Quantitative determination and identification of phenolic compounds of three Tunisian legumes: *Vicia faba*, *Lens culinaris* and *Phaseolus vulgaris*

Mohamed Journi 1, Hédi Hammouda 1,2*, Malika Trabelsi-Ayadi 1 and Jamila Kalthoum Chérif 1,2

1 Laboratoire d’Application de la Chimie aux Ressources et Substances Naturelles et à l’Environnement (LACReSNE). Faculté des Sciences de Bizerte, 7021 Zarzouna- Bizerte Tunisie.  
2 Institut Préparatoire aux Études d’Ingénieurs de Tunis, Monfleury 1008 Tunis-Tunisie.

**ARTICLE INFO**

Article history:
Received: May 13, 2015  
Revised: June 30, 2015  
Accepted: August 10, 2015  
Available online August 26, 2015

Keywords:
*Vicia faba* L.  
*Lens culinaris* L.  
*Phaseolus vulgaris* L.  
Phenolic contents  
LC-MS*

* Corresponding Author;  
E. Mail: hedi.hammouda@yahoo.fr  
Tel: +216 25124568

**ABSTRACT**

The polyphenol content and chromatographic profiles of three legumes extracts of *Vicia faba* L., *Lens culinaris* L. and *Phaseolus vulgaris* were found and compared. In addition, the total phenol content (TPC) in the cited legumes was measured by colorimetric method while the identification was carried out by chromatography LC-ESI-MS technique. The results of TPC, determined by the Folin-Ciocalteu reagent, had the maximum value in the acidified methanol legume extracts of *P. vulgaris* (3.201±0.012 mg EAG/g), *L. culinaris* (2.591 ± 0.02 mg EAG/g) and *V. faba* (0.991 ± 0.01 mg EAG/g). Fragmentation behavior of polyphenol compounds were investigated using ion trap mass spectrometry in negative electrospray ionization. The MS, MSn of polyphenols allowed structural characterization of these compounds. Based on the obtained chromatograms, six phenolic compounds were identified in *L. culinaris* extract as catechin glucoside, catechin gallate, isomer 1 kaempferol tetraglycoside, isomer 2 kaempferol tetraglycoside, kaempferol-3-robinoside-7-rhamnoside and Kaempferol-3-glycoside. In *P. vulgaris* extract five compounds were identified: methyl catechin gallate, Myricetin-3-pentoside, Kaempferol 3-o-glycoside, Apigenin glycoside and Kaempferol hexose malic acid. Finally, *V. faba* extract contains also five compounds: catechin gallate, quercetin arabinoside, epicatechin glucoside, methyl epicatechin gallate and kaempferol glucoside sulfate.

Copyright © 2015 Journi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

The Leguminosae and the Fabaceae are a large family of plants used for human and animal nutrition. Legumes are widely distributed and contain between 670 and 750 genera and around 19,000 different species (Lewis et al. 2005; Polhill et al. 1981). These plants occupy an important place in our diet and with cereals constitute the backbone of the food system. Many species of legumes are used as protein sources in human diet (bean, soy, pea, lentils etc.) (Khelil.
Vicia faba (fava bean), Lens culinaris (lentils) and Phaseolus vulgaris (common beans) are among the plants which are very known and take an important place in human diet in Tunisia. It is now recognized that a high consumption of natural products such as fruits, vegetables and legumes is associated with the reduction risk of several diseases as cancer, cardiovascular disease, coronary heart disease and atherosclerosis, as well as neurodegenerative and chronic inflammatory diseases (Prior et al. 2000). In addition, numerous results of scientific research showed the link between these beneficial effects and antioxidants compounds (polyphenols, carotenoids, terpenoids, vitamins etc.) (Soto-Vaca et al. 2012). Polyphenols are considered to be the most important compounds involved in different physiological processes since they can modulate a variety of biological events. (El Modafar et al. 2001). Polyphenols are secondary metabolites widely distributed in the plant kingdom. They are characterized by the presence of phenol structural units linked with hydroxyl groups or with sugars or organic acids. Polyphenol compounds are divided into several classes: phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids, proanthocyanidins, anthocyanins etc. Additionally, polyphenols act as metal chelators, antimutagens or anticarcinogens, antimicrobial and clarifying agents (Proestos et al. 2005).

