Identification of phenolic compounds in leaves of some olive cultivars

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ABSTRACT

Leaves of olive tree (*Olea europaea* L.) cultivars were collected from Siwa Oasis namely: Maraqi (MS), Wattigen(WS) and Hamid (HS). Leaves of the same cultivars were also collected from the orchard of the Faculty of Agriculture, Saba Basha, University of Alexandria and were assigned as (MM), (WM) and (HM), respectively. All leaves were collected from sixteen years old trees in the middle of December 2009. The fresh olive leaves were washed with distilled water and dried at **37 °C** for 3 days. The dried leaves were pulverized in a blender in order to decrease the particle size of the leaves to 90 – 150 µm. Data revealed that cultivars of the faculty of Agriculture had moisture content higher than that of Siwa Oasis. The total solid contents were vice versa. Olive leaves contained high amounts of carbohydrate and crude fiber, while it contained trace amount of crude protein and crude fat. The extracted yield % was determined by different solvents. Methanol extraction gave the highest yield, meanwhile, hexane gave the lowest yield as compared the other solvents.

The total phenolic content of all cultivars were determined using folin-ciocalteau. The solvent mixture (ethyl acetate: methanol: water) gave the highest total phenolic contents as compared to the other solvents for the same cultivar. The HS cultivar had the highest total polyphenols content (TPP) followed by the WS cultivar, while, the HM had the lowest polyphenol content. On the other hand, the MS cultivar had the highest total flavonoids content (TFD) while the HM cultivar had the lowest total flavonoids content (TFD) as compared to the other cultivars; the same trend was occured for the total flavanols content (TFL). The MS cultivar had the highest total flavanols content and the HM had the lowest total flavanols content. The phenolic compounds of olive leaf powders were extracted with 70% ethanol and analyzed using High Performance Liquid Chromatography (HPLC). Hydroxytyrosol, tyrosol, catechin, caffiec acid, vanillic acid, vanillin, rutin, luteolin-7-glucoside, verbascoside, apigenin-7-glucoside, diosmetin-7-glucoside, oleuropein, luteolin have been identified.

Key words: phenolic compounds, olive leaves, Siwa Oasis.

INTRODUCTION

Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet. The food and agricultural products generate substantial quantities of phenolics-rich by-products, which could be valuable as natural sources of antioxidants. Some of these by-products have been the subject of investigations and have proven to be effective

sources of phenolic antioxidants (Balasundram *et al.*, 2006). For many centuries, olive leaves have been associated with health and preservation. Ancient Egyptians used them to mummify their pharaohs (Solar-Rivas *et al.*, 2000). Later, olive leaves were used as a folk remedy to fight various diseases.

Olive oil industry generates large amounts of by-products such as crude olive cake, vegetation water, twigs and leaves (10% of the total olives weight) (Guinda *et al.*, 2004). Olive leaves were always used only as animal feed (Molina *et al.*, 1996; Martin *et al.*, 2003) but they can be used for others applications such as cosmetic, therapeutic and food industries. Recently, olive leaves extract has been investigated as an additive supplemented to food products, such as oils (Bouaziz *et al.*, 2008) and meats (Hayes *et al.*, 2010) to extend shelf life and impart the image of wholesomeness to consumers.

There is an increasing interest in the phenolic compounds in olive by-products due to their biological properties. Olive oil polyphenols possess good antioxidant activities (Gordon *et al.*, 2001 and Paiva-Martins *et al.*, 2003). Also, olive leaves are a source of several antioxidants (Benavente-Garcia *et al.*, 2000; Savournin *et al.*, 2001, Briante *et al.*, 2002a and b; Bouaziz and Sayadi, 2005; Meirinhos *et al.*, 2005; Ranalli *et al.*, 2006. Phenolic compounds are found in all parts of the plant but their nature and concentration varies greatly between the various tissues.

