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# Separation and Quantification of Phenolic Compounds of Wild Edible Plants

## Research article

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**Abstract**

Ten wild edible plants were subjected to analysis. Individual phenolic compound concentration and the percentage from total phenolic compounds were pursued using RP-HPLC. Results showed that ten phenolic compounds (gallic, protocatechuic, catechin, gentisic, chlorogenic, vanillic, syrnigic, caffeic, epicatechin and benzoic acid) have been identified in the investigated plants. The highest catechin (300.9 mg/100g) (*Ruta chalepensis*L.) which is equivalent to 27.18% of total phenolic compounds, gallic acid (361.2 mg/100g) (*Ruta chalepensis* L.) which counts for 33.65% of total phenolics, chlorogenic acid (17.2 mg/100g) (*Ruta chalepensis* L.) (1.29%) and caffeic acid (137.5 mg/100g) (*Centaurea iberica* Trev.ex.Spreng) (36.3%). The lowest concentrations were found in *Ruta chalepensis* L. (7.69%). *Centaurea iberica* Trev.ex.Spreng contains the highest concentrations of protocatechuic acid (32.5 mg/100g) (6.19%). Caffeic acid (105.8 mg/100g) (*Ruta chalepensis* L.) (7.96%). No vanillic acid, caffeic acid and benzoic acid were detectable in *Arum palaestinum* Boiss.

**Keywords:** Wild edible plants; Total phenolics; phenolic compounds; RP-HPLC

## Introduction

Fats, oils and lipid-based foods deteriorate through several degradation reactions, while the main deterioration processes are oxidation reactions and the decomposition of oxidation products, which result in decreased nutritional value and sensory quality. Retardation of these oxidation processes is important, and can be achieved using specific additives have the ability to inhibit oxidation, known as antioxidants [1].

Natural antioxidants are in high demand because of their potential in health promotion and disease prevention, and consumer acceptability [2]. Great efforts have been made in finding naturally occurring antioxidants of plant origin, thus many plant phenolic compounds exhibiting antioxidant properties have been studied and proposed for protection against oxidation [3].

Wild plants play an important role in the diet of inhabitants in different parts of the world. The investigated plants tend to be drought-resistant, gathered both in times of abundance and times of need and used in every day cooking [4]. Many of these plants are nutritionally important because of their high vitamin, mineral and fiber contents.

In Jordan, large numbers of wild edible plants are widely distributed and consumed in various ways [5]. The soft pods and seeds of *Tetragonolobus palaestinus* Boiss. (Jalaton is the local name)


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which is a wild dry legume species found in the highlands of Jordan, are consumed by Jordanian people before it reaches the dry stage [6].

Wild plants are also used in folk medicine as antiscorbutic, antispasmodic, carminative agents against bronchitis and as diuretics [7]. In Saudi Arabia, the aerial parts of *Ruta chalepensis*L. are used as an analgesic, antipyretic and for the treatment of rheumatism and mental disorders [8].

The overall objective of our research was to identify types and concentrations of these phenolic compounds using RP-HPLC in some wild edible plants from Jordan.

## Materials and Methods

### Plant Material

About 3 kg of each of wild edible plant (*Arum palaestinum* Boiss., *Centaureaiberica* Trev. ex. Spreng., *Cichorium intybus* L., *Coriandrum sativum* L., *Gundelia tournefortii* L., *Malva parviflora* L., *Rumex acetosella* L., *Ruta chalepensis* L., *Salvia hierosolymitana* Boiss., *Salvia hierosolymitana* Boiss. and *Tetragonolobus palaestinus*Boiss.) were purchased from local stores, or has been collected them from various regions in Ajloun region [9]. All plants were obtained at the time of optimal growing conditions during the months of March and April. The scientific and local names were identified according to two references [6,10]. The plants were washed and rinsed with tap water and followed by rinsing with distilled water and left for drying at room temperature. The dried plants were mined into an average 0.4 mm diameter particle. The plants grounded and preserved in ziploc bags and kept at -18 °C, until further use.