Several studies focused on the identification and characterization of polyphenols in some legumes (Baginsky et al. 2013) and have shown the presence of polyphenols in extracts of V. faba. In L. culinaris extracts, researchers have shown that flavonoids are the major phenolic subclass. The main identified compounds in the analyzed extracts are catechin and the procyanidins (Duenas et al. 2002; Long-Ze lin et al. 2008). Recently, in the United States, they were characterized in P. vulgaris extracts the flavonols and anthocyanins subclasses. In this context the objective of our study is to determine quantitatively the levels of total polyphenols and flavonoids (sensu strict) and to identify or characterize the phenolic compounds in these three Tunisia legumes by liquid chromatography coupled with mass spectrometry (LC - MSn).

2. Materials and Methods

2.1 Plant materials

The studied food legumes harvesting was conducted in June 2013 in a verge in Menzel Temime (Nabeul gouvernorate) northeast Tunisia southeast of the peninsula of Cap Bon, located at 36° 46' 56" N latitude, 10° 59' 15" E longitude. The protocol for plant materials preparation was performed in triplicate: Three batches of 100 g were randomly constituted for three Tunisian leguminous. All studied food legumes crops; V. faba, P. vulgaris and L. culinaris are lyophilized (freeze LEYBOLD, model LYOVAC GT 2), then ground using a stainless crush (Moulinex) in order to obtain a fine and homogeneous powder and stored at -20°C until analyses.

2.2 Solvents and reagents

Glacial acetic acid was purchased from Biosolve Ltd (Valkenswaard, The Netherlands). HPLC gradient grade quality acetonitrile and methanol were purchased from Biosolve Ltd (Valkenswaard, The Netherlands). Formic acid and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Folin-ciocalteu reagent and sodium carbonate were obtained from Fluka (Germany). Ultrapure water was obtained using a Milli-Q water system (Millipore, Bedford, MA).

(-)-Epicatechin (EC), (+)-catechin (CAT), gallic acid, chlorogenic acid and quercetin were purchased from Sigma (Sigma-Aldrich, Germany). Kaempferol, chrysoeriol, quercetin 3-O-rutinoside (rutin), quercetin 3-O-glucoside (isoquercitrin), isorhamnetin 3-O-rutinoside, kaempferol 3-O-rutinoside and luteolin 3-O-glucoside were
purchased from Extrasynthese (Lyon, France).

### 2.3 Preparation of the phenolic extracts

Each crushed legumes material *V. faba, L. culinaris* and *P. vulgaris* (20 mg) were macerated separately at room temperature in darkness for 15 min in an ultrasonic bath (Brasson 2200, USA) respectively in 10 mL of methanol/water (80:20, v/v), ethanol/water (80:20, v/v) and acidified methanol 1% acetic acid (99/1 : v/v). Filtrated extracts were evaporated to dryness and dispersed in 15 mL methanol, passed through 0.45 μm filters (Uptidisc Interchim, Montluçon, France) and stored at 4°C until analysis.

### 2.4 Total phenols content

The Determination of total phenols content of different polarities crude extracts of three leguminous were quantified using the Folin-Ciocalteu reagent according to the modified method described by Singleton et al. (1965). Approximately, 200 μL of the different legumes extracts obtained by the three organic solvents were taken in a test tube. 1 mL of 10% Folin-Ciocalteu reagent solution was added to each tube and vortexed rigorously during 2 min. After incubation in dark for 3 min at room temperature, 1 mL of saturated Na₂CO₃ (20%) was added to the different solution mixtures. The absorbance at λ= 760 nm was determined at room temperature using a Cary UV/Vis spectrophotometer (UV-1800 Shimadzu spectrophotometer, Tokyo, Japan). Results were expressed as gallic acid equivalent per gram of dry matter.