In *O.europaea L.* oleuropein, demethyloleuropein, ligstroside and oleoside represent the predominant phenolic oleosides (Solar-Rivas *et al.*, 2000), whereas verbascoside (Ryan *et al.*, 1999) is the main hydroxycinnamic derivative of olive fruit (Servili *et al.*, 1999).

Oleuropein is generally the most prominent phenolic compound in olive cultivars and may reach concentrations of up to 140 mg g^{-1} on a dry matter basis. Oleuropein occurs not only in the Olea genus but also in many other genera belonging to the Oleaceae family. Oleuropien, the main constituent of olive leaf extract is a complex phenol present in large quantities in olive tree leaves and in low quantities in olive oil (Angerosa *et al.*, 1999).

The polyphenol content in the olive leaf is still relatively unknown, but it could range from 1.5 to 7.0 g per 100 g in fresh leaves (Niaounakis and Halvadakis, 2004). However, it is known that olive leaf polyphenol composition is similar to that of olive oil.

The bitter compound oleuropein, the major constituent of the secoiridoid family in the olive trees, has been shown to be a potent antioxidant

endowed with anti-inflammatory properties (Le Tutour and Guedon, 1992.; Benavente-Garcia *et al.*, 2000; Savoumin *et al.*; 2001).

This phenolic profile can drastically change according to the variety, the geographical location, and the agro- ecological conditions, especially seasons. Fabbri *et al.* (2008) observed that cultivar and collection time caused large variations in the total amount of the different components. The maximum content of polyphenols and secoiridoids was found in December (winter).

The aim of this work was to study the proximate chemical composition, extracted yield%, total phenolic content, chemical composition of polyphenols and the profile of phenolic compounds by HPLC of olive leaves samples collected from two different locations.

MATERIALS AND METHODS

Plant material, reagents, and standards

Leaves of olive tree (*Olea europaea* L.) cultivars were collected from Siwa Oasis namely: Maraqi Siwa (MS), Wattigen Siwa (WS) and Hamid Siwa (HS). Leaves of the same cultivars were also collected from the orchard of the Faculty of Agriculture, Saba Basha, University of Alexandria and were assigned as Maraqi Mazra (MM), Wattigen Mazra (WM) and Hamid Mazra (HM). All leaves were of sixteen years old plants and collected at the same time in the middle of December 2009. The selected trees were of uniform size, planted at 5 x 5 meters. The leaves were collected from different parts of each tree, so as to minimize the effect of sun exposure and differences related to different maturation stage in each region, the chosen cultivars were those predominating in the respective area. All solvents and chemicals used were of analytical grade and obtained from El- Gomhouria Company, Alexandria, Egypt.

Preparation of olive leaf samples

Olive leaves were washed with deionized water and dried at 37 °C for 3 days. Then the dried leaves were pulverized in a blender in order to decrease the particle size of the leaves to 90–150 μm. Finally the olive leaf powder was stored in light protected glass bottles for further use. (Altiok *et al.*, 2008).

Proximate chemical composition

Chemical analyses of olive leaves were realized according to the Association of Official Analytical Chemists (AOAC, 1990). Moisture content was determined in air drying oven at 105 °C up to constant weight (24 h).

Total protein was determined by the Kjeldahl method. Fat content was determined by using the Soxhlet method, using petroleum ether (60 – 80 °C for 6 hrs as a solvent. Crude fiber content was determined by digested the samples with diluted acid and alkali, then dried at 105 °C for 12 hrs, placed in the furnace at 550 °C for 3 hrs. Ash content was measured by using a muffle furnace at 550 °C up to constant weight. Nitrogen free extract content (carbohydrate) was estimated by difference of mean values,

Extraction of olive leaf polyphenols

Fatty materials were extracted from the dried samples with petroleum ether (60–80 °C for 6 hrs) using soxhlet apparatus. The total phenolics were separately extracted from the defatted samples (100 g each) according to the method described by (Altiock *et al.*, 2008). Different solvents such as acetone, hexane, ethanol, methanol, mixture of acetone: water: acetic acid (90:9.5:0.5 v/v), and mixture of ethyl acetate: methanol: water (60:30:10) were used at room temperature. After 24 hr extraction times the extracts were filtered and centrifuged for 5 min at 500 rpm. The yield, and total phenol content of all extracts were determined. In order to obtain olive leaf extract OLE, the extraction solvent was removed by using rotary evaporator at 38 °C with 120 rpm rotation under vacuum. Then, solvent free olive leaves extract (OLE) was dried by using a freeze drier system at -18 °C and 0.2 mbar. Dried OLE powder was stored in light protected glasses until further analysis.

