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Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Different Parts of *Malus domestica* L. in Iraq

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Abstract

The current study was designed to estimate the qualitative and quantitative analyses of total phenolic and flavonoid compounds extracted by two techniques (maceration and soxhlet) for three aerial parts (leaves, stems, and petioles) of *Malus domestica* grown naturally in Iraq. A general phytochemical investigation of the three parts of the plant had been carried out before the extraction process. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined by colorimetric methods and by HPLC method. The free radical scavenging assay for (DPPH) was carried out to measure antioxidant activity for all extracted portions. Phytochemical screening of the three parts of *Malus domestica* showed the presence of different active constituents such as saponins, tannins, alkaloids, terpenoids, flavonoids, polyphenols, and glycosides. The maceration extraction technique resulted in a higher yield for all fractions than the Soxhlet technique. The results also showed a variation in the phenolic compounds among the different parts, revealing that the leaves of *Malus domestica* had a high content of phenolic acids and flavonoids compared to the other parts. In contrast, the petioles (extracted by soxhlet) showed the strongest antioxidant activity (86.01 %) compared to other parts. Based on the HPLC assay, the components obtained from leaves, stems, and petioles ethanolic extract were declared to contain seven phenolic acids (including Ascorbic acid, Gallic acid, Vanillic acid, Chlorogenic acid, p-coumaric acid, Caffeic acid, and Ferulic acid). Ten flavonoids (including Rutin, Quercetin, Myricetin, Catechin, Kaempferol, Hesperetin, Isorhamnetin, Apigenin, and Luteolin) were identified.

Keywords: Antioxidant Activity, Flavonoids , Iraqi *Malus domestica*, Phenolic compounds.

Introduction

Apple (*Malus domestica*) is a deciduous tree (3 - 12 meters) belonging to the family Rosaceae [(1)]. The tree grows in temperate zones [(2)]. Traditionally, the dried leaves and flower infusion were used to control blood pressure, and the fruit was used as a laxative, diuretic, and antidiarrheal. Miscellaneous classes of phenolic compounds and flavonoids, such as rutin, quercetin, catechin, and epicatechin, and phenolic acids, such as gallic acid, chlorogenic acid, caffeic acid and coumaric acid, were present in Malus domestica fruit, peel, and leaves [(3)]. These compounds have numerous biological activities such as anti-inflammatory [(4)], antioxidant [(5)], anticancer [(6)], neuroprotective effect [(7)], antiulcer [(8)], increase the immunity, and improve human health [(9)].

Due to the beneficial effects of secondary metabolites, the interest in quantifying and characterizing the secondary metabolites of plants is growing [(10)].

In recent years , researchers have been most focused on the extraction of metabolites and their use in pharmaceutical, pharmacological, and food studies for their beneficial properties [(11)]. Researchers have focused their attention on the bioactive components of vegetables and fruits, considering their health benefits for the prevention and treatment of different human diseases [(12)]. Among the significant *M. domestica* secondary metabolites, which are biologically active components, the plant is rich in organic acids, polyphenols, terpenes, and carotenoids, the main phytochemicals [(13)]. Most previous studies focused on studying the fruits, while the plant's leaves and other parts should have been highlighted. Although apples are rich in critical bioactive compounds, data concerning the phenolic compounds in the aerial parts (leaves, stems, and petioles) of *Malus domestica* grown in Iraq have yet to be found or authorized in this country. Thus, the present study was designed to evaluate phenolic compounds in Iraqi *M. domestica* plants quantitatively by HPLC.

Besides, to compare phenolic content within the different parts (leaves, stems, and petioles) of the plant, as well as a comparison between two extraction techniques, the cold maceration and hot contentious extraction methods by Soxhlet apparatus for estimation of total phenolic and total flavonoid contents from different *M. domestica* parts have been carried out in the current study.

It was hoped that this study would help understand these bioactive compounds from aerial parts of Iraqi apple trees, shedding light on new drugs possessing pharmacological importance and providing unique treatment developments.

Materials and Methods

Plant material Collection

Healthy and fresh aerial parts (leaves, stems, and petioles) of Iraqi *Malus domestica* were collected from the Ala'bara area, Diyala Governorate, Iraq, in November 2022 (early winter). Leaves were collected from different places of each tree; the aerial parts were washed and dried in the shade and air circulation at room temperature[14] for (leaves 15 days, stems 25 days and petioles 20 days) Figure (1).

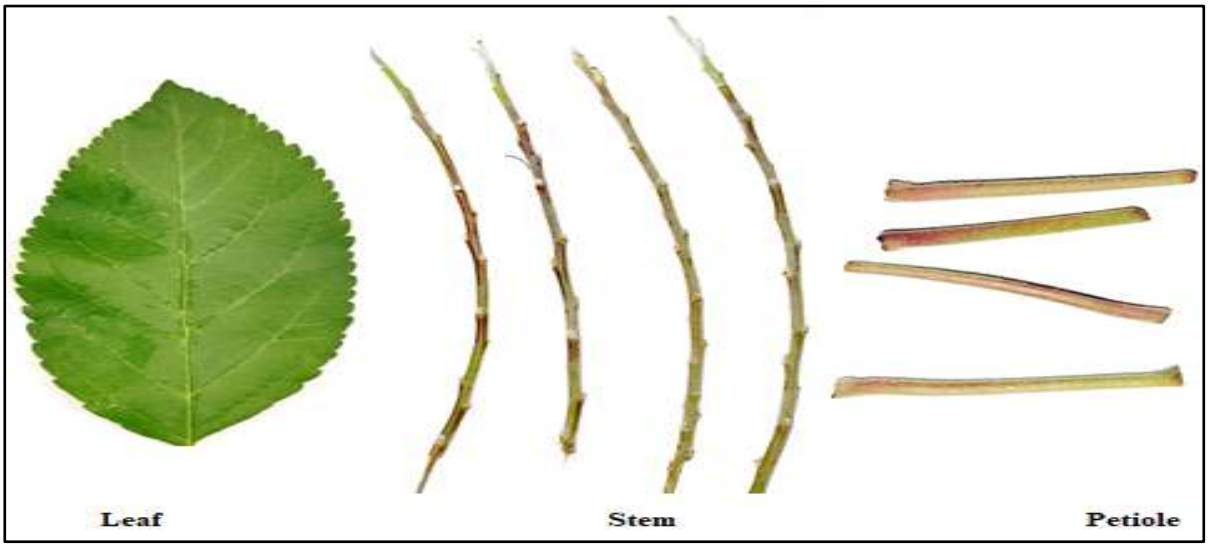

Figure 1. Aerial parts of Iraqi *Malus domestica* (leaf, stems and petioles)

Preliminary phytochemical screening

Plant material extracts were prepared by macerating (leaves 10gm, stems 10gm and petioles 10gm) in 100 ml of ethanol 70% separately for seven days, then filtered using (Whatman No. 1) filter paper. The filtrate is stored in a cool, dry place till screening [15]. Different chemical tests were analysed to check the absence or presence of different secondary metabolites such as Saponin, tannins, polyphenols, flavonoids, alkaloids, and reducing sugars of Iraqi *Malus domestica* extracts.

Table 1. Phytochemical screening tests of extracts of aerial parts of Iraqi *Malus domestica*

Constituent class	Chemical Tests	Experimental procedure	Reference
Saponins	Foam test	1 ml of extract + 9 ml of distilled water, agitated in a test tube for 15 seconds and left to stand for 15 minutes	[16]
Tannins	1%lead acetate test	2ml of extracts + a few drops of lead acetate (1%) solution	[17]
Polyphenols	Ferric chloride test	2 ml of extract + 2 ml 5% ferric chloride solution	[18]
Flavonoids	Alkaline reagent test	2 ml of extract + a few drops of sodium hydroxide solution	[17]
Alkaloids	Dragendroff's test	5 ml of extract + a few drops of Dragendroff's reagent (solution of potassium bismuth iodide)	[18]
Reducing sugars	Banedict's test	5 ml of extract + a few drops of Banedict's reagent + boiling on water bath	[16]

Defatting

A hundred grams of (leaves, stems, and petiole) were macerated in 1000 ml of n-hexane for 72h as a defatting step. The plant material was filtered using Whatman paper No.1, and the filtrate was dried by a rotary evaporator to obtain an n-hexane layer for chemical estimation, weighted and stored at 4°C until use. The Defatting plant material was exposed to another extraction process after removing all n-hexane solvent residues [19].

Preparation of plant extracts

Two different extraction methods were followed to prepare crude extracts from defatting aerial parts of Iraqi *Malus domestica* **,** Maceration method and Soxhlet methods.