### Chemicals

Acetonitrile and all chemicals were of HPLC analytical grade. HPLC standards, caffeic acid (3-4-Dihydroxycinnamic acid), benzoic acid, vanillic acid (4-hydroxy-3-methoxybenzoic acid), gentisic acid (2,5-dihydroxybenzoic acid), gallic acid, chlorogenic acid, protocatechuic acid (3,4-dihydroxybenzoic acid), syrnigic acid, (+)-catechin, (-)-epicatechin, and all other chemicals and reagents were of HPLC grade and were purchased from local agent. All solutions were prepared using deionized water.

### Phenolic compounds extraction

Phenolic compounds extraction method was done on according to previously reported method [11]. Approximately a replicate of 5 g of each individual plant were subjected to extraction with 50 ml of methanol. The extraction conditions were performed at 60 °C and 60 minutes under continuous stirring. A Whatman No. 3 filter paper was used to filter the extract into a 50 ml volumetric flask and placed in a dark place until analysis.

### Determination of total phenolics

The Folin-Ciocalteu procedure was adopted in this work for total phenolic content in the extracts [12]. A tow replicates (50 µL of the plant extract) were transferred and mixed into a glass test tube containing 0.4 ml of 10 % Folin-Ciocalteu reagent for three minutes, followed by addition of 0.8 ml of a 10 % sodium carbonate ($Na_2CO_3$). The mixture in the tubes were allowed to stand for 1 hour at ambient temperature, and the light absorption was measured at 725 nm using spectrophotometer (CELL, model CE 1020, England) against a blank containing 50 µl methanol in place of plant extract. A gallic acid was used as calibration standard, and the results were calculated according to gallic acid equivalent (GAE) (mg/100g dry weight basis).

### RP-HPLC separation and quantification of phenolic compounds

The content of phenolic compounds in plant samples was determined by High-Performance Liquid Chromatography (HPLC). About five grams of each plant sample were weighed out, and extracted with 50 ml of methanol. Extraction was carried out under stirring for 60 minutes, at 60 °C. Each extract was filtered out using Whatman No. 3 filter paper, and filled accordingly in a 50 ml volumetric flask. Each extract was vacuum-concentrated at 40 °C up to dryness and then re-dissolved in 25 ml of mobile phase solvent B (0.1 % triflouroacetic acid in water). Each solution was centrifuged to get the supernatant. 25 µL aliquot of the supernatant were injected into the High Performance Liquid Chromatography (HPLC), and the analysis was performed after.

The chromatographic equipment consisting of an interface D-7000, diode array detector L-7455, autosampler L-7200, Pump L-7150, solvent degasser L-7612 and a reversed phase column (C18 RP 0.5 µ column). All of these parts belong to Merck Hitachi. Thermo GOH, 150 × 4.6 mm. The chromatographic conditions were modified [13]. The mobile phase consisted of solvents A and B. Solvent A was 0.1% triflouroacetic acid in 10 % acetonitrile, solvent B was 0.1% triflouroacetic acid in water. Initial solvent conditions and the flow rate were set as follow: time started from zero, 50, 51, 65, 66 and 85 minutes. Solvent A started at 100, 100, zero, zero, 100 and 100% corresponding to time. Solvent B started at zero, zero, 100,100, zero and zero% corresponding to time. The flow rate (mL/min) started at 1.0, 1.0, 1.5, 1.5, 1.0 and 1.0 mL/min at the corresponding time. Column temperature was maintained at ambient temperature throughout the run. Phenolic compounds peaks were identified by their HPLC retention times at 234 nm.