Total phenol content analysis

Total phenol content of extracts was determined by the method Folin-Ciocalteu assay. An aliquot (0.5 ml) of each extract was reacted with the freshly prepared 1.25 ml of 20% sodium carbonate and 0.5 ml of 1N Folin reagent in a screw-capped test tube. Required dilutions were prepared with distilled water. Test tubes were vortexed and after 40 min, absorbance readings were recorded at 725 nm. The phenol content was expressed as mg gallic acid equivalent per gram of extract.

Determination of total flavonoids

Total flavonoids were measured according to a colorimetric assay described by Zhishen *et al.*, (1999). A 1 ml aliquot of appropriately diluted sample was added to a 10 ml volumetric flask containing 4 ml of 10% H_2O. At zero time, 0.3 ml of 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 of 10% $AlCl_3$ was added. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the reaction flask were diluted to volume with addition of 2.4 ml of H_2O and thoroughly mixed. Absorbance of the mixture was determined at 510 nm versus prepared water blank. The content of total flavonoids in the olive leaves extract was expressed as catechin equivalents per 100g of dry samples (CTE/100 g dw). All samples were analyzed in triplicate.

Determination of Total flavanols

The amount of total flavones was assayed calorimetrically by the vanillin method using catechin as a standard (Price *et al.*, 1978) 5.0 ml of 0.5% vanillin in MeOH (methanol) was added to 1.0 ml of methanolic olive leaves extracts and mixed well. Similarly a blank was prepared by adding 5.0 ml of 4 % HCl in methanol to 50 ml of 0.5% vanillin MeOH. The absorbances of sample and blank were measured at 500 nm in the dark at room temperature. The absorbance of the blank was subtracted from the absorbance of the sample. The content of total flavanols in the olive leaves extract was expressed as catechin equivalents per 100g of dry sample (CTE/100 g dw). All samples were analyzed triplicate.

High Performance Liquid Chromatography of phenolic compounds

The HPLC was used for the quantification of phenolic compounds. The HPLC equipment used was Agilent technologies Series 1200 equipped with UV detector. The stationary phase was C18 Eclipse-XBD, C18 (5 µm, 4.6 * 150 mm). The injection volumes of samples were 20µl. The flow rate was 0.8 ml/min and the absorbance changed was monitored at 280 nm. The mobile phase for chromatographic analysis were: (A) acetic acid / water (2.5: 97.5) and (B) Acetonitrile. A linear gradient was run from 95% (A) and 5% (B) to 75% (A) and 25% (B) during 20, min ; it changed to 50% (A) and (B) in 20 min (40 min, total time); in 10 min it changed to 20% (A) and 80% (B) (50 min, total time), after re-equilibration in 10 min (60 min, total time) to initial composition.

RESULTS AND DISSCUSION

Proximate chemical composition

Table (1) shows the proximate chemical compositions of six olive leave cultivars collected from two locations in Egypt. Three cultivars were collected from Siwa Oasis including: Maraqi (MS), Wattigen (WS) and Hamid (HS). The other three cultivars were collected from the Orchard of Faculty of Agriculture namely Maraqi Mazra (MM), Wattigen Mazra (WM) and Hamid Mazra (HM). Data generally revealed that moisture content were significantly difference among olive leaves cultivars, the moisture content of Siwa olive leaves cultivars had low content (46.50 , 47.84 , 47.24 %) for MS, WS and HS respectively, The moisture content of Orchard cultivars were (50.45, 51.08, 52.02%) for MM, WM and HM, respectively. On the other hand, the cultivars from Siwa Oasis had higher total solid contents than that of Orchard of Faculty. The MS showed the highest total solid content (53.50%), while the HM had the lowest total solid content (47.67%). Protein contents were significantly differences among the cultivars. Nevertheless, the MM had the highest protein content (17.67 %),