Maceration extraction

Fifty grams of defatting aerial parts material (leaves, stems, and petiole) were macerated separately with 500ml Ethanol 70% for seven days, occasionally stirring. The mixture was filtered using Whatman paper No.1, and the filtrate was separated from the extracted plants. The extracted plants (leaves, stems and petioles) were macerated again with 250ml of Ethanol 70% for another three days. The combined liquids (filtrate) were dried as a final step with a rotary evaporator at 40°C to get viscous concentrate dried extracts, which were weighted and stored at 4 °C until use. The Marc was thrown out[20].

Soxhlet extraction

Fifty grams of defatting aerial parts material (leaves, stems and petioles) were placed separately in the Soxhlet apparatus and subjected to extraction in 500ml Ethanol at 70% till exhaustion. The Ethanol extract was filtered using (Whatman paper No.1) and evaporated by rotary evaporator, weighted and stored at 4°C until use[21].

Quantitative estimation of total phenolic content (TPC) in ethanolic extracts

Total Phenolic Content (TPC) was determined by the Folin – Ciocalteu method with slight modification [22]. Gallic acid standard solutions had been prepared in different concentrations (30, 40, 60, and 80) µg /ml. Samples were prepared by dissolving 1 mg of each extract in 10 ml of distilled water. 1 mL of the folin reagent was mixed with 1 ml from each standard solution and each of the extract in separate tubes. After 5 min, 5 mL of distilled water was added and mixed, followed by 1 mL of Na2CO3 10%, incubated at room temperature, and kept in the dark for 60 min. Finally, the absorbance was read at 760 nm against a blank. TPC was expressed as µg gallic acid equivalent (GAE)/mg of dry extracts. Gallic acid was prepared using the calibration curve of gallic acid.

Quantitative estimation of total flavonoid content (TFC) in ethanolic extracts

Total flavonoids content (TFC) was determined according to the procedure described by Verzelloni *et al.* [23]. Rutin standard solutions were prepared to get the following concentrations (0.15625, 0.3125, 0.625, and 1.25) mg/ml for the rutin standard curve. Samples were prepared by dissolving 1 mg of each extract in 10 ml of distilled water. An Aliquot of 1 ml from each extract and each standard solution were mixed with 4 mL distilled water each in a separated tube, and 0.3 ml from NaNO2 5% was added respectively. After 5 min, 0.3 mL of the 10% AlCl3 was added to all tubes to stand then for another 5 min. Finally, 2 mL NaOH (1M) was added and incubated at room temperature for 30 min; the absorbance was measured against the blank solution at 510 nm. Rutin standard curve was drawn by plotting each concentration against their Absorption reading. TFC was expressed as µg of Rutin equivalents/mg dry plant extract.

Estimation of antioxidant activity by (DPPH) reagents

DPPH (2,2′-diphenyl-1-picrylhydrazyl) free radical scavenging activity assay was carried out according to the method described by Brand-Williams *et al.* [24] with simple modification · DPPH solution was prepared in methanol (o.1 mM). Samples were prepared in methanol (2mg/ml). An aliquot of 2 ml from each sample (leaves, stems, and petioles) was mixed with 2 ml of prepared DPPH solution separately to be kept in the dark for 30 minutes at room temperature. Absorbance was determined at a wavelength of 517 nm using a UV spectrophotometer. The antioxidant activity of extracts was calculated from the Ascorbic acid calibration curve, and the percentage of radical scavenging was calculated using the following formula.

Percentage of radical scavenging

$$= \left[\frac{A0 - A1}{A0}\right] \times 100$$

Where, A_0 = the absorbance of DPPH solution,A_1 = the absorbance of the sample

Analysis the ethanolic extracts (maceration method) by HPLC technique

HPLC conditions for the analysis of ethanolic extracts (maceration method) of aerial parts (leaves, stems, and petiole) of Iraqi *M. domestica* were shown in Table(2).

Table2. HPLC conditions analysis for ethanolic extracts (maceration method)

Phenolic compounds	**Flavonoids**		**Phenolic acids**	
Instrument	Shimadzu, Japan		Shimadzu, Japan	
Mobile phase	A= Methanol (70%) B= 0.1% Formic acid (30%)		A= 0.1% Acetic acid (20%) B= Methanol (80%)	
Column	ODS_{C18} (250× 4.6 Id) mm/5µm partial size		ODS_{C18} (250× 4.6 Id) mm/5µm partical size	
Flow rate	0.7 ml / min		0.8 ml / min	
Injection Volume	20 µl		20 µl	
Concentration of sample	50 mg / 1 ml		50 mg / 1 ml	
Detection wavelength	UV-Vis at λ 338nm		UV-Vis at λ 254nm	
Column Temperature	Room Temperature		Room Temperature	
Standards used	Flavonoid	Injection concentration (ppm)	Phenolic acid	Injection concentration (ppm)
	Rutin	5	Ascorbic acid	2
	Quercetin	2.5	Gallic acid	5
	Myricetin	5	Vanillic acid	5
	Catechin	1	Chlorogenic acid	1
	Kaempferol	2.5	p-Coumaric acid	5
	Hesperetin	5	Caffeic acid	1
	Isorhamnetin	5	Ferulic acid	2
	Apigenin	5		
	Coumarin	2.5		
	Luteolin	2.5		

Results and Discussion

The phytochemical screening of plant Ethanolic extract from different parts of Malus domestica

Phytochemical screening of (leaves, stems, and petioles extracts) of Iraqi *Malus domestica* showed the presence of varied degrees of saponins, tannins, alkaloids, terpenoids , flavonoids, polyphenols and reducing sugars. These tests are low-cost and quick processes. Results are summarized in Table (3), A preliminary screening of phytochemicals is crucial for identifying secondary metabolites with medicinal and physiological properties, such as anticarcinogenic, antidiabetic, antioxidant and antimicrobial activities[25]. Similar results were obtained in a study by Pandey *et al*., who used leaves, stem bark, pulp and peels of apples [26].

Table 3. Preliminary phytochemical screening of Iraqi Malus *domestica* leaves, stems and petioles

phytochemicals	**Observation**	**Leaves**	**stems**	**petioles**
Saponins	Formation of bubbles	+ +	+	+
Tannins	White precipitate	+ +	+	+
Polyphenols	Deep blue or deep green	+++	++	+
Flavonoids	Bright yellow color	+++	++	++
Alkaloids	orange brown precipitate	++	+	–
reducing sugars	Reddish brown precipitate	+++	+++	+
*The symbols + = less present / ++= adequately present /+++: abundantly present / – = absent				

The yield of ethanolic extract

The extraction yield measures the efficiency of the solvent in extracting specific components from the original material. It is calculated as the ratio of the extracted mass to the dry weight of the plant part [27]. The yield residue of aerial parts (leaves, stems, and petioles extracts) of Iraqi *Malus domestica* for the two extraction techniques is shown in Table (4). According to the results, the maceration method was higher in yield for all fractions than the Soxhlet method. The efficiency of bioactive component extraction depends on various factors, including the chemical nature of the components, extraction method, plant sample size and weight, solvent type, pH, extraction time, and temperature [28]. In the present study, the extraction yield was greatly affected by the extraction method.

Similar behaviour was observed in *Osteospermum ecklonis* Extraction [29] by Petroleum ether and chloroform. The cold maceration process is simple and requires no complex equipment but still produces high-yield extracts[30].

Table 4. The yield of Ethanolic extracts of aerial parts of Iraqi *Malus domestica* by two different Methods

Plant part	Weight of plant powder (Raw material)	Yield	
		Maceration / Ethanol 70%	Soxhlet /Ethanol 70%
Leaves	50 g	19.842 g	16.675 g
stem	50 g	10.495 g	8.512 g
petiole	50 g	14.086 g	10.264 g

Quantitative estimation of total phenolic content (TPC)and total flavonoids content (TFC) in different part of the plant extracted by two techniques

Total phenolic content in the ethanolic extract by two extracted techniques (Maceration and Soxhlet) for different parts of Iraqi *Malus domestica* plant(leaves, stems, and petioles) was calculated according to the straight line equation obtained from the Gallic acid standard curve, Figure (2).