**Preparation of blank and positive control**

Briefly, 20 mg of gallic acid was taken in a 10 mL volumetric flask and dissolved with methanol. Serial doubling dilutions of stock solution of gallic acid were prepared. About 200 μL of each gallic acid prepared concentrations and 200 μL of MeOH was used as blank (instead of the extract) were prepared with the same protocol cited previously. Finally, the absorbance of each concentration of gallic acid and methanol were measured by UV-visible spectrophotometer (UV-1800 Shimadzu spectrophotometer, Tokyo, Japan) at fixed wavelength of 760 nm. The standard curve was prepared by plotting concentrations versus absorbance of Gallic acid.

### 2.5 Flavonoid (stricto sensu) content

Total flavonoids content was generally determined using the aluminium chloride colorimetric assay Zhishen et al. (1999). This method is based on the formation of a yellowish complex between the aluminum cation, Al (III), and the carbonyl and hydroxyl groups of flavonoids compounds (Lamaison et al. 1991). Briefly, 250 μL of different obtained legumes extracts were taken in separate sealed tubes. Then 125 μL of water and 75 μL of sodium nitrate solution were added to each tube. The mixtures were vortexed vigorously and kept in dark for 6 min in room temperature. 1.5 mL of a 2% methanol solution of AlCl₃.6H₂O was added to each test tube and kept for 2 h in a dark place. The absorbance was measured by UV-visible spectrophotometer (UV-1800 Shimadzu spectrophotometer, Tokyo, Japan) at fixed wavelength of 510 nm.

**Preparation of quercetin standard**

Total flavonoids were calculated using the calibration curve of quercetin standard. A total of 2 mg of quercetin was dissolved in methanol (10 mL) and then diluted to prepare different concentrations 12.5-200 μg/mL. Each prepared solution of quercetin standard and methanolic AlCl₃, used as blank, were prepared and assayed with the same procedure cited previously. Finally, total flavonoids content of the crude extracts were calculated using quercitin calibration curve and the results were expressed in mg rutin equivalent flavonoid/100 g
dry matter (Al-Saeedi et al. 2015).

2.6 LC-ESI-MS for specific characterization of phenolic compounds.

For identification of the phenolic compounds, LC–MS/MS analysis was performed on a 4000 Q-TRAP® LC/MS/MS mass spectrometer (MDS SCIEX, Applied Biosystems, Carlsbad, CA, USA) connected to an Agilent 1200 HPLC system with a G 1315D DAD, G1312A binary pump, G1379B degasser, G1316A thermostatted column compartment, and a G1329A autosampler (Agilent Technologies, Palo Alto, CA, USA). HPLC separation was performed on a Symmetry® C18 column (250×4.6 mm, 5 μm particle size) with a Novapak® C18 guard column (10×4.6 mm, 4 μm particle size, Waters, Mississauga, ON, Canada) at 25 °C and at a flow rate of 1.0 mL/min. The phenolic compounds were separated using a linear elution gradient: initial, 3% B; 0-5 min, 9% B linear; 5-15 min, 16% B linear; 15-45 min, 50% B, linear followed by washing and reconditioning of the column. The solvents were continuously degassed with helium. The injection volume was 10 μL. MS/MS analysis was carried out in the negative ion mode using the following mass spectrometer conditions: high-purity nitrogen gas (99.995%) as nebulising gas (GS1) at 50 psi, heating gas (GS2) at 30 psi, and curtain gas (CUR) at 25 psi for the electrospray probe. Ion spray source temperature was 600 °C and ion spray voltage was 4 kV. Collision-induced dissociation (CID) spectra were acquired using nitrogen as the collision gas under collision energy (CE) of 20 eV. The other MS parameters used were as follows: declustering potential (DP), 70 V; entrance potential (EP), 10 V; and collision exit potential (CXP), 7 V. The MS spectra were firstly acquired in Full Scan negative ionization mode in the 50-2000 m/z range in order to obtain the signals corresponding to the deprotonated [M-H]– molecular ions (Hammouda et al. 2013).