The stock solution of ten phenolic compounds was prepared at 1.0 mg/100 mL in methanol. From this solution, four standard solutions were prepared by dilution. These were used to prepare the calibration curves. Ten calibration curves were constructed covering the concentration range from 5-50 ppm, with (r) values for gallic acid amounting 0.9980, protocatechuic acid and syrnigic acid 0.9973, catechin and vanillic acid 0.9975, gentisic acid 0.9979, chlorogenic acid 0.9978, caffeic acid 0.9974, epicatechin 0.9972 and for benzoic acid 0.9957. The calibration graphs were used to calculate the phenolic compounds concentrations in plant samples. Phenolic compounds were identified by comparing their retention time with that of the standard. For each phenolic compound, the concentration in mg/100g of dry sample was computed according the following:

Concentration (mg/100g) = Concentration in ppm × (1/1000) × (F.V/ S. wt.) × 100,

Where: F.V = Final Volume, S. wt. = Sample weight.

### Statistical analysis

The statistical analysis was performed in SAS software Version 8.2 software package using the general linear model procedure and the data are presented as means of two replicates [14]. Significant


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**Table 1:** Concentrations of phenolic compounds found in wild edible plants from in Jordan.[a a]Values (mg/100g) are computed on dry weight basis and average of two replicates.

| Wild Edible Plant | Gallic acid | Protocatechuic acid | Catechin | Gentisic acid | Chlorogenic acid | Vanillic acid | Syringic acid | Caffeic acid | Epicatenchin | Benzoic acid |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Arum palaestinum Boiss*** | 1.3±0.05$^{e}$ | 0.1±0.01$^{c}$ | 1.1±0.01$^{b}$ | 0.9±0.15$^{c}$ | ND | ND | 0.1±0.01$^{c}$ | ND | 0.6±0.01$^{c}$ | ND |
| ***Centaurea iberica Trev. ex Spreng.*** | 2.5±0.06$^{d}$ | 0.2±0.01$^{c}$ | 2.3±0.01$^{b}$ | 18.85±0.05$^{a}$ | 0.4±0.01$^{c}$ | 0.3±0.01$^{d}$ | 1.0±0.02$^{b}$ | 36.4±0.09$^{a}$ | 0.6±0.01$^{c}$ | 0.0±0.01$^{b}$ |
| ***Cichorium intybus L.*** | 16±0.09$^{b}$ | 0.2±0.01$^{c}$ | 0.5±0.02$^{c}$ | 13.3±0.01$^{b}$ | ND | 49.5±0.01$^{a}$ | 1.2±0.01$^{b}$ | 0.2±0.01$^{d}$ | 2.0±0.01$^{b}$ | ND |
| ***Coriandrum sativum L.*** | 1.1±0.08$^{e}$ | 0.2±0.01$^{c}$ | 0.1±0.02$^{c}$ | 0.0±0.01$^{c}$ | ND | 11.3±0.01$^{b}$ | 0.1±0.01$^{c}$ | 0.5±0.01$^{d}$ | 33.8±0.01$^{a}$ | ND |
| ***Gundelia tournefortii L.*** | 0.3±0.01$^{e}$ | 1.2±0.01$^{b}$ | 0.5±0.01$^{c}$ | 0.5±0.01$^{c}$ | ND | 3.4±0.01$^{c}$ | 3.9±0.01$^{a}$ | 2.3±0.02$^{c}$ | ND | ND |
| ***Malva parvijlora L.*** | 10.0±0.04$^{c}$ | 1.2±0.01$^{b}$ | 1.4±0.01$^{b}$ | 0.6±0.02$^{c}$ | 0.3±0.01$^{c}$ | 2.7±0.01$^{c}$ | 0.5±0.01$^{c}$ | 1.9±0.01$^{c}$ | ND | ND |
| ***Rumex acetosella L.*** | 3.8±0.09$^{d}$ | 0.5±0.01$^{c}$ | 0.5±0.01$^{c}$ | 0.3±0.01$^{c}$ | 0.1±0.01$^{c}$ | 1.0±0.01$^{d}$ | 0.2±0.01$^{c}$ | 0.7±0.01$^{d}$ | ND | ND |
| ***Ruta chalepenszs L.*** | 27.9±0.06$^{a}$ | 0.3±0.01$^{c}$ | 22.7±0.01$^{a}$ | 0.1±0.01$^{c}$ | 1.0±0.33$^{b}$ | 0.3±0.01$^{d}$ | 0.4±0.16$^{c}$ | 8.0±0.01$^{b}$ | 3.1±0.67$^{b}$ | 3.0±0.01$^{a}$ |
| ***Salvia hierosolymitana Boiss*** | 0.7±0.01$^{e}$ | 0.0±0.01$^{c}$ | 0.1±0.01$^{c}$ | 1.0±0.04$^{c}$ | 0.1±0.01$^{c}$ | 1.0±0.04$^{d}$ | 0.8±0.09$^{c}$ | 0.3±0.01$^{d}$ | 0.4±0.02$^{c}$ | 0.1±0.01$^{b}$ |
| ***Tetragonolobus palaestinum Boiss*** | 9.5±0.08$^{c}$ | 4.3±0.01$^{a}$ | 2.9±0.09b | 1.8±0.01$^{c}$ | 7.0±0.01$^{a}$ | 1.3±0.09$^{d}$ | 1.4±0.01$^{b}$ | ND | 3.2±0.01$^{b}$ | ND |