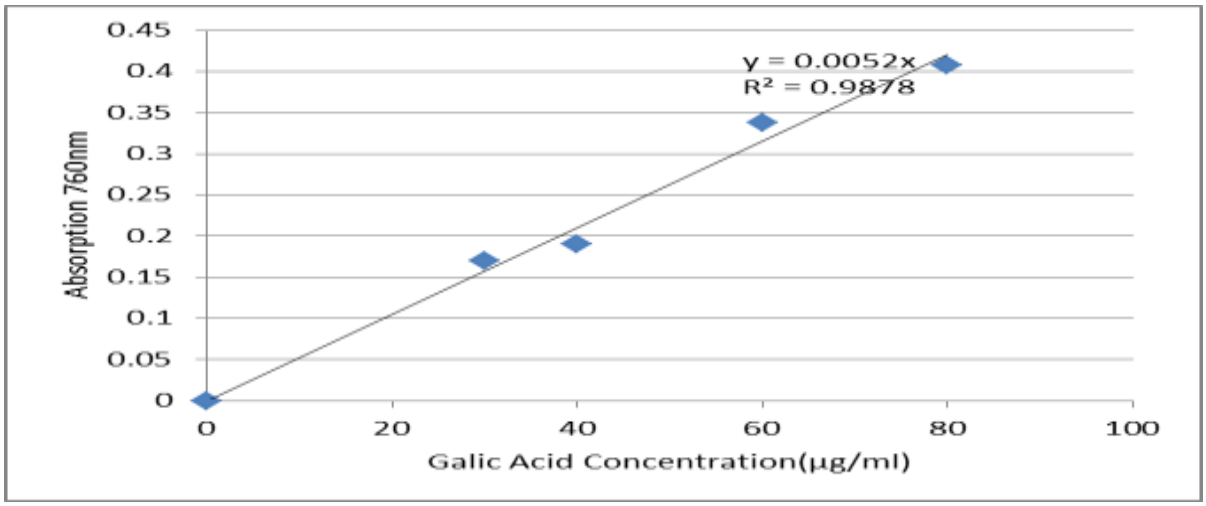

Figure 2. Calibration curve of gallic acid for determination of total phenolic compounds in Ethanolic extracts (leaves, stems, and petiole) maceration and soxhlet methods

While the Total Flavonoid Content (TFC) was calculated according to the straight line equation obtained from the Rutin standard curve, Figure (3).

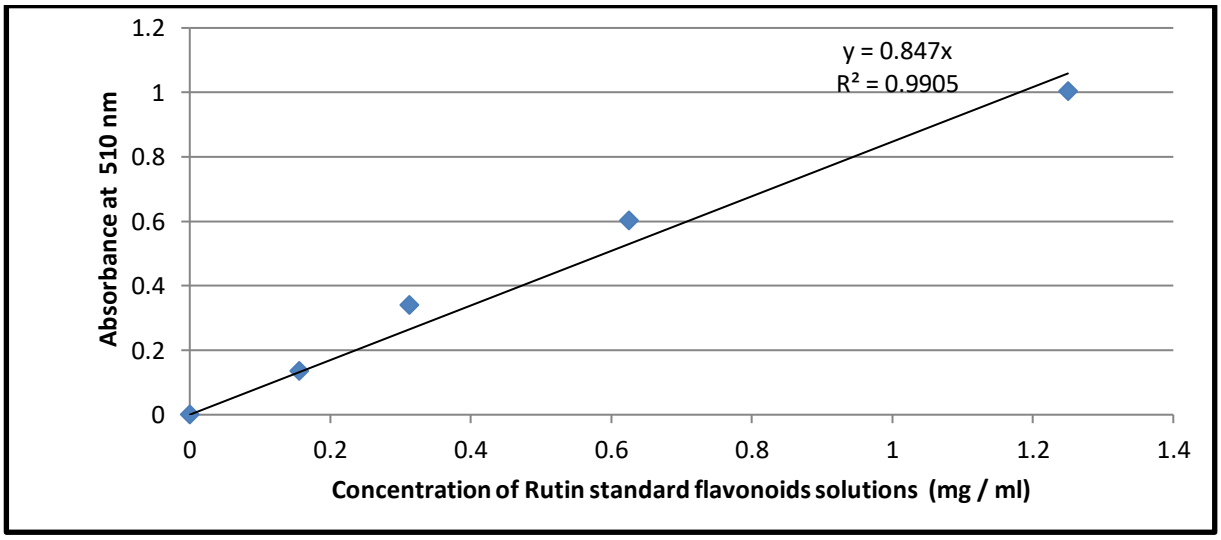

Figure 3. Calibration curve of rutin for determination of total flavonoid content in ethanolic extracts (leaves, stems, and petiole) maceration and soxhlet methods

The equation was applied as [Y= 0.847 X], where Y= the absorbtion reading, and X = the concentration, to get the Total flavonoid concentration(X)in mg/ml in each part of the plant and each extraction method.

The equation was applied as[Y= 0.0052X] where Y= the absorbtion reading, and X = the concentration, to get the Total phenolic concentration(X) in mg/ml in each part of the plant and each extraction method.The total phenolic content in the samples obtained with the two techniques tested was significantly different. The highest phenolic content in leaves extraction by Soxhlet (62 µg /mg plant) and by Maceration (50 µg /mg plant), whereas the lowest phenolic content was found in the stems for both extraction methods by Maceration (40 µg /mg plant) and by Soxhlet (32 µg /mg plant), results are summarized in Table (5). The highest total Flavonoids content was found in leaves in both extraction methods, where leaves were extracted by Maceration (155 µg /mg plant) and by Soxhlet (137 µg /mg plant). In contrast, the lowest Flavonoids content was found in stems extracted by soxhlet (79 µg /mg plant). Results are summarized in Table (5). Through the results, it is clear that there is a direct relationship between the total phenolic content and the content of flavonoids. . It is important to note that extracts with a higher phenolic content do not necessarily have a higher flavonoid content [14]. TFC obtained through Soxhlet extraction is lower than the TFC obtained through maceration. The Soxhlet system has observed that the main problem with this technique is its application to thermolabile compounds, such as phenols, which are susceptible to high-temperature degradation [31], efficiently changes the properties of solvents, affecting the selectivity and extractability toward target components. However, high extraction temperatures may deteriorate some sensitive molecules and cause a decrease in flavonoid content compared to maceration[32].The stem has the lowest phenolic and flavonoid content because it has the lowest extraction yield.

A study by Jitendra P. *et al.* [26] in Nepal found that the stem bark had the highest content of Phenolic and Flavonoids content among different parts (Peel, Leaf and Stem bark) of 6 apple cultivars. Petkovska *et al*. [33] measured the phenolic content in the pulp, peel, and leaves of apples. They found that the leaves contained the highest amount, followed by the peel and then the pulp.

Estimation of antioxidant activity by (DPPH) reagents

Among six different extracts of Iraqi *Malus domestica*, petiole (extracted by soxhlet) showed a vigorous antioxidant activity (86.01 %), whereas the lowest antioxidant activity was in petiole extracted by maceration (75.76%). Plants contain phenolic compounds, which have antioxidant and therapeutic properties, including anti-inflammatory, anti-carcinogenesis, cardiovascular protection, and anti-ageing due to their hydroxyl group scavenging capacity [34]. The antioxidant activity of *Malus domestica* is attributed to phenolic compounds such as flavonoids and phenolic acid [35.36].Flavonoids are the most widespread and diverse group of natural phenolic compounds. Hydroxyl position in the flavonoid molecule determines antioxidant properties, and it depends on the ability to donate electrons or hydrogen to free radicals; each type of Flavonoids differs in its antioxidant potential and concentration in the plant part[20]. However, the comparison results were split into two clusters: maceration extracts had more phenolic compounds. In contrast, Soxhlet ones had higher antioxidant capacity, closely tied to the extraction procedure Because Each extraction procedure successfully brought out the characteristic compounds[34].The results of HPLC show that the petiole contains the highest amount of ferulic acid compared to the leaves and stems. It may be the reason for making the petiole more efficient in scavenging free radicals, as many previous studies have shown it is a powerful antioxidant [37,38]. Maceratoin extracts and their total phenolic content does not necessarily incorporate all the antioxidants that may be present in an extract [39]. The apparatus used for soxhlet extraction combines percolation and immersion techniques [40]. As a result, the extracts obtained are enriched with components of medium and low volatility as well as thermal stability, such as lipids and polycyclic aromatic hydrocarbons, these components may contribute to the antioxidant efficiency of the extracts[41].A study by de Oliveira *et al.* [44] found significant variations in antioxidant capacities among different parts of apples, including the peel, pulp, and seeds.

Analysis the Ethanolic extracts residue by HPLC technique

HPLC technique was used for identifying Phenolic acids and flavonoids in ethanolic extracts of leaves, stems and petioles of Iraqi *Malus domestica*. A total of 7 different phenolic acids and ten flavonoids were identified in apple samples.

The HPLC chromatograms of the standards of Phenolic compounds and extracted Phenolic acids from leaves, stems and petioles respectively are shown in Figures(4,5,6,7) and Table(6,7,8,9), flavonoids Standards and extracted flavonoids from leaves, stems and petioles respectively are shown in Figures (8,9,10,11) and Table(10,11,12,13).An apparent variation was found in the concentrations of phenolic acids in the aerial parts, where p-coumaric acid is the most abundant phenolic in the leaves (0.240 mg /g. Leave), followed by gallic acid(0.178 mg /g. Leave). The petioles recorded slightly lower concentrations **(**0.207 mg /g and 0.123 mg /g, respectively), while the stems recorded the lowest

levels of phenolic acids. In petiole, Ferulic acid was the highest concentration of the rest of the parts, and for the rest of the acids in concentration (0.248 mg /g), Chloragenic acid was detected in leaves only results are summarized in Table (14) As shown in Table (15), the highest concentration of flavonoids detected was in the leaves and the lowest in the stems and petioles. Isorhamnetin (0.104 mg /g), Myricetin (0.101 mg /g) and Apigenin (0.101 mg /g) is predominant in the samples compared to other flavonoids.