while the WS had the lowest protein content (15.06 %) compared to the other cultivars. Olive leaves of all cultivers generally showed traces of crude fat content (less than 4 %), however the WS showed the highest crude fat content (3.04 %), while the MM showed the lowest crude fat content (2.44 %) as compared to the other olive leaves cultivars, crude fat contents, however, were not significantly differences for all olive leaves cultivars. Nitrogen Free Extract (NFE) contents which calculated by difference constituted the most dominant components in olive leaves cultivars. Nevertheless the Orchard of Faculty had the highest content of nitrogen free extract than that of Siwa Oasis cultivars; the WM had the highest carbohydrate content as compared to other cultivars (55.64%), while the MS had the lowest carbohydrate content (50.74%). Fiber content represented the second most dominant components after carbohydrates. The Siwa Oasis cultivars had the highest fibers content than that of Orchard of Faculty. Nevertheless, the MS had the highest content of fiber (21.74%), while the WM had the lowest fiber content (17.37%). Ash contents constituted the third most dominant components after carbohydrates and crude fiber in total solid of olive leaves, there were a variance in ash contents for the two locations of cultivars. Nevertheless, the WS showed the highest content of ash (10.75%), while MM had the lowest ash content (6.90 %). Erbay and Icier, (2009) reported that the chemical composition of olive leaves (g/100g) were moisture: 49.83, protein: 5.45, oil: 6.54, ash 3.61, crude fiber 7.00, carbohydrates 27.58 (g/100gm). On the other hand, Boudhrioua *et al.*, (2008) studied the proximate composition of four olive leaves cultivars in Tunisia (Chemlali, Chemchali, Zarrazi and Chetoui and they found that the fresh olive leaves were intermediate moisture products; the moisture content expressed in wet basis (g/100 g fresh fresh leaves) varied from 46.24% (Zarrazi) to 49.75% (Chemlali). Protein and fat contents of the leaves varied respectively from 5.04% (Chetoui) to 7.61% (Chemlali) and from 1.05 % (Chemlali) to 1.30% (Zarrazi), respectively. Ash content varied from 2.86% (*Zarrazi*) to 4.45% (Chemlali). Accordingly, carbohydrates content varied from 37.14% (Chemlali) to 42.60% (Chetoui).

Table (1): Proximate chemical composition of olive leaves from different cultivars (based on dry weight)

Components	MS	WS	HS	MM	WM	HM	LSD(0.05)
Total solid	53.50a ± 1.08	52.16a ± 1.00	52.76a ± 0.92	49.55b ± 0.96	48.92b± 0.90	47.98b± 0.86	1.69
moisture	46.50d± 0.90	47.84d± 0.81	47.24d ±1.02	50.45bc ±0.99	51.08abc± 0.81	52.02ab± 0.94	1.58
Crude protien	15.94b± 0.25	15.06b ±0.44	15.95c ±0.32	17.67a± 0.30	16.57b± 0.49	16.25b± 0.54	0.73
Crude fat	2.63± 0.18	3.04± 0.63	2.46 ±0.51	2.44 ± 0.33	2.51 ± 0.54	2.54 ± 0.54	NS
ash	8.95bc± 0.24	10.75bcd± 0.25	8.32a ± 1.07	6.90e ± 0.24	7.91cd± 0.28	8.60bcd± 0.29	0.88
Crude fiber	21.74a± 0.33±	20.11b± 0.26	19.56b 0.82	18.28c± 0.54	17.37c± 0.64	18.11c± 0.61	0.92
carbohydrate	50.74e ±0.51	51.04e ±0.48	53.71cd ± 0.65	54.71abc ±0.61	55.64ab ±0.39	54.50bcd ±0.54	0.95