Means ± SEM (standard error of the mean) in the same column followed by the same letter are not significantly different at P≤ 0.05. ND = not detected.

differences were defined at $p \leq 0.05$ and the analysis of Variance (ANOVA) was used to compare the means of the presented data.

## Results and Discussion

### Total phenolics

The total phenolic compounds concentration found in the plants *Arum palaestinum* Boiss., *Centaureaiberica* Trev. ex Spreng., *Cichorium intybus* L., *Coriandrum sativum* L., *Gundelia tournefortii* L., *Malva parviflora* L., *Rumex acetosella* L., *Ruta chalepensis* L., *Salvia hierosolymitana* Boiss. and *Tetragonolobus palaestinus* Boiss. was 1010.6, 379.8, 598.6, 936.0, 375.5, 204.4, 543.2, 1328.8, 911.1, 163.1 mg GAE/100g dry weight, respectively [9].

*Ruta chalepensis* showed significantly higher concentrations of total phenolics (1328.8 mg GAE/100g) as compared with *Coriandrum sativum*L. (936 mg GAE/100g) and *Salvia hierosolymitana* (911.1 mg GAE/100g). *Arum palaestinum* Boiss. contained higher amount of total phenolics as compared with *Cichorium intybus*L., *Coriandrum sativum*L., *Rumex acetosella*L. and *Salvia hierosolymitana* Boiss.

### HPLC identification and quantification of phenolic compounds

Figures 1, 2, 3, 4 and 5 show the HPLC chromatograms of the principal phenolic compounds for ten wild edible plants from Jordan. The phenolic compounds which were identified were; gallic acid, protocatechuic acid, catechin, gentisic acid, chlorogenic acid, vanillic acid, syrnigic acid, caffeic acid, epicatechin and benzoic acid. The peaks of these compounds were recorded at 234 nm and identified by comparison with internal standards. Figures 1, 2, 3, 4 and 5 also show the HPLC chromatogram for the standard phenolics and the identified phenolic compounds in the investigated plants. Table 1 shows data on the principal phenolic compounds concentrations. Data shown in Table 1, indicated variability in quantitative and qualitative composition of phenolic compounds in all plants. Total phenolic compounds concentration was higher in *Ruta chalepensis*L. as compared with the other plants. As shown in Table 1 gallic acid concentrations ranged between 1.1 and 361.2 mg/100g. The lowest concentration was found in *Gundelia tournefortii*L. and the highest in *Ruta chalepensis*L. Gallic acid was the main component in the extracts of *Arum palaestinum* Boiss, *Malva parviflora*L., *Rumex acetosella*L., *Ruta chalepensis*L. and *Tetragonolobus palaestinus*Boiss. These data agree well with data reported on gallic acid as a major phenolic compound in *Barringtonia racemosa*, *Cassia auriculata L.*, *Euphorbia hirta L.* and *Feronia elephantum* Correa [15]. Protocatechuic acid concentrations ranged between 0.3 (*Salvia hierosolymitana*Boiss) and 23.5 mg/100g (*Centaurea iberica*Trev.ex.Spreng). All other plants had intermediate values. These values were lower than those detected in Sumac (*Rhus coriaria* L.) (40 mg/100g) [16].