This is the first study to report the presence of phenolic compounds in the stems and petioles. A similar study by Liaudanskas *et al*. [20] used raw material from apple leaves to prepare ethanol extracts (70%) from lyophilized leaf powder. The extracts contained quercetin, isoquercitrin, hyperoside, rutin, phloridzin, epicatechin, catechin avicularin, and lesser amounts of caffeic and chlorogenic acid. Similar results from the quantitative analysis of a high amount of phloridzin, lower amounts of quercitrin, isoquercitrin, and hyperoside, and traces of chlorogenic acids and p-hydroxybenzoic were reported by Alina *et al*. [14]. Petkovska *et al*. [33]observed Peel, flesh, and leaves of 21 apple varieties were extracted with water/methanol (10:90 v/v) and analyzed for 27 quantified phenolic compounds, including phenolic acids, dihydrochalcones, procyanidins flavones and flavonols. De Oliveira *et al*. [42] found phenolic compounds in the peel, pulp, and seeds of apples, including Rutin, Catechin, Luteolin, Quercetin, Hydroxycinnamic acid, Chlorogenic acid, and 4-p-coumaroylquinic acid.

Table 5. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH) of Iraqi *Malus domestica* leaves, stems and petioles extracts

Test	Leaves Concentration (µg /mg leaves)		Stems Concentration (µg /mg stems)		petioles Concentration (µg /mg petioles)	
	Maceration	Soxhlet	Maceration	Soxhlet	Maceration	Soxhlet
Total Phenolic Content (TPC)	50	62	40	32	47	37
Total Flavonoid Content (TFC)	155	137	105	79	124	86.2
Antioxidant Activity (DPPH)%	76.92%	80.19%	76.30%	81.43%	75.76%	86.01%

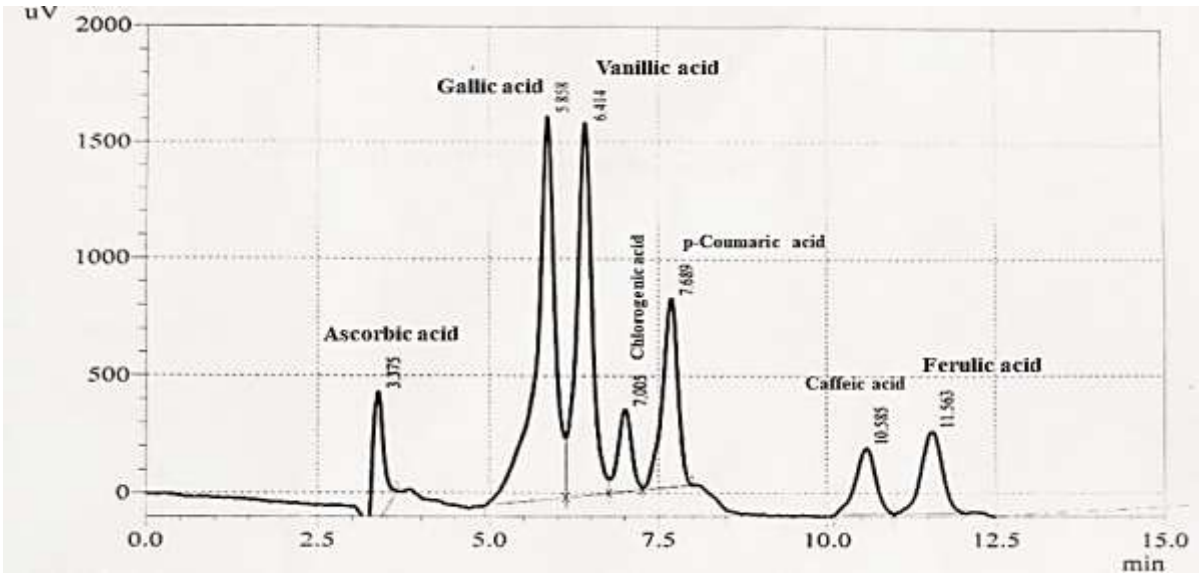

Figure 4. HPLC chromatogram of phenolic acids standards

Table 6. The retention time and Area of Phenolic acids Standards analyzed by HPLC

Peak	Ret. Time	Area	Height	Area %	Height %
Ascorbic acid	3.375	6138	555	6.482	9.932
Gallic acid	5.858	31843	1639	33.628	29.329
Vanillic acid	6.414	25150	1597	26.560	28.572
Chlorogenic acid	7.005	4904	353	5.179	6.312
p-coumaric acid	7.689	12970	808	13.697	14.460
Caffeic acid	10.585	5955	284	6.288	5.085
Ferulic acid	11.563	7732	353	8.165	6.311
Total		94693	5589	100.000	100.1000

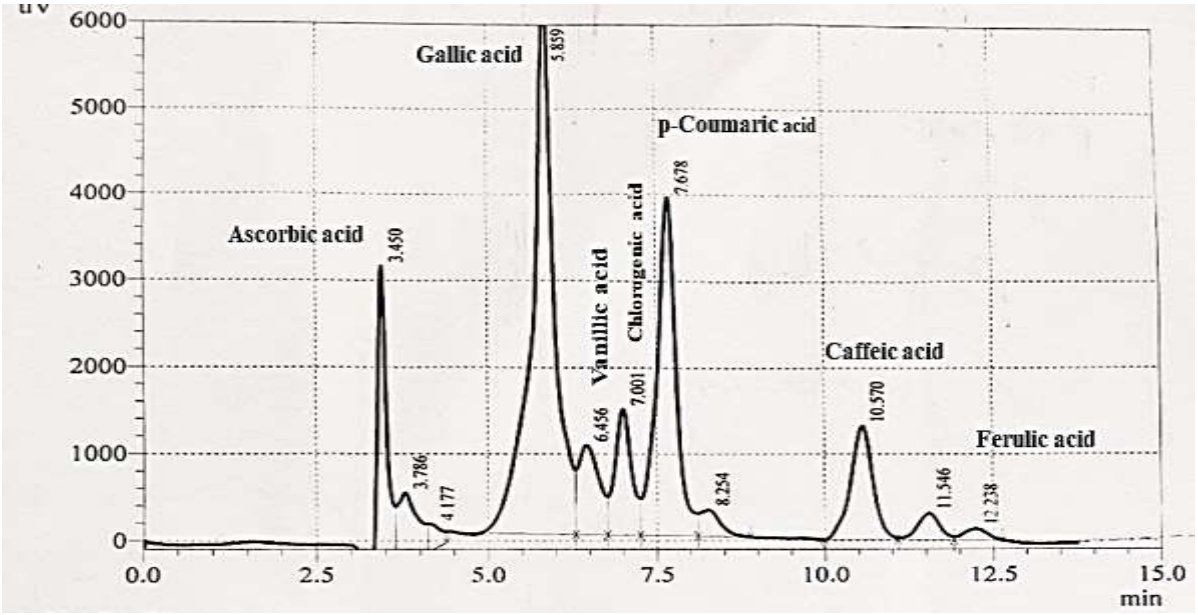

Figure5. HPLC chromatogram of phenolic acids of leaves(Maceration method)

Table7. The retention time and Area of Phenolic acids of leaves(Maceration method) analyzed by HPLC

Peak	Ret. Time	Area	Height	Area %	Height %
Ascorbic acid	3.450	34551	3598	9.307	18.509
Unknown	3.786	17148	830	4.619	4.271
Unknown	4.177	3722	302	1.003	1.553
Gallic acid	5.859	142652	6253	38.427	32.170
Vanillic acid	6.456	22407	1033	6.036	5.314
Chlorogenic acid	7.001	25270	1452	6.807	7.472
p-coumaric acid	7.678	78478	3908	21.140	20.103
Unknown	8.254	6371	307	1.716	1.578
Caffeic acid	10.570	30340	1315	8.173	6.765
Ferulic acid	11.546	7109	308	1.915	1.585
Unknown	12.238	3178	132	0.856	0.681
Total		371226	19438	100.000	100.1000