MS = Maraqi Siwa WS=Wattigen Siwa HS= Hamid Siwa
MM = Maraqi Mazra WM= Wattigen Mazra HM= Hamid Mazra
NFE= Nitrogen Free Extract

Extraction yield

The extraction yield is a measure of the solvent to extract specific components from the original material, the extraction method must allow complete extraction of the compounds of interest, and it must avoid their chemical modification. Extraction yield is dependent on the solvent and the method of extraction. The activity of natural extract has been found to depend on the active components of the raw material, the type and polarity of extraction solvent, and the extraction procedure (Kouri *et al.*, 2007). The extracted yield % of different olive leaves cultivers using different solvents is shown in Table (2). Data revealed that the yield extracted from olive leaves cultivars were not significantly different except that extracted with ethanol. Methanol, however, gave the highest yield extract (11.32%) for the HS cultivar as compared to other solvents, while the MM gave the lowest yield extract (7.09 %) as compared to the other cultivars when extracted with hexane.

Total phenolic content

Table (3) shows the phenolic content which was expressed as mg gallic acid equivalent per /g of extract (mg GAE/g extract) of different cultivars by different solvents. The solvent mixtures gave the highest total phenolic content as compared to the other solvent for the same cultivar. The order of different solvent in extracted total phenolic content was: (ethyl acetate methanol: water) > (acetone: water: acetic acid) > methanol > acetone > ethanol > hexane for the same cultivar. The solvent mixtures of (ethyl acetate: methanol: water) gave the highest total phenolic content extracted from MS cultivar, (12.34 mg GAE/g extract), while hexane gave the lowest total phenolic contents for HM cultivar (8.70 mg GAE/g extract) when extracted with hexane. All the different olive leaf cultivars extracted with different solvents showed significant differences.

Table (2): Extracted yield (%) of leaves in different olive cultivars by different solvents

Solvents	Olive Cultivars						LSD(0.05)
	MS	WS	HS	MM	WM	HM	
Hexane	7.54 ±0.63	7.23 ±0.53	7.16 ±0.51	7.09 ±0.12	7.58 ±0.68	7.19 ±0.11	NS
Acetone	8.45 ±0.16	9.06 ±0.50	9.21 ±0.45	8.76 ±0.61	8.81 ±0.56	9.17 ±0.10	NS
Ethanol	8.85b ±0.105	9.15a ±0.45	9.19ab ±0.86	8.90ab ±0.73	8.79b	8.73b ±0.79	1.01
Methanol	10.73 ±0.89	10.42 ±0.65	11.32 ±0.77	11.16 ±0.72	10.76 ±0.52	10.57 ±0.74	NS
Acetone: H_2O: CH_3COOH	8.84 ±0.58	9.14 ± 0.62	9.35 ±0.64	9.02 ±0.45	8.86 ±0.89	8.95 ±0.95	NS
Ethyl acetate: Methanol: H2O	8.76 ±0.83	8.41 ±0.48	8.71 ±0.59	8.55 ±051	8.27 ±0.67	8.19 ±0.61	NS

MS = Maraqi Siwa WS=Wattigen Siwa HS= Hamid Siwa
MM = Maraqi Mazra WM= Wattigen Mazra HM= Hamid Mazra

Table (3): Total phenolic content (mg GAE/g extract) of leaves in different olive cultivars by different solvents