Catechin concentration represented an important share to the phenolic compounds in *Ruta chalepensis* (300.9 mg/100g) (Table 1). Catechin concentration of *Ruta chalepensis*L. was comparable to that detected in the leaves of max red bartlett cultivar of pears (*Pyrus communis* L.) (305 mg/100g) (17). Chlorogenic acid concentrations ranged between 0.5 (*Salvia hierosolymitana*Boiss.) and 17.2 mg/100g (*Ruta chalepensis*L.). *Centaurea iberica* Trev.ex.Spreng, *Malva parviflora*L., *Rumex acetosella*L. and *Salvia hierosolymitana* Boiss. concentrations of chlorogenic acid were all higher than those reported on the peels and flesh of apple fruit (*Malus domestica* Borkh., cv. Aroma) (6.5 and 15.1 mg/100g, respectively) [18]. Chlorogenic acid was not detected in *Arum palaestinum*Boiss., *Cichorium intybus*L., *Coriandrum sativum*L. and *Gundelia tournefortii*L..

Vanillic acid was the main constitutive component in *Cichorium intybus* L. (296.4 mg/100g), with a value higher than that found in sumac (*Rhus coriaria* L.) (50 mg/100g) [16]. Caffeic acid was the main component in the extract of *Centaurea iberica*Trev.ex.Spreng (137.5 mg/100g) (Table 1). The concentration of other phenolic compounds in *Centaurea iberica*Trev.ex.Sprengwas not as high as caffeic acid. The content of gentisic acid was 71.6 mg/100 g, protocatechuic


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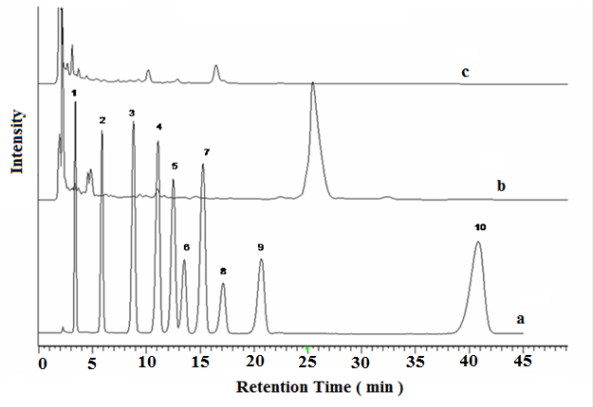


**Figure 1:** HPLC chromatograms for (a) standards, (b) *Salvia hierosolymitana*Boiss, (c) *Rumex acetosella*L., 1=gallic acid, 2=protocatechuic acid, 3=catechin, 4=gentisic acid, 5=chlorogenic acid, 6=vanillic acid, 7=syrnigic acid, 8=caffeic acid, 9=epicatechin, 10=benzoic acid.

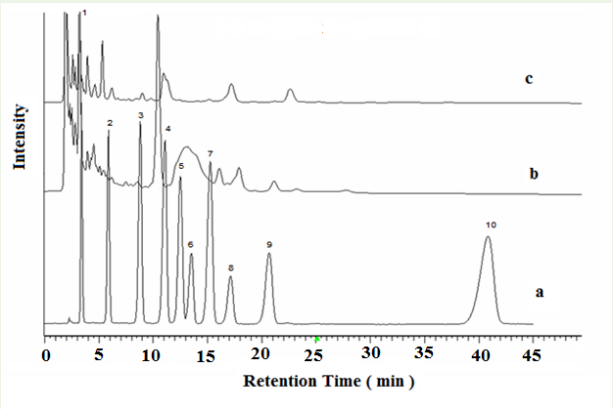


**Figure 2:** HPLC chromatograms for (a) standards, (b) *Cichorium intybus*L., (c) *Centaurea iberica*Trev. Ex.Spreng.1=gallic acid, 2=protocatechuic acid, 3=catechin, 4=gentisic acid, 5=chlorogenic acid, 6=vanillic acid, 7=syrnigic acid, 8=caffeic acid, 9=epicatechin, 10=benzoic acid.