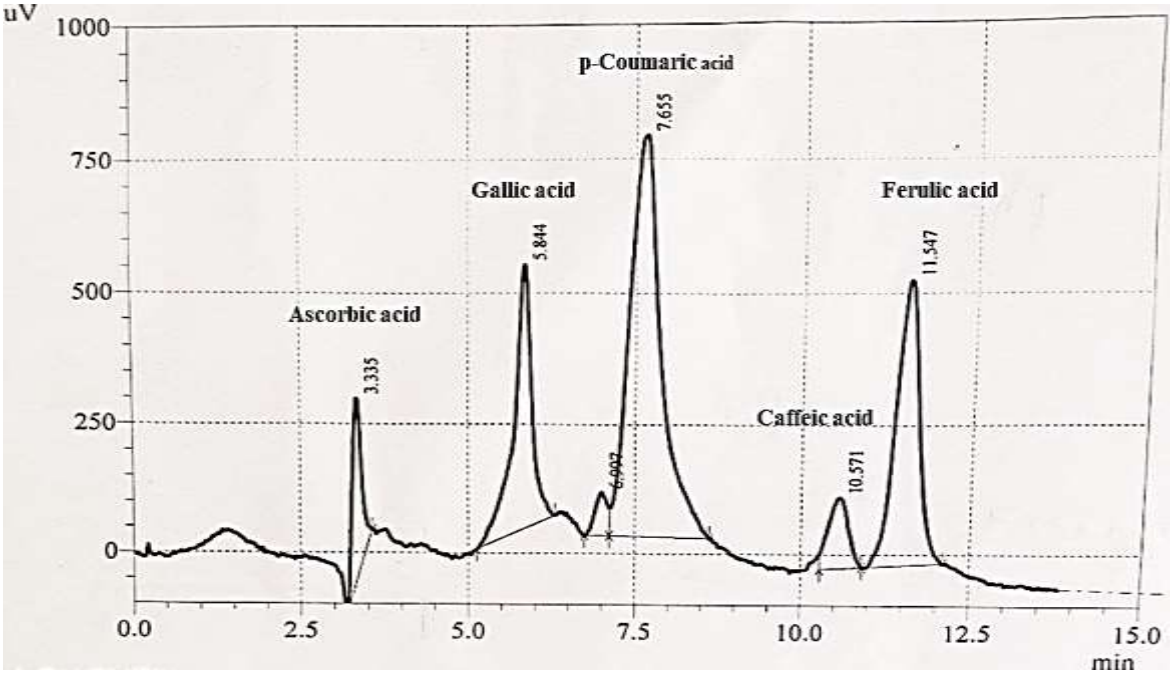

Figure 6. HPLC chromatogram of Phenolic acids of stems (Maceration method)

Table8. The retention time and Area of Phenolic acids of stems(Maceration method) analyzed by HPLC

Peak	Ret. Time	Area	Height	Area %	Height %
Ascorbic acid	3.335	3514	356	6.360	14.893
Gallic acid	5.844	9523	506	17.235	21.164
Unknown	6.997	1079	84	1.952	3.528
p-coumaric acid	7.655	25238	761	45.676	31.830
Caffeic acid	10.571	2864	138	5.183	5.776
Ferulic acid	11.547	13037	545	23.594	22.809
Total		55255	2390	100.000	100.1000

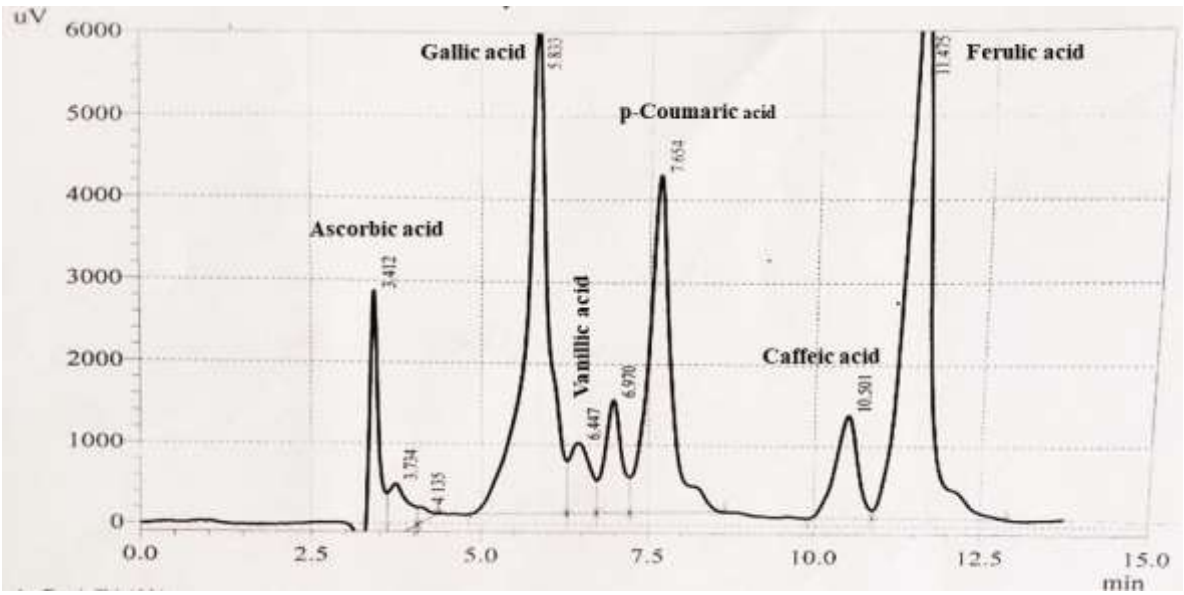

Figure 7. HPLC chromatogram of Phenolic acids of petioles (Maceration method)

Table 9. The retention time and Area of Phenolic acids of petioles (Maceration method) analyzed by HPLC

Peak	Ret. Time	Area	Height	Area %	Height %
Ascorbic acid	3.412	29855	3190	5.714	13.043
Unknown	3.734	12573	660	2.406	2.699
Unknown	4.135	1982	183	0.379	0.746
Gallic acid	5.833	139094	6067	26.621	24.803
Vanillic acid	6.447	18681	884	3.575	3.614
Unknown	6.970	24985	1405	4.782	5.744
p-coumaric acid	7.654	95481	4139	18.274	16.920
Caffeic acid	10.501	29820	1282	5.707	5.241
Ferulic acid	11.475	170036	6651	32.542	27.190
Total		522507	24461	100.000	100.1000

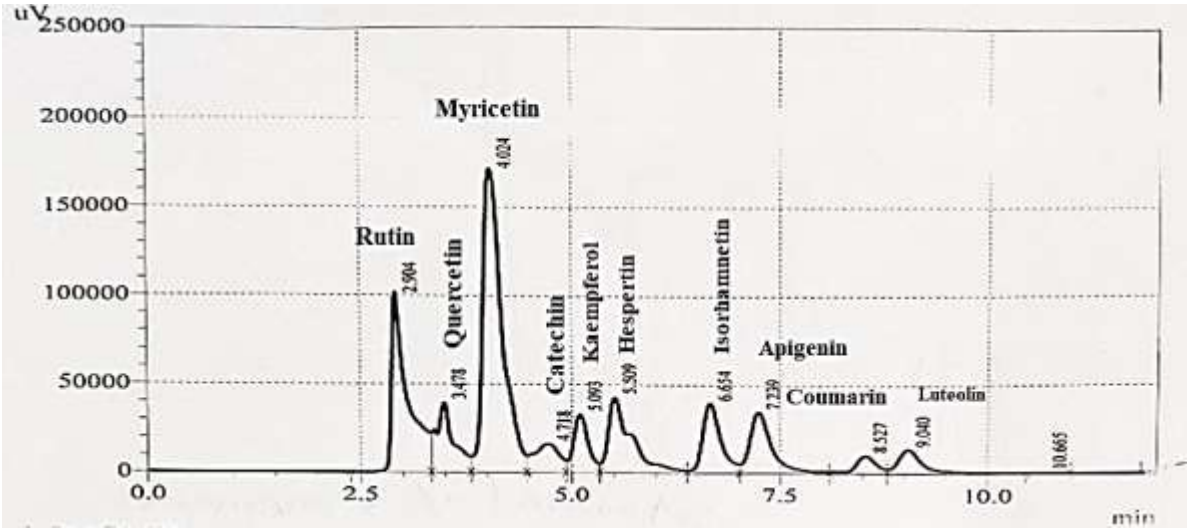

Figure 8. HPLC chromatogram of flavonoids standards

Table10. The retention time and Area of flavonoids Standards analyzed by HPLC

Standard	Ret. Time	Area	Height	Area %	Height %
Rutin	2.904	1355260	101779	17.038	20.222
Quercetin	3.478	564329	39536	7.095	7.855
Myricetin	4.024	2748297	171901	34.551	34.155
Catechin	4.718	338059	16056	4.250	3.190
Kaempferol	5.093	409177	33077	5.144	6.572
Hesperetin	5.509	820419	42778	10.314	8.499
Isorhamnetin	6.654	605300	39685	7.610	7.885
Apigenin	7.239	639678	34854	8.042	6.925
Coumarin	8.527	177051	10008	2.226	1.989
Luteolin	9.040	295227	13524	3.711	2.687
Total		7954413	503303	100.000	100.1000