Solvents	Olive Cultivars						LSD(0.05)
	MS	WS	HS	MM	WM	HM	
Hexane	10.82a ±0.78	9.15b ±0.70	9.48b ±0.37	9.76ab ±0.76	8.97b ±0.84	8.70b ±0.76	1.27
Acetone	11.35a ±0.72	10.32ab ±0.72	9.88ab ±0.62	10.05b ±0.60	9.47b ±0.59	9.37b 0.61	1.14
Ethanol	11.09a ±0.84	9.91b ±0.81	9.65ab ±0.69	9.94ab ±0.71	9.18b ±0.53	8.98b 0.60	1.25
Methanol	12.14a ±0.58	10.78b ±0.68	10.07b ±0.82	10.46b ±0.70	9.93b ±0.75	9.73b ±0.54	1.21
Acetone: H_2O: CH_3COOH	12.27ab ±0.41	11.31bcd±0.47	10.71abc±0.61	10.98bcd±0.60	10.35bcd±0.46	10.06cd±0.75	1.00
Ethyl acetate: Methanol: H2O	12.34ab ±0.58	11.69bcd ±0.95	10.91abc 0.79±	11.28abcd±0.49	10.67 bcd± 0.59	10.38cd± 0.36	1.16

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hexane. All the different olive leaves cultivars extracted by different solvent had significantly differences.

Polyphenolic composition of olive leaves

Table (4) showed the polyphenolic composition of olive leaf extracts in terms of total polyphenol compounds (TPP), total flavonoids (TFD), total flavanols (TFL). Data revealed that Siwa Oasis cultivars showed the highest total polyphenol (TPP) content as compared to the orchard of Faculty, except the MM cultivar had total polyphenol content (13.29 mg GAE/100g) higher than the MS cultivar which showed total polyphenol content (13.25 mg GAE/100g). No significant differences were found between the olive leaves cultivars. On the other hand, the HS cultivar had the highest polyphenols content (13.37 mg GAE/100 g) followed by WS cultivar (13.34 mg GAE/100g), while the HM cultivar had the lowest polyphenol content (12.78 mg GAE/100g).

The total flavonoids (TFD) had a significant differences between the olive leaves cultivars, Siwa Oasis cultivars had the highest total flavonoids content than Orchard of Faculty except MM cultivar (4.89 mg CTE/100g) which belongs to Orchard of Faculty was higher than HS cultivar (4.76 mg CTE/100g) which belongs to Siwa Oasis cultivar, MS had the highest total flavonoid content as compared to other olive leave cultivars (5.74 mg TCE/100g), while HM had the lowest total flavonoids content (4.47 mg CTE/100g) . The total flavanols (TFL) content of olive leaves extracts had no significant differences between all cultivars and take the same trend as happened in total flavonoids, the MS cultivar had the highest total flavanol (1.79 mgCTE/100g), while the HM cultivar had the lowest total flavanol content (1.52 mg CTE/100g).

Identification of phenolic compounds in olive leaves

For the characterization of olive leaf extracts, quantification of major polyphenolic compounds present in olive leaf extracts were determined by using HPLC analyzes. Figure (1) shows the typical HPLC profile of olive leaf extracts from different cultivars. Data showed that the olive leaves extracts exhibited several peaks corresponding to different major polyphenols which were identified as hydroxytyrosol, tyrosol, catechin, caffiec acid, vanillic acid, vanillin, rutin, luteolin-7-glucoside, verbascoside, apigenin-7-glucoside, oleuropein, and luteolin).

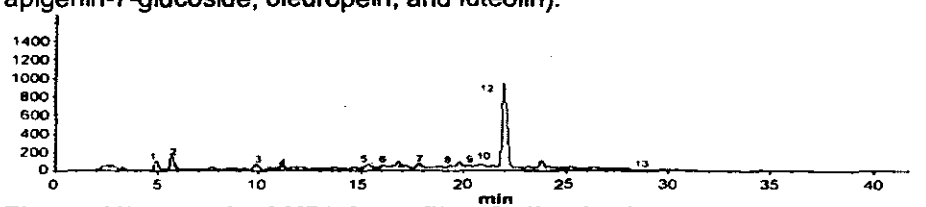

Figure (1): A typical HPLC profile of olive leaf crude extracts and peak number

Table (4): Chemical composition of polyphenols of leaves in different olive cultivars