acid concentration was 23.5 mg/100g. *Ruta chalepensis* found to be high in caffeic acid (105.8 mg/100g) as compared with other plants. *Centaurea iberic* Trev.ex.Sprenga and *Ruta chalepensis*L. caffeic acid concentrations were higher than those reported on *Silybum marianum*, *Taraxacum officinale*, *Archangelica officinalis* and *Herniara glebra* (92.8, 72.6, 85.3 and 78.1 mg/100g, respectively) [19]. All other plants contain lower concentrations of caffeic acid (0.9 to 8.6 mg/100g) than those detected in different tomatoes (*Lycopersicon esculentum* Mill.) cultivars (13.9 to 24.1 mg/100g) [20].

Epicatechin was the main phenolic in *Coriandrum sativum*L. (316.0 mg/100g), a value that is higher than that detected in the leaves of Williams cultivar of pears (*Pyrus communis* L.) (219 mg/100g) [17]. Epicatechin was not detected in *Gundelia tournefortii*, *Malva parviflora*L. and *Rumex acetosella*L.. Epicatechin concentrations of *Arum palaestinum*Boiss, *Centaurea iberica* Trev.ex.Spreng., *Ruta chalepensis*L, *Salvia hierosolymitana*Boiss and *Tetragonolobus palaestinus*Boiss were lower than those found in *Filipendula vulgaris* (35 mg/100g), *Aruncus silvester* (50 mg/100g), *Waldsteinia geoides* (54 mg/100g), *Potentilla alba* (132 mg/100g) and *Geum rivale* (4 mg/100g) [21]. *Cichorium intybus* L. (11.6 mg/100g) found to have similar concentration of epicatechin as that of the Red Globe cultivar of grapes (11.6 mg/100g) [22].

It was found that the highest levels were for gallic acid (27.81%, *Ruta chalepensis* L.), prutocatechuic acid (6.19 %, *Centaurea iberica* Trev.ex.Spreng), Catechin (22.65%, *Coriandrum sativum* L., gentisic acid (18.85%, *Centaurea iberica* Trev.ex.Spreng), chlorogenic acid

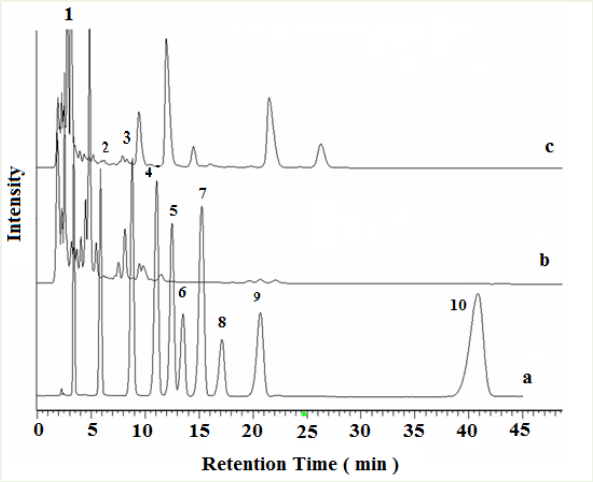


**Figure 3:** HPLC chromatograms for (a) standards, (b) *Arum palaestinum*Boiss., (c) *Coriandrum sativum*L..1=gallic acid, 2=protocatechuic acid, 3=catechin, 4=gentisic acid, 5=chlorogenic acid, 6=vanillic acid, 7=syrnigic acid, 8=caffeic acid, 9=epicatechin, 10=benzoic acid.