Accurately weighed aliquots of powder (10 mg) were extracted using 1 mL of pure methanol containing 1% v/v acetic acid for 15 min in an ultrasonic bath (Brasson 2200, USA). The mixture was then filtered on PTFE filters (0.45 μm, Uptidisc Interchim, Montluçon, France). The filtrate was then ready for LC-MS analysis.

The polyphenols were identified by comparison of LC–MS/MS spectrum, HPLC retention time, and DAD absorbance spectra with available standards.

3. Results and discussion

3.1. Total phenolics content

Phenolic content of different organic solvents extracted according to the Folin-Ciocalteu method of the three legumes species are presented in figure (1). As seen in the cited figure, we show different solubility degrees of the polyphenols in each solvent. However, acidified methanol extracts (1% v/v acetic acid) of all analyzed legume powders present the higher amounts of polyphenols as compared to the other used solvents. Total phenolic content decreased in the following order: acidified methanol extracts > hydro-methanolic extracts > hydro-ethanolic extracts. The obtained data roughly divide our materials into three classes: (1) high phenolic content: P. vulgaris; (2) medium content: L. culinaris and (3) low content: V. faba.

Figure 1. Total phenols content of the analyzed legume extracts expressed in mg of GAE/g DM.
The total phenolic contents of the analyzed *P. vulgaris* legume extract are very similar to those found by Marathe et al. (2011), they use methanol/water in 80/20 (v/v). However phenolic content TPC varied between 2.406±0.055 mg EAG/g and 3.21±0.07 mg EAG/g in the same legume cultivated in India, these results are very similar to our results. Recently, Boudjou et al. (2013) show higher phenolic contents (2.2 mg EAG/g) of *L.culinaris* cultivated in Algeria extracted by the same extraction method ethanol/ water (80/20 v/v). Our amounts are less than the cited work; this difference is due to genotype, geographic and climatic conditions. Fratianni et al. (2014) use a different extraction solvent (acetone/water 80/20) found a low phenolic amounts in Italian *L. culinaris* with values ranging from 1.098 to 1.594 mg EAG/g this difference is due to polarity of the extraction solvent.

The total phenolic contents of the analyzed *V. faba* legumes are very low compared to the results mentioned by Boudjou et al. (3.21 mg EAG/g) in hydro-ethanolic extracts of Algeria *V. faba* (Boudjou et al. 2013). Whereas the observed results are near those mentioned by Baginsky et al. (2013) in Chilean *V. faba* values ranging from 0.81 mg EAG/g to 1.33 mg EAG/g.

### Table 1. Flavonoid contents of the analyzed legume extracts expressed in mg of QE/g Dry Matter

<table>
<thead>
<tr>
<th>Legume crude extracts</th>
<th>Total flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. Vulgaris</em></td>
<td>0.346±0.423a</td>
</tr>
<tr>
<td><em>L. culinaris</em></td>
<td>0.190±0.212</td>
</tr>
<tr>
<td><em>V. faba</em></td>
<td>0.128±0.187</td>
</tr>
</tbody>
</table>

a: standard deviation (n=3).

![Figure 2](image-url)

**Figure 2.** Base peak Reversed phase LC-ESI-MS chromatogram of *L. culinaris* methanol extract.
3.2 Flavonoids

In order to avoid anthocyanin interference, if present, in some legumes powders, flavonoids were only determined in the methanol fractions. In all three legume species, total flavonoid contents in methanolic extracts were expressed in mg eq. rutin/100 g dry weight. The data ranged from 0.128 to 0.346 mg of QE/g Dry Matter (Table 1).

Referring to Table (1), flavonoids content were identified in larger quantities in *P. vulgaris* extract (0.346±0.423 mg of QE/g D M), followed by *L. culinaris* methanol extracts. The lowest amount of flavonoids content was registered in *V. faba* methanolic extract (0.128 mg of QE/g D M).

3.3 Identification of phenolic compounds by LC-ESI-MS in the negative mode

The qualitative polyphenol profiles of three Tunisian leguminous were characterized by combining several approaches including LC-MS/MS of acidified methanol extracts.

