were comparable to those (mean = 1161 mg/kg) reported by Beretta, et al. (2009) for eight Italian chestnut honeys, but around 3x higher

than reported those by Truchado, et al. (2009). The presence of 3-PKA (187 ± 3 mg/kg) and gamma-Lact-3-PKA (43 ± 2 mg/kg) were also detected. Truchado et al. (2009) identified and quantitated alkaloids in chestnut honeys for the first time. We can confirm that the tautomerization (94 ± 2 mg/kg) of kynurenic acid

occurs based on its identical molecular weight and UV spectrum to that reported by Truchado et al. (2009) and that the major terpenoids are 4-(1'-dimethyl)cyclohexana-1,3-diene-1-carboxylic acid (179 ± 11 mg/kg) and its gentobioside derivative (305 ± 4 mg/kg).

The data presented in this report shows that the content of phenolic compounds detected in Moroccan Jujube honeys compares favourably with that of other countries. However, future larger studies incorporating Jujube honey samples from further Moroccan provinces are recommended. An evaluation of the potential health-benefits of Jujube honey extracts in comparison to a range of other monofloral honeys are also urgently recommended in a range of in-vitro antioxidant and cell culture assays as well as in preclinical models.

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