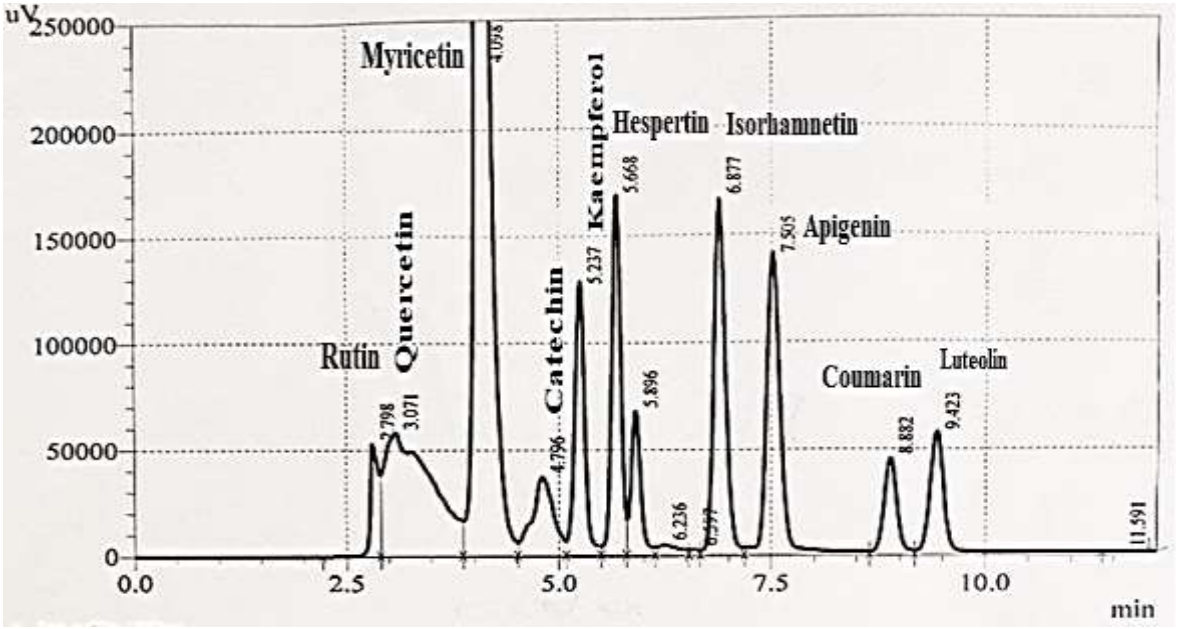

Figure 9. HPLC chromatogram of flavonoids Leaves (Maceration method)

Table 11. The retention time and Area of flavonoids of leaves(Maceration method) analyzed by HPLC

Peak	Ret. Time	Area	Height	Area %	Height %
Rutin	2.798	377766	52879	2.106	3.367
Quercetin	3.071	2249943	58093	12.545	3.699
Myricetin	4.098	7057974	640354	39.354	40.777
Catechin	4.796	657628	36868	3.667	2.348
Kaempferol	5.237	1054059	128672	5.877	8.194
Hesperetin	5.668	1270053	168250	7.082	10.714
Unknown	5.896	531675	67387	2.964	4.291
Unknown	6.236	91589	5079	0.511	0.323
Unknown	6.597	20581	2603	0.115	0.166
Isorhamnetin	6.877	1588671	165738	8.858	10.554
Apigenin	7.505	1643715	140494	9.165	8.947
Coumarin	8.882	483895	44618	2.698	2.841
Luteolin	9.423	861172	57025	4.802	3.631
Unknown	11.591	1025	62	0.006	0.004
Unknown	12.860	29428	1551	0.164	0.099
Unknown	13.990	3859	233	0.022	0.015
Unknown	14.987	1327	71	0.007	0.005
Unknown	15.544	1103	46	0.006	0.003
Unknown	17.062	2988	154	0.017	0.010
Unknown	24.232	3864	117	0.022	0.007
Unknown	24.981	2420	70	0.013	0.004
Total		17934735	1570362	100.000	100.1000

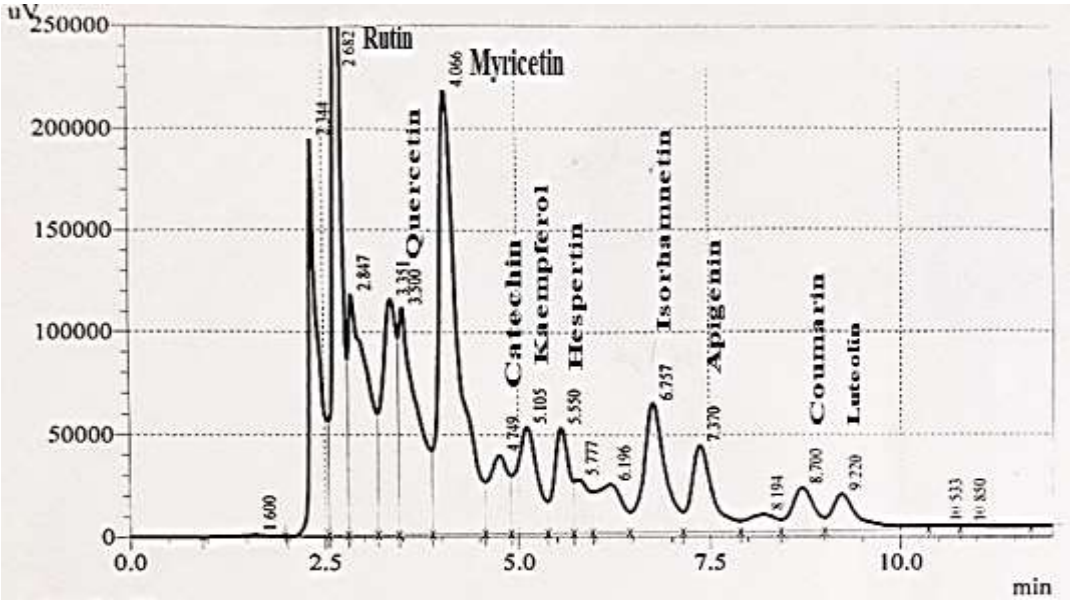

Figure 10. HPLC chromatogram of flavonoids stems (Maceration method)

Table 12. The retention time and Area of flavonoids of stems(Maceration method) analyzed by HPLC

Peak	Ret. Time	Area	Height	Area %	Height %
Unknown	1.600	21418	793	0.104	0.051
Unknown	2.344	1618590	193922	7.855	12.471
Unknown	2.682	3103247	446574	15.061	28.718
Rutin	2.847	2000189	117653	9.707	7.566
Unknown	3.351	1509833	115134	7.328	7.404
Quercetin	3.500	18011355	110682	8.742	7.118
Myricetin	4.066	3792864	217226	18.408	13.969
Catechin	4.749	651177	38626	3.160	2.484
Kaempferol	5.105	992095	52399	4.815	3.370
Hesperetin	5.550	706816	51575	3.430	3.317
Unknown	5.777	354499	26351	1.720	1.695
Unknown	6.196	559082	23827	2.713	1.532
Isorhamnetin	6.757	1258209	63581	6.106	4.089
Apigenin	7.370	836961	42609	4.062	2.740
Unknown	8.194	226035	8363	1.097	0.538
Coumarin	8.700	439048	21159	2.131	1.361
Luteolin	9.220	490308	17840	2.380	1.147
Unknown	10.533	47222	1931	0.229	0.124
Unknown	10.850	83380	1763	0.405	0.113
Unknown	12.079	41156	1294	0.200	0.083
Unknown	12.522	57802	1278	0.281	0.082
Unknown	13.567	12377	384	0.060	0.025
Unknown	14.183	1012	72	0.005	0.005
Total		20604677	1555035	100.000	100.1000