Initially, acidified methanol extracts of samples were qualitatively analyzed using LC-DAD-MS. The main phenolic compounds in the samples were identified by comparison with available standards and interpretation of the MS and UV-visible data corresponding to the main chromatographic peaks.

3.3.1 *Lens culinaris* phenolic compounds

The chromatography data obtained for *L. culinaris* methanol extracts are illustrated in figure (2) and summarized in Table (2). The combination of retention time, UV spectra, absorbance detection (λ max nm) and the molecular and fragment ions data show a total of 6 tentatively identified phenolic compounds.

Catechin glucoside, catechin gallate, isomer (1) and (2) of kaempferol tetraglycoside, kaempferol-3-
robinoside-7-rhamnmoside, and kaempferol-3-glucoside (peaks 1 to 6, respectively (Figure 2)) were positively identified by comparing their retention time and UV spectra with those of the corresponding commercial standards, and the results were confirmed by LC-MS (Table 2).

The compounds corresponding to peaks 1 and 2 (Figure 2) showed deprotonated molecules \([M - H]^-\) at m/z 451 and 441, respectively. Their UV spectra are similar to the flavan-3-ols (a single band at 278 nm). However, their early retention times (8.9 and 10.41 min for compounds 1 and 2, respectively) indicate that these compounds are highly polar, which is much more consistent with the presence of a sugar moiety in the structure. In addition, MS/MS spectra of these ions showed the typical fragmentations of flavanols, including the retro-Diels–Alder fragmentation of the heterocycles (loss of 152 amu), the loss of the phloroglucinol A-ring (loss of 126 amu). These compounds were thus identified as catechin glucoside and catechin gallate that have already been mentioned in the literature by Amarowicz et al. (2010).

Peaks 3 to 6 (Figure 2) were unambiguously identified as Isomer 1 and 2 of Kaempferol tetraglycoside, \([M - H]^- = 933\), Kaempferol-3-robinoside-7-rhamnoside \([M - H]^- = 739\), and kaempferol 3-glucoside \([M-H]^- = 447\), respectively (Figure 3), according to their chromatographic, UV-visible, and MS characteristics by comparison with authentic standards.

Peak 6 showing a molecular ion at m/z 447 and a main product ion at m/z 285 was partially identified as a kaempferol- 3-glucoside. The kaempferol moiety was confirmed by observing the close similarity between the 447→285 MS³ spectrum and the m/z 285 MS² spectrum of the kaempferol standard (data not shown).

These phenolic compounds were previously detected in lentils and these flavanols have already been investigated by Zhang et al. (2015) using LC-ESI-MS/MS in negative mode.

### 3.3.2 Phaseolus vulgaris phenolic compounds

Reversed phase HPLC coupled with UV–visible and MS detection in negative mode was used to detect and characterize the phenolic compounds in the crude methanol extracts prepared from the seeds of *P. vulgaris*. The identification or partial characterization of individual compounds is presented in Table (3). Five compounds eluted in the 14-25 min zone of the chromatogram (Figure 4), numbered 1-5, and were identified as flavanols and flavonols phenolic class.

![Figure 4. Base peak Reversed phase LC-ESI-MS chromatogram of *P. vulgaris* methanol extract.](image-url)

Compound corresponding to peak 1 showed deprotonated molecules \([M - H]^-\) at m/z 455. A main product ion at m/z 304 corresponding to methylated catechin was observed on the MS/MS spectra. This loss of 151 amu corresponded to the molecular weight of gallate unit. This compound was thus identified as the methyl catechin gallate. Compound corresponding to peak 2 showed deprotonated molecules \([M - H]^-\) at m/z 449. This compound was thus identified as the myricetin 3-pentoside. This compound was considered as formally identified when all data (retention time, UV spectrum, and MS/MS spectrum) correctly matched
Journi et al., / Advances in Chemistry and Biochemistry Sciences, 02 (2015), 03, 01-12