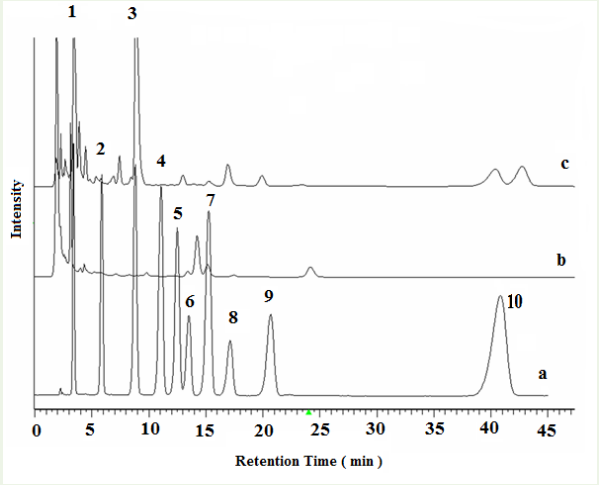


**Figure 4:** HPLC chromatograms for (a) standards, (b) *Gundelia tournefortii*L., (c) *Ruta chalepensis*L.1=gallic acid, 2=protocatechuic acid, 3=catechin, 4=gentisic acid, 5=chlorogenic acid, 6=vanillic acid, 7=syrnigic acid, 8=caffeic acid, 9=epicatechin, 10=benzoic acid.


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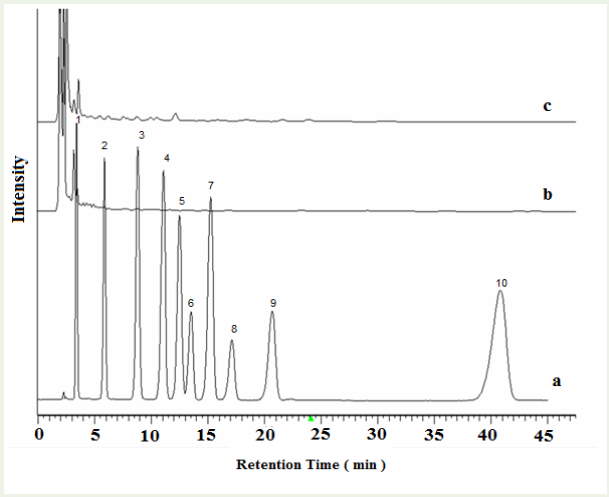


**Figure 5:** HPLC chromatograms for (a) standards, (b) *Malva parviflora*L. (c) *Tetragonolobus palaestinus*Boiss. 1=gallic acid, 2=protocatechuic acid, 3=catechin, 4=gentisic acid, 5=chlorogenic acid, 6=vanillic acid, 7=syrnigic acid, 8=caffeic acid, 9=epicatechin, 10=benzoic acid.

(6.99%, *Tetragonolobus palaestinus*Boiss), vanillic acid (49.52 %, *Cichorium intybus* L.), syringic acid (3.84%, *Gundelia tournefortii*L.), caffeic acid (36.2%, *Centaurea iberica* Trev.ex.Spreng), epicateuchin acid (33.76%, *Coriandrum sativum* L.) and benzoic acid (3.03%, *Coriandrum sativum* L.). Moreover,*Ruta chalepensis* L. has the highest levels of gallic acid, catechin and benzoic acid. These data agree very well with data reported previously on some Mediterranean plants [23].

In conclusion, RP-HPLC results showed the plants contained several phenolic compounds: gallic acid, protocatechuic acid, catechin, gentisic acid and syrnigic acid presented the predominating compounds. Jordanian wild plants are valuable in antioxidant components, which can be applied in food systems and pharmaceutical products. More work should be done to evaluate phenolic compounds and their antioxidants activity for greater number of plants grown in the wilderness of Jordan, in order to create nutritional and medicinal reference for these plants and to evaluate their health benefits.

## Acknowledgment

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