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RELATIONSHIP BETWEEN TOTAL PHENOLIC, TOTAL FLAVONOID AND OLEUROPEIN IN DIFFERENT AGED OLIVE (*OLEA EUROPAEA* L.) CULTIVAR LEAVES

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Abstract

Background: Olive leaves have recently gained attention owing to its antioxidant antibacterial, antifungal and anti-inflammatory effects. Bioactive contents of olive leaves differ according to cultivation area, ecological conditions, age of tree, agronomical practices, cultivar, leaf growth stage and other abiotic and biotic stress factors.
Material and Method: In this study, *Olea europaea* L. cultivar (Kilis Yaglık) at different age grown in Kilis were examined. In this context, total phenolic content, total flavonoid content and oleuropein content of young and old tree leaves were determined.
Results: Correlations between total phenolic, total flavonoid and oleuropein in young and old trees of Kilis Yaglık cv. were found to be significant with respect to the content of each other, cultivars and age under irrigated and non-irrigated conditions.
Conclusion: In the current study, more phenolic compounds and oleuropein were determined in young trees of cv. Kilis Yaglık whereas the higher total amounts of flavonoids were obtained in old trees. There were positive strong-correlation in terms of total phenolic and oleuropein contents and positive-moderate correlation in relation to the total flavonoid content between old and young trees of cv. Kilis Yaglık.

Key words: *Olea europaea* L., Oleuropein, Flavanol, Total Phenolic

Introduction

Olive trees (*Olea europea*) are native to the eastern Mediterranean basin that spreads westwards beyond Turkey into Europe. Although ripe fruits and edible oil extracted from the fruits of olive trees are the main parts used in Mediterranean cousins. Due to its physiologically active polyphenols, olive has a considerable place in the Mediterranean diet (Ryan et al. 1999; Perreira et al. 2007). People have also used olive leaf extracts in folk medicine in these areas. Because of the polyphenol and flavonoid contents, leaf extracts can be potent sources that are used in medicine industry in the future (Myriam Ben, 2012). Olive fruit, olive oil and its leaves have pharmacological properties and they have been realized as important medicinal components and a healthy diet because of their phenolic content (Visioli et al, 2002).

Olive leaves have been extensively used as a medicine for the treatment of fever and other diseases such as malaria in the past (Gucci et al. 1997; Fernandez-Escobar et al., 1999; Ciafardini and Zullo, 2002). The leaves have been used up as a dietary component, in the form of an extract or a whole powder (Karakaya, 2009) Olive leaves have many pharmaceutical properties because of many potentially bioactive compounds that may have anti-hypertensive, antioxidant, hypoglycaemic, anti-inflammatory and hypocholesterolemic properties (Karakaya, 2009). Besides these features, many reports indicated that olive leaves can decrease blood pressure and arrhythmia, increase blood flow in the coronary arteries, decrease and prevent intestinal muscle spasms (Khayyal et al., 2002; Zarzuelo, 1991, Samuelsson, 1951; Pereira et al., 2007).

The leaves also have some properties against some microorganisms as a antimicrobial effect (Somova et al., 2003; Lee and Lee, 2010; Andrikopoulos et al., 2002; Benavente-Garcia et al., 2000; Furneri et al., 2002; Briante et al., 2003; Skerget et al., 2005). All these potential health utility of olive leaves are mostly related to polyphenols such as oleuropein, hydroxytyrosol, tocopherol, tyrosol, caffeic acid, elenolic acid derivatives, p-coumaric acid, vanillic acid and flavonoids (Ryan et al., 2003; Bianco and Uccella, 2000; Tasioula-Margari and Ologeri, 2001). In these phenolics, the combined phenolic compounds have substantial high antimicrobial activity than the others (Lee and Lee, 2010). Usage of whole olive leaf and olive leaf extract has increased rapidly in both the pharmaceutical and food industries owing to precious biophenol compounds and effects to health (Fernandez-Escobar et al., 1999; Delgado-Pertinez et al., 2000) Due to the presence of additive and/or synergistic effects of their phytochemicals, the whole leaf extract is recommended to achieve health benefits (Pereira et al., 2007). In the other hand, some properties of oleuropein have been reported such as protecting against pathogens (Uccella, 2001) and repelling insects (Lo Scalzo et al., 1994).

Material and Methods

Plant Material

An experiment was conducted on two different aged Kilis Yaglık cultivars under Kilis ecological conditions between 2011-2012 years. 9 and 65 year old tree groups of Kilis Yaglık were used in the current study. The experiment was designed as a randomized block with three replications and each replication was represented with two trees. The trees were subjected to different water regimes for long term: Control (irrigated) and Drought (non-irrigated) for one year. To monitor seasonal variations between the different aged trees with respect to the bioactive contents, the leaf samples were collected six times in a year and then dried at laboratory conditions for subsequent analysis.

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Determination of Total Phenolic Content

Total phenolic content was determined according to the Folin-Ciocalteu reagent method (Singleton et al., 1999). The amount of total phenol was calculated as mg/g (Gallic Acid Equivalents) from calibration curve of Gallic acid standard solution (R^2=0.9993). An aliquot of each sample (0.1 ml) was diluted to 1 ml with distilled water. Briefly, 0.5 ml of Folin-Ciocalteu reagent (1:1 v/v) and 1.5 ml of 20 % (w/v) sodium carbonate were added to the diluted sample solution, and the mixture was then vortexed and allowed to stand for 2 hour at room temperature for colour development. The volume was completed to 10 ml with distilled water and their absorbance was measured at 765 nm (Evolution 201 UV-Visible Spectrophotometer). The total phenolic content was expressed as mg/g gallic acid equivalents (GAE). All samples were analyzed in triplicate.

Determination of Total Flavonoid Content

The flavonoids content was determined by aluminum chloride method using quercetine as a reference compound (Kumaran and Karunakaran, 2006). This method based on the formation of a complex flavonoid-aluminum. The amount of total flavonoid was calculated from calibration curve of quercetine standard solution (R^2 =0.9815). 1ml of olive leaf extracts or standards quercetine solution (500 µg/ml) was added to 4 ml distilled water and 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10 % $AlCl_3$ was added. After 6 min, 2mL of 1 mol L^- NaOH was added and final total volume was completed to 10 mL with distilled water. The solution was thoroughly mixed. Afterwards, the absorbance of the mixture was measured at 510 nm against prepared water as a blank. Total flavonoid content of plant leaves was expressed as mg quercetine equivalents (CE)/g of dried olive leaf material.

Determination of Oleuropein Content

The oleuropein content was determined according to the method described by Altinyay and Altun (2006) with slight modifications. Briefly, 5 grams of the dried and powdered materials were macerated with 50 ml methanol for 4 hours at room temperature using a magnetic stirrer. The extracts were filtered and evaporated to dryness at 40 ºC. Then, the residues obtained after methanol extraction were dissolved in 50 ml of HPLC grade Merck methanol. Solutions were passed through a 0.45 µm filter and 20 µl extracts were directly injected into the HPLC column. The results were obtained as a mean value of three replicates. Concentrations of 10-100 ppm of standard solutions were prepared in methanol (R^2=0.99).

Statistical Analysis

All measurements were replicates. The data were subjected to the two-ANOVA and means comparison was analyzed using Duncan's multiple range tests. Statistical analysis was performed using MSTATC (Michigan State University, East Lansing, MI). Differences were considered to be statistically significant at a level of $P < 0.05$.

Results and Discussion

The data obtained from this study in order to investigate the relations between total phenolic, total flavonoid and oleuropein contents in the leaves of old and young trees of Kilis Yaglık cultivar with respect to age factors are presented in Table 1.

Table 1: Monthly changes Total Phenolic contents, Total Flavonoid contents and Oleuropein content in leaves of olive at different aged Kilis Yaglık cultivars

	Total Phenolic (mg/g GAE)			**Total Flavonoid** (mg/g QE)			**Oleuropein** (%)		
Months	**Old**	**Young**	**Average**	**Old**	**Young**	**Average**	**Old**	**Young**	**Average**
February	133.60	135.95	134.78	75.71	63.23	67.92	7.47	7.79	7.63
April	123.39	126.85	125.13	77.81	84.27	77.42	7.16	7.33	7.25
June	99.99	100.64	100.32	75.87	73.05	73.30	7.08	7.27	7.17
August	81.10	79.46	80.28	87.24	81.60	83.88	6.63	6.58	6.60
October	85.38	92.35	88.87	65.42	61.05	64.67	5.92	6.52	6.22
December	102.16	102.90	102.53	78.70	64.51	75.07	5.63	6.29	5.96
LSD:	101.20 **b**	103.30 **a**	105.30	77.87 **a**	73.23 **b**	73.71	6.65 **b**	6.96 **a**	6.81

Means in the same column by the same letter are not significantly different to the test of Duncan (α=0.05)

Correlation Leaf Content According to Age

Variations between two different age group (Old and Young) of cv. Kilis Yaglık in relation to the total phenolic, total flavonoid and oleuropein content were determined. In this context, more phenolic compounds and oleuropein were determined in young trees of cv. Kilis Yaglık whereas the higher total amount of flavonoids was obtained in old trees. There were positive strong-correlation in terms of total phenolic (R^2 = 0.99045) and oleuropein contents (R^2 = 0.93959) and positive-moderate correlation in relation to the total flavonoid content (R^2=0.66554) between old and young trees of cv. Kilis Yaglık (Figure 1-3). However, decline in total phenolic content was determined in different species and genotypes under stress conditions (Dixon et al., 1995; Naczk et al., 2004; Ksouri et al., 2007).

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The effects of maturity on total phenolic contents have been revealed (Seemannová et al., 2006; Achakzai et al., 2009). Total phenolic contents were influenced with the age factors and important decreases were determined with increasing periods (Padda and Picha, 2007).

Oleuropein content in unprocessed olive fruit and its leaves decline with fruit maturity and processing (such as oil extraction) through consequences of some chemical and enzymatic reactions and then the concentration of primary degradation product, hydroxytyrosol, increases. The oleuropein content is high at beginning of first stages of ripening and equals to 14 % of fruit dry matter (Amiot et al., 1986; Amiot et al., 1989). At the beginning of green-ripening, oleuropein content decrease and glycosidic derivatives such as elenolic acid glycoside and dimetil oleurope, of oleuropein occurs. The accumulation of dimetil oleurope continues until becoming the major constituent of black olives (Bianco et al., 1999).

Correlation Leaf Content to Each Other

In the current study, there was a weak-negative correlation (R^2 = 0.28572) between total phenolic and total flavonoid contents. However, a positive-strong correlation (R^2 = 0.71199) was obtained between total phenolic content and oleuropein content. Also, a week-negative correlation (R^2 = 0.0549) was determined between total flavonoid and oleuropein content (Figure 4-6).

In this study, changes in oleuropein content in leaves harvested in different periods were investigated. Herein, genetic factors elicited significant variations in oleuropein contents of leaves. The oleuropein content in leaves harvested in October was lower than the samples harvested in March. This difference may be attributed to the high degradation of this glucoside and low production rate of young shoots. Age factors influenced the oleuropein content beyond harvest methods, quantitative methods or harvest periods. Dark green leaves (during development period) contained more oleuropein content than the yellowish leaves (during leaf-pruning) (Ranalli et al., 2006). The oleuropein in the leaf degrades via β- glucosidase mediated biochemical process (Briante et al., 2000; 2001; 2002; Moracci et al., 1995).

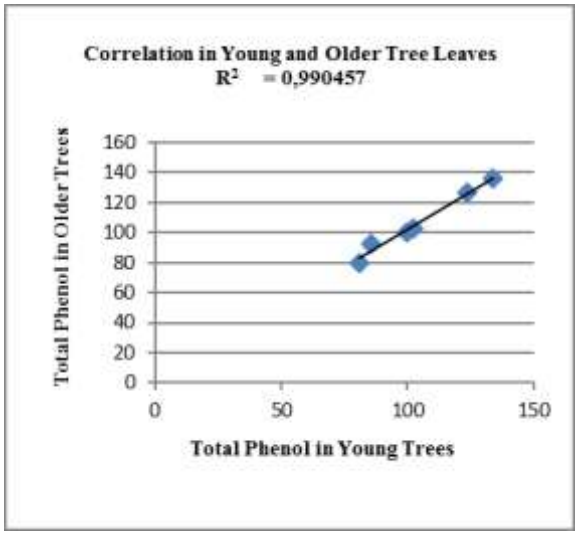

Figure 1: Correlation total phenolic content in young and older olive leaves

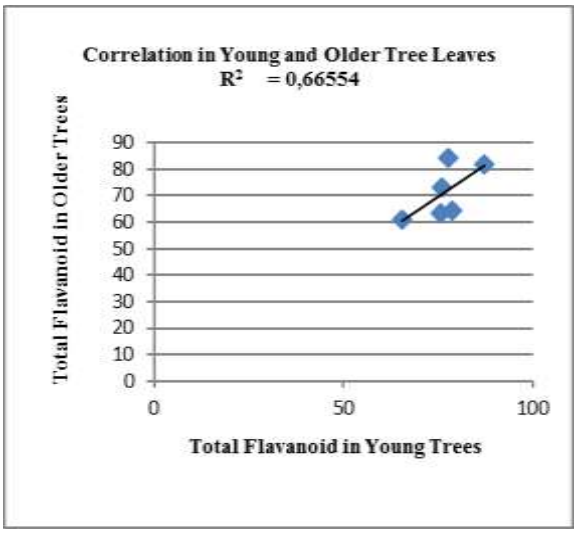

Figure 2: Correlation total flavanoid content in young and older olive leaves.