Table 2. LC-UV-visible-MS and mass spectral characteristics of some phenolic compounds in L. culinaris methanol extract

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Tentative identification</th>
<th>RT (min)</th>
<th>Molecular weight</th>
<th>[M-H]⁺ (m/z)</th>
<th>λ_max (nm)</th>
<th>Major MS/MS product ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catechin glucoside</td>
<td>8.90</td>
<td>452</td>
<td>451</td>
<td>279</td>
<td>451;289</td>
</tr>
<tr>
<td>2</td>
<td>Catechin gallate</td>
<td>10.41</td>
<td>442</td>
<td>441</td>
<td>279</td>
<td>441;289</td>
</tr>
<tr>
<td>3</td>
<td>Isomer 1 Kaempferol</td>
<td>13.55</td>
<td>934</td>
<td>933</td>
<td>266;347</td>
<td>609;447;285</td>
</tr>
<tr>
<td></td>
<td>tetrarglycoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Isomer 2 Kaempferol</td>
<td>14.26</td>
<td>934</td>
<td>933</td>
<td>266;347</td>
<td>609;447;285</td>
</tr>
<tr>
<td></td>
<td>tetrarglycoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Kaempferol-3-robinoside-7-</td>
<td>18.90</td>
<td>740</td>
<td>739</td>
<td>267;353</td>
<td>739;431;285</td>
</tr>
<tr>
<td></td>
<td>rhamnoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Kaempferol-3-glycoside</td>
<td>20.86</td>
<td>448</td>
<td>447</td>
<td>267;343</td>
<td>447;285</td>
</tr>
</tbody>
</table>

Figure 5. MS² mass spectrum of [M-H]⁺, m/z 563 from Kaempferol hexose malic acid.

those of the available standard.

Peaks 3 and 5 (RT = 18.65 and 24.17, Figure 4) revealed molecular ions at m/z 447 and 563, respectively. Their MS/MS spectrum showed a main product ion at m/z 285, which was consistent with Kaempferol conjugate molecules. Peak 3 showing a molecular ion at m/z 447 and a main product ion at m/z 285 was partially identified as a kaempferol-3-glucoside gallate that have already been mentioned in the literature Long-Ze et al. (2008).

Peak 5 showing a molecular ion at m/z 563 and a main product ion at m/z 285 corresponded to
Kaempferol. The loss of 134 amu corresponded to the molecular weight of malic acid. The m/z 447 corresponded to Kaempferol hexosyl unit. Finally, MS and MS/MS data were collected to complete the identification of kaempferol hexose malic acid (Figure 5). However, as far as we know, this is the first mention of their probable presence in *P. vulgaris* methanol extract.

Peak 4 (Figure 4) was identified as apigenin 3-O-glycoside by LC-ESI/MS/MS. UV spectra of this peak demonstrates \( \lambda_{\text{max}} \) at 269 and 336 nm (Table 3), which are characteristic of apigenin. LC-ESI/MS/MS spectra of this peak suggest that it is an O-glycoside. Fragmentation of O-glycosides results in the loss of the glycosyl units and produces dominate fragment ion corresponding to the aglycone.

### 3.3.3 *Vicia faba* seeds phenolic compounds

Additional flavonoids including catechin, epicatechin, quercetin and kaempferol were identified in *V. faba* seeds. Figure (6) shows typical HPLC chromatogram base peak of the crude extract of *V. faba* seeds.

The retention time, UV spectra, the molecular and...
fragment ions of a total of 5 confirmed and tentatively identified phenolic compounds presented in Table (4). Catechin gallate, quercetin arabinoside, epicatechin glucoside, methyl epicatechin gallate, methyl epicatechin gallate and kaempferol glucoside sulfate (peaks 1 to 5) were identified by comparing their retention time and UV spectra with those of the corresponding commercial standards, and the results were confirmed by MS (Table 4). Peaks 1, 3 and 4 showed a $\lambda_{\text{max}}$ of 279 nm which were similar to that of UV spectrum characteristic of flavanol monomers. Peak 1 it exhibited a molecular ion [M-H]$^-$ at m/z 441 corresponding to catechin gallate, this compound as detected in V.faba seeds after phloroglucinolysis reaction by Jin et al. (2012). The LC-MS showed a molecular ion [M-H]$^-$ at an m/z 451 (Peak 3) and a fragment ion at m/z 289; the difference was loss of a hexose molecule. This compound was thus identified as the epicatechin glucoside. Peak 4 presented a similar UV spectrum, but different retention time to that of methyl catechin gallate which has been positively identified. It showed a negative molecular ion [M-H]$^-$ at m/z of 455 and a fragment ion [M-H]$^-$ at m/z of 289. This

Table 4. LC-UV-MS and mass spectral characteristics of phenolic compounds in V. faba methanol extract

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Tentative identification</th>
<th>RT (min)</th>
<th>Molecular weight</th>
<th>[M-H]$^-$ (m/z)</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Major MS/MS product ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catechin gallate</td>
<td>10.20</td>
<td>442</td>
<td>441</td>
<td>279</td>
<td>441;289</td>
</tr>
<tr>
<td>2</td>
<td>Quercetin arabinoside</td>
<td>13.90</td>
<td>434</td>
<td>433</td>
<td>293 ; 358</td>
<td>433;301</td>
</tr>
<tr>
<td>3</td>
<td>Epicatechin glucoside</td>
<td>14.20</td>
<td>452</td>
<td>451</td>
<td>278</td>
<td>451;289</td>
</tr>
<tr>
<td>4</td>
<td>Methyl epicatechin gallate</td>
<td>14.90</td>
<td>456</td>
<td>455</td>
<td>279</td>
<td>455;441;289</td>
</tr>
<tr>
<td>5</td>
<td>Kaempferol glucoside sulfate</td>
<td>21.06</td>
<td>528</td>
<td>527</td>
<td>263 ; 351</td>
<td>527;447;285</td>
</tr>
</tbody>
</table>

Figure 7. MS$^2$ mass spectrum of [M-H]$^-$, m/z 527 from Kaempferol glucoside sulfate.
compound was then tentatively identified as methyl epicatechin gallate. Peak 2 showed a molecular ion \([M-H]\) of m/z 433 and a quercetin aglycone fragment ion \([M-H]\) at m/z 285 (The loss of 132 amu corresponded to the molecular weight of [arabinose-H\(_2\)O], this compound was tentatively identified as quercetin arabinoside. The molecular ion of the compound corresponding to peak 5 (m/z 527) revealed a mass difference of 80 amu compared to the molecular ion observed for kaempferol glucoside (m/z 447; detected and identified in P. vulgaris seeds sample) and similar UV spectrum to the kaempferol (\(\lambda_{\text{max}} = 263; 351\)). The loss of 80 amu was observed for some compounds on the MS/MS spectra and was interpreted as an indication of the presence of a sulfate group in the molecular structure. This compound was tentatively identified as kaempferol glucoside sulfate (Figure 7).

4. Conclusion

Comparison of the different Tunisian legume extracts shows the positive influence of extraction solvents in the yields of total phenols and flavonoids. The richest extracts were the methanol acidified 1% acetic acid (99/1, v/v). The basis of the HPLC data are, to our knowledge, the most complete estimate of the average phenolics composition of these three legumes (\(V.\ faba\), \(L.\ culinaris\) and \(P.\ vulgaris\)) presently consumed in Tunisia considering all the main monomers, flavonols and flavones. Totally, six compounds are tentatively identified by the combination of LC-UV-ESI/MS and mass spectral characteristics.

5. Acknowledgement

This work was supported by a studentship from the Tunisia Ministry of Higher Education, Scientific Research and Technology. The authors would like to acknowledge the Tunisian Ministry for the scholarship. A special thanks to Doctor Farouk Mraihi (Sciences faculty of Bizerte) for the helpful contribution in the preparation and the revision of the manuscript. We are also grateful to anonymous reviewers who helped, through their constructive comments and suggestions to improve the quality of the manuscript.

### References