Solvents	Olive Cultivars						LSD(0.05)
	MS	WS	HS	MM	WM	HM	
Total Polyphones TPP (mg GAE/ 100g)	13.25 ±0.46	13.34 ±0.64	13.37 ±0.54	13.29 ±0.40	12.95 ±0.44	12.78 ±0.43	NS
Total flavonolds TFD (mg CTE/100g)	5.74ab ±0.27	5.29bcd ±0.30	4.76abc±0.40	4.89 bcd ±0.54	4.60 bcd ±0.47	4.47cd ±0.39	0.73
Total flavanols TFL (mgCTE/100g)	1.79 ±0.82	1.73 ±0.72	1.66 ±0.47	1.68 ±0.44	1.58 ±0.69	1.52 ±0.55	NS
(TFD/TPP %)	43.32	39.65	35.60	36.79	35.52	34.97	

MS = Maraqi Siwa WS=Wattigen Siwa HS= Hamid Siwa
MM = Maraqi Mazra WM= Wattigen Mazra HM= Hamid Mazra

Table (5) shows the identified phenolic compounds in different olive leaves extracts (OLE) from Siwa Oasis and from Alexandria. Data revealed that all cultivars showed considerable percentage of hydroxytyrosol.The (OLE0 from Hamid Siwa (HS) cultivar showed the highest percentage of hydroxytyrosol content (21.62%) and the (OLE) of MS showed the lowest. Nevertheles, cultivars planted in mazra orchard showed high content of hydroxytyrosol than those cultivated in Siwa (6.52, 10.04 and 21.62%). Data also showed that the cultivars of mazra orchard had higher content of tyrosol (3.44 – 6.87%) compared to those cultivated in Siwa (2.41-3.63%).The HS cultivar showed the highest content of catechin (13.81%) compared to all other cultivars. Caffiec acid, on the other hand, showed the highest content (33.69%) in the WS followed by (18.24%) in MS as compared to other cultivars. However, the (OLE) of mazra cultivar showed the lowest contents of caffiec acid. Also, vanillic acid content was higher in Siwa cultivars (15.30%) in the MS and (18.47%) in the WS as compared to those of mazra cultivars. Vanillin contents, on the other hand, was higher in mazra cultivars (13.38, 16.27 and 13.78%) than that of Siwa cultivars (6.65, 1.28, and 10.50%). Rutin and Oleuropien contents were major components in all cultivars and varied from (6.49- 16.95%) for rutin and for Oleuropien from (4.93 - 21.09%) being higher in mazra cultivars. Luteolin also ranged from (1.19%) in WS to (13.83%) in HM.

It could be concluded that the leaves of olive tree cultivars collected from Siwa Oasis HS had the highest total phenolic compounds, MS had the highest total flavonoids and total flavanols contents. Thirteen phenolic compounds have been identified.

Table (5): The peak area and % of the phenolic compounds extracted from leaves in different olive cultivars.

Peak No	Ph cmpd	MS		WS		HS		MM		WM		HM	
		Area	%	Area	%	Area	%	Area	%	Area	%	Area	%
1	OHtyrosol	0.2684	6.52	0.274	10.04	0.3506	21.62	0.3852	15.87	0.1670	15.19	0.4708	14.07
2	Tyrosol	0.0993	2.41	0.0694	2.54	0.0589	3.63	0.0973	4.01	0.0379	3.44	0.2300	6.87
3	Catechin	0.1818	4.42	0.1538	5.63	0.2239	13.81	0.2908	11.98	0.0657	5.97	0.2351	7.02
4	Caffiec	0.750	18.24	0.919	33.69	0.0506	3.12	0.0688	2.83	0.0203	1.84	0.1196	3.57
5	Vanillic	0.629	15.30	0.504	18.47	0.0683	4.21	0.2404	9.90	0.0375	3.41	0.2628	7.85
6	Vanillin	0.2736	6.65	0.0351	1.28	0.1703	10.50	0.3248	13.38	0.1789	16.27	0.4611	13.78
7	Rutin	0.2669	6.49	0.2873	10.53	0.1759	10.84	0.3097	12.76	0.1864	16.95	0.4736	14.15
8	Lut-7-glu	0.0687	1.67	0.0694	2.54	0.0619	3.81	0.0802	3.30	0.0404	3.67	0.1596	4.77
9	Verbascoside	0.322	7.83	0.0344	1.26	0.0208	1.28	0.0533	2.19	0.0298	2.71	0.0405	1.21
10	Apig-7-glu	0.357	8.68	0.0494	1.81	0.0205	1.26	0.0430	1.77	0.0279	2.53	0.0318	0.95
11	Dios-7-glu	0.322	7.83	0.0525	1.92	0.0301	1.85	0.1295	5.33	0.0405	3.68	0.0982	2.93
12	Olue	0.2028	4.93	0.2381	8.73	0.1853	11.42	0.3343	13.77	0.2319	21.09	0.2989	8.93
13	Luteln	0.369	8.97	0.0409	1.19	0.2041	12.58	0.0690	2.84	0.0352	3.20	0.4628	13.83
	Sum	4.1105	99.94	2.7273	99.63	1.6212	99.93	2.4263	99.93	1.0994	99.95	3.3448	99.93