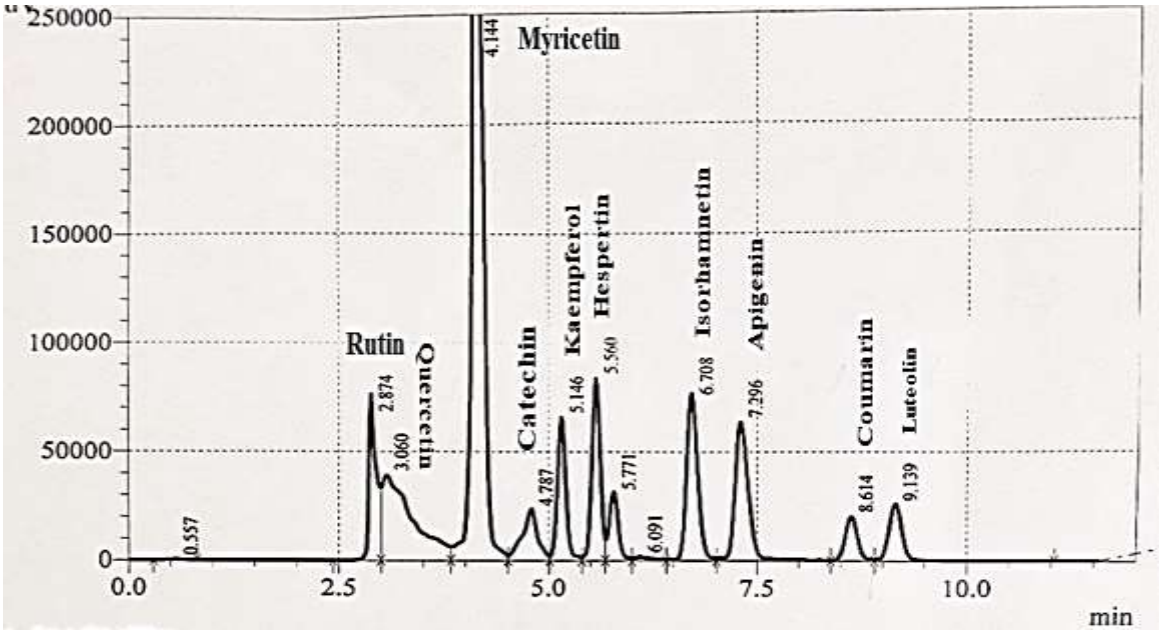

Figure 11. HPLC chromatogram of flavonoids petioles(Maceration method)

Table 13. The retention time and Area of flavonoids of petioles (Maceration method) analyzed by HPLC

Peak	Ret. Time	Area	Height	Area %	Height %
Unknown	0.557	3586	240	0.043	0.027
Rutin	2.874	488425	76411	5.839	8.500
Quercetin	3.060	978543	38984	11.699	4.336
Myricetin	4.144	3316325	390010	39.648	43.383
Catechin	4.787	302620	23405	3.618	2.604
Kaempferol	5.146	472611	65719	5.650	7.310
Hesperetin	5.560	598633	83951	7.157	7.157
Unknown	5.771	237692	31376	2.842	3.490
Unknown	6.091	31227	1879	0.373	0.209
Isorhamnetin	6.708	719320	77140	8.600	8.581
Apigenin	7.296	716834	63776	8.570	7.094
Coumarin	8.614	206171	20076	2.465	2.233
Luteolin	9.139	292494	26020	3.497	2.894
Total		8364481	898987	100.000	100.1000

Table14. The retention time of extracted Phenolic acids from (leaves, stems,petioles), corresponding standards and Concentration(mg/g) analyzed by HPLC

Phenolic acids	Concentration mg /g. Leaves	Concentration mg /g. Stems	Concentration mg /g. Petioles
Ascorbic acid	0.089	0.005	0.054
Gallic acid	0.178	0.006	0.123
Vanillic acid	0.035	-	0.021
Chlorogenic acid	0.040	-	-
p-coumaric acid	0.240	0.041	0.207
Caffeic acid	0.040	0.002	0.028
Ferulic acid	0.015	0.014	0.248

Table15. The retention time of extracted Flavonoids from (leaves, stems,petioles), corresponding standards and Concentration(mg/g) analyzed by HPLC.

Flavonoids	Concentration mg /g. Leaves	Concentration mg /g. Stems	Concentration mg /g. Petioles
Rutin	0.011	0.031	0.010
Quercetin	0.079	0.033	0.024
Myricetin	0.101	0.028	0.033
Catechin	0.015	0.008	0.005
Kaempferol	0.051	0.025	0.016
Hesperetin	0.061	0.018	0.020
Isorhamnetin	0.104	0.043	0.033
Apigenin	0.101	0.027	0.031
Coumarin	0.054	0.025	0.016
Luteolin	0.058	0.017	0.014

Conclusion

The present study showed that ethanolic extract of leaves, stems, and petioles of Iraqi *M. domestica* exhibited potent antioxidant activity due to phenolic acids and flavonoids. The study provides new evidence about the presence and composition of high phenolic acids and flavonoids content in leaves and petioles relative to stems of *M. domestica* of Iraqi origin. Moreover, the content of phenolic and flavonoid contents may vary among the different plant parts and be influenced by the extraction technique. The results in this study prompt further research on the phytochemical composition and biological activity of apple aerial parts by evaluating the antioxidant activity of individual polyphenolic compounds in vitro and in vivo and confirming the potential of apple trees as a raw material in medical practice as well as the production and development of cosmetic preparations and dietary health supplements rich in biologically active contents.

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Conflicts of Interest

There is no conflict of interest regarding the publication of this manuscript.

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Author Contribution

All authors confirm contribution to the paper as follows: study conception: Rihab Jumaah.; and the study design was by Zainab Yaseen and Yasir Hussein; data collection, analysis and interpretation of results by ; Rihab Jumaah and Zainab Yaseen. All authors reviewed the results and approved the final version of the manuscript.

References

1. Spengler, R.N. Origins of the apple: The role of megafaunal mutualism in the domestication of Malus and rosaceous trees. Front. Plant Sci. 2019, 10, 617.
2. Boyer, J.; Liu, R.H. Apple phytochemicals and their health benefits. Nutr. J. 2004, 3, 5–20.
3. Eberhardt, M.; Lee, C.; Liu, R.H. Antioxidant activity of fresh apples. Nature 2000, 405, 903–904.
4. D. R. Lauren, W. A. Smith, A. Adaim et al., Chemical composition andin vitroanti-inflammatory activity of apple phenolic extracts and of their sub-fractions. International Journal of Food Sciences and Nutrition, 2009,vol. 60, no. 7, pp. 188–205.
5. Can, Z.; Dincer, B.; Sahin, H.; Baltas, N.; Yildiz, O.; Kolayli, S. Polyphenol oxidase activity and antioxidant properties of Yomra apple (Malus communis L.) from Turkey. J. Enzyme. Inhib. Med. Chem. 2014, 29, 829–835.
6. Schiavano, G.F.; De Santi, M.; Brandi, G.; Fanelli, M.; Bucchini, A.; Giamperi, L.; Giomaro, G. Inhibition of breast cancer cell proliferation and in vitro tumorigenesis by a new red apple cultivar. PLoS ONE 2015, 10, e0135840.
7. H. J. Heo, D. O. Kim, S. J. Choi, D. H. Shin, and C. Y. Lee. Apple phenolics protect in vitro oxidative stress-induced neuronal cell death. Journal of Food Sciences. 2004,vol. 69, no. 9, pp. 357–360 .
8. Y. Hamauzu, M. Irie, M. Kondo, and T. Fujita, "Antiulcerative properties of crude polyphenols and juice of apple, and Chinese quince extracts," Food Chemistry, 2008,vol. 108, no. 2, pp. 488–495.
9. D. Lin, M. Xiao, J. Zhao et al.,An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules, 2016,vol. 21, no. 10, p. 1374.

10. Bevilacqua, M.; Bro, R.; Marini, F.; Rinnan, Å.; Rasmussen, M.A.; Skov, T. Recent chemometrics advances for foodomics. TrAC Trends Anal. Chem. 2017, 96, 42–51.

11. Kschonsek, J.; Wolfram, T.; Stöckl, A.; Böhm, V. Polyphenolic compounds analysis of old and new apple cultivars and contribution of polyphenolic profile to the in vitro antioxidant capacity. Antioxidants 2018, 7, 20.

12. Wu, J.; Gao, H.; Zhao, L.; Liao, X.; Chen, F.; Wang, Z.; Hu, X. Chemical compositional characterization of some apple cultivars. Food Chem. 2007, 103, 88–93.

13. Feliciano, R.P.; Antunes, C.; Ramos, A.; Serra, A.T.; Figueira, M.E.; Duarte, C.M.M.; de Carvalho, A.; Bronze, M.R. Characterization of traditional and exotic apple varieties from Portugal. Part 1—Nutritional, phytochemical and sensory evaluation. J. Funct. Foods .2010, 2, 35–45.

14. Alina Sowa, Grażyna Zgórka, Aleksandra Szykuła, Roman Franiczek, Beata Żbikowska, Andrzej Gamian, Zbigniew Sroka,. Analysis of Polyphenolic Compounds in Extracts from Leaves of Some *Malus domestica* Cultivars: Antiradical and Antimicrobial Analysis of These Extracts. *BioMed Research International*. 2016, , 12 pages.

15. Q Fardiyah, Suprapto, F Kurniawan, T Ersam4, A Slamet, Suyanta . Preliminary Phytochemical Screening and Fluorescence Characterization of Several Medicinal Plants Extract from East Java Indonesia. The 2nd International Conference on Chemistry and Material Science (IC2MS) Materials Science and Engineering.2020, 833.

16. Morsy N. Phytochemical analysis of biologically active constituent of medicinal plants. Main Group Chemistry 2014; 13: 7-21.

17. Kokate C.K., Gokhale S.B. and Purohit A.P.: A text book of pharmacognasy 29th Ed, Nirali Prakashan 2009; Pp 635.

18. Bargah R.K.: Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertu. Journal of Pharmacognosy and phytochemistry. 2015; 4(1): 7-9.

19. Niran A.A. Phytochemical Investigation, Molecular Docking and Cytotoxic Activity of Some Active Compounds of Iraqi Achillea santolina L. (Asteraceae).thesis, University of Mustansiriya ,College of Pharmacy.2022,Pp.240.

20. M. Liaudanskas, P. Viˇskelis, R. Raudonis, D. Kviklys, N. Uselis, and V. Janulis, .Phenolic composition and antioxidant activity of Malus domestica Leaves. Fe Scientific World Journal, vol. 2014, pp. 1–11.

21. Vieira SF, Ferreira H, Neves NM. Antioxidant and Anti-Inflammatory Activities of Cytocompatible Salvia officinalis Extracts: A Comparison between Traditional and Soxhlet Extraction. Antioxidants (Basel). 2020;9(11):1157.

22. Beertoncelj J. , Doberˇsek U. , Jamnik M. , and Golob T., Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chemistry.2007,vol. 105, no. 2, pp. 822–828.

23. Verzelloni E. , Tagliazucchi D. , and Conte A., .Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsamic vinegar. Food Chemistry. 2007, vol. 105, no. 2, pp. 564–571.

24. Brand-Williams W. , Cuvelier M. E. , and Berset C. , Use of a free radical method to evaluate antioxidant activity. LWT—Food Science and Technology. 1995, vol. 28, no. 1, pp. 25–30.

25. N. Savithramma, M. L. Rao, and D. Suhrulatha, .Screening of medicinal plants for secondary metabolites. Middle East Journal of Scientific Research. 2011, vol. 8, no. 3, pp. 579–584.

26. Pandey, J., Bastola, T., Tripathi, J., Tripathi, M., Rokaya, R. K., Dhakal, B., ... & Poudel, A. Estimation of total quercetin and rutin content in Malus domestica of Nepalese origin by HPLC method and determination of their antioxidative activity. Journal of Food Quality. 2020, 1-13.

27. Aspe, Estrella & Fernández, Katherina. The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from Pinus radiata Bark. Industrial Crops and Products - IND CROPS PRODUCTS. (2011). , 34. 838-844.

28. Negrean OR, Farcas AC, Pop OL, Socaci SA. Blackthorn-A Valuable Source of Phenolic Antioxidants with Potential Health Benefits. Molecules. 2023 Apr 14;28(8):3456.

29. Hind Mohammed Jewely and Thukaa Zuhair.Evaluation of Antileishmanial Activity of Osteospermum ecklonis Extract of Aerial Parts against Leishmania donovani: in vitro. Iraqi J Pharm Sci., 2022, Vol.31(Suppl.)

30. Sankeshwari, Roopali M; Ankola, Anil V; Bhat, Kishore; Hullatti, Kirankumar. Soxhlet versus Cold Maceration: Which Method Gives Better Antimicrobial Activity to Licorice Extract Against Streptococcus Mutans?. Journal of the Scientific Society . May–Aug 2018,45(2):p 67-71,.

31. Spigno, G., Trarnelli, L., De Faveri, D.M., Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J. Food Eng. , 2007, 81, 200–208.

32. Jallali, I., Megdiche, W., M'Hamdi, B. et al. Changes in phenolic composition and antioxidant activities of the edible halophyte Crithmum maritimum L. with physiological stage and extraction method. Acta Physiol Plant.2012, 34, 1451–1459.

33. Petkovska A, Gjamovski V, Stanoeva JP, Stefova M. Characterization of the Polyphenolic Profiles of Peel, Flesh and Leaves of Malus domestica Cultivars Using UHPLC-DAD-HESI-MS. Nat Prod Commun. 2017 Jan;12(1):35-42.

34. D. Lin, M. Xiao, J. Zhao et al., An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules.2016, vol. 21, no. 10, p. 1374.

35. M. V. Eberhardt, C. Y. Lee, and R. H. Liu,.Antioxidant activity of fresh apples, Nature.2000, vol. 405, no. 6789, pp. 903-904.

36. K. W. Lee, Y. J. Kim, D.-O. Kim, H. J. Lee, and C. Y. Lee, .Major phenolics in apple and their contribution to the total antioxidant capacity. Journal of Agricultural and Food Chemistry.2003,vol. 51, no. 22, pp. 6516–6520.

37. JANUS, Ewa, et al. New Ferulic Acid and Amino Acid Derivatives with Increased Cosmeceutical and Pharmaceutical Potential. Pharmaceutics. 2022, 15.1: 117.

38. Rezaei A, Varshosaz J, Fesharaki M, Farhang A, Jafari SM. Improving the solubility and in vitro cytotoxicity (anticancer activity) of ferulic acid by loading it into cyclodextrin nanosponges. Int J Nanomedicine. 2019 Jun 24;14:4589-4599.

39. Tawaha K, Alal FQ, Gharaibeh M, Mohammad M, El-Elimat T . Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem.2007, 104:1372–1378.

40. Suhaj M., Spice antioxidants isolation and their antiradical activity: a review. J Food Compos Anal.2006,19:531–537

41. Romanik G, Gilgenast E, Przyjazny A, Kamin´ski M . Techniques of preparing plant material for chromatographic separation and analysis. J Biochem Biophys Methods.2007, 70: 253–261

42. De Oliveira Raphaelli, C., Azevedo, J. G., dos Santos Pereira, E., Vinholes, J. R., Camargo, T. M., Hoffmann, J. F., ... & Nora, L.,Phenolic-rich apple extracts have photoprotective and anti-cancer effect in dermal cells. Phytomedicine plus.2021, 1(4), 100-112.

المحتوى الفينولي والفلافونويدي الكلي و الفعالية المضادة للأكسدة لأجزاء مختلفة من التفاح *Malus domestica* L. في العراق

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الخلاصة

صممت الدراسة الحالية للتقدير النوعي والكمي للمركبات الفينولية والفلافونويدية المستخلصة بتقنيتين مختلفتين (النقع والسكسوليت Soxhlet) لثلاثة أجزاء هوائية (الأوراق والسيقان والسويقات) من نبات التفاح *Malus domestica* المزروع بشكل طبيعي في العراق، تم تحديد إجمالي محتوى الفينول (TPC) ومحتوى الفلافونويد الكلي (TFC) من خلال الطرق اللونية وطريقة .HPLCتم إجراء اختبار كسح الجذور الحرة لـ (DPPH) لقياس نشاط مضادات الأكسدة لجميع الأجزاء المستخلصة. و أظهر الفحص الكيميائي النباتي للأجزاء الثلاثة من التفاح *Malus domestica* وجود مكونات نشطة مختلفة مثل (الصابونين والعفص والقلويدات والتربينات والفلافونات و الفينولات المتعددة والكلايكوسيدات. سجلت تقنية الاستخلاص بالنقع إنتاجية أعلى yield (مردود residue (لجميع الأجزاء مقارنة بتقنية Soxhlet. أظهرت النتائج أيضًا تباينًا في المركبات الفينولية بين الأجزاء المختلفة ، حيث أظهرت أن أوراق *Malus domestica* تحتوي على نسبة عالية من الأحماض الفينولية والفلافونات مقارنة بالأجزاء الأخرى ، بينما أظهرت السويقات (المستخرجة بواسطة soxhlet) أقوى نشاط مضاد للأكسدة (٨٦,٠١٪) مقارنة بالأجزاء الأخرى. بناءً على اختبار HPLC ، تم الكشف عن احتواء المستخلصات التي تم الحصول عليها من الأوراق والسيقان والسويقات على ٧ أحماض فينولية (تشمل حمض الأسكوربيك ، حمض الغاليك ، حمض الفانيليك ، حمض الكلوروجينيك ، حمض الكوماريك ، حمض الكافيك وحمض الفيريوليك) و ايضاً تم تحديد ١٠ مركبات فلافونويد (تشمل روتين ، كيرسيتين ، ميريستين ، كاتشين ، كايمبفيرول ، هسبارتين ، إيزورهامنتين ، أبجينين ، كومارين ولوتولين).

الكلمات المفتاحية: الفاعلية المضادة للأكسدة ، الفلافونات، التفاح العراقي ، المركبات الفينولية .