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MM = Maraqi Mazra WM= Wattigen Mazra HM= Hamid Mazra

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الملخص العربى

التعرف على المركبات الفينولية فى اوراق بعض أصناف الزيتون

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تم تجميع اوراق الزيتون *Olea europaea* L., من اشجار عمرها 16سنه لاصناف (مراقى- وطيجن - حامض) من واحة سيوة ونفس الاصناف من مزرعة كلية زراعة سابا باشا فى منتصف شهر 2009/12، حيث تم غسل الاوراق الخضراء بماء مقطر وتجفيفها عند 37 م° وبعد ذلك تم طحن الاوراق. واظهرت النتائج بان اصناف مزرعة الكلية بها محتوى اعلى مقارنة باصناف واحة سيوة، والعكس من ذلك كان فى حالة المواد الصلبة الكلية ، من جهة اخرى فان اوراق الزيتون تحتوى على كميات مرتفعة من الكربوهيدرات ويليها الالياف الخام بينما كان محتواها من البروتين الخام والاليياف الخام منخفضا جدا. وعند تقدير الناتج (المتحصل عليه من المستخلص) باستخدام عدة مذيبات، كان الناتج المتحصل باستخدام الميثانول هو الاعلى بينما اعطى الهكسان اقل ناتج، وفى حالة تقدير الفينولات الكلية كان لخليط المذيبات المتكون من (خلات الايثايل : ميثانول : ماء) اعلى كمية من الفينولات بينما تم الحصول على اقل كمية باستخدام الهكسان. اما فى حالة تقدير البولى فينولات، كان صنف حامض سيوة اعلى محتوى متبوعا بصنف وطيجن سيوة ، بينما كان صنف حامض مزرعة الكلية اقل محتوى من البولى فينولات الكلية. وفى حالة تقدير البولى فلافونويدات الكلية اعطى صنف مراقى سيوة اعلى محتوى بينما مان صنف حامض مزرعة الكلية اقل محتوى من البولى فلافونويدات الكلية، وهو ماحدث فى حالة تقدير البولى فلافانولات الكلية. وعندما تم حقن مستخلصات العينات بنفس الطريقة التى اتبعها Altiok *et al* ., 2008)، وتم التعرف على المركبات الفينولية بكل الاصناف حيث اتضح تواجد نفس المركبات الفينولية وهى (hydroxytyrosol, tyrosol, catechin , caffiec acid , vanillic acid , vanillin , rutin, luteolin-7-glucoside, verbascoside, apigenin-7-glucoside, oleuropein, luteolin.)، كما ان الريوتين واليوروبين يعتبران من اكثر المركبات الفينولية تواجدا فى مستخلصات اوراق الزيتون حيث كان محتوى الريوتين يتراوح بين ((6.49– 16.95%) بينما كان محتوى الاليوروبين يتراوح بين (4.93 to 21.09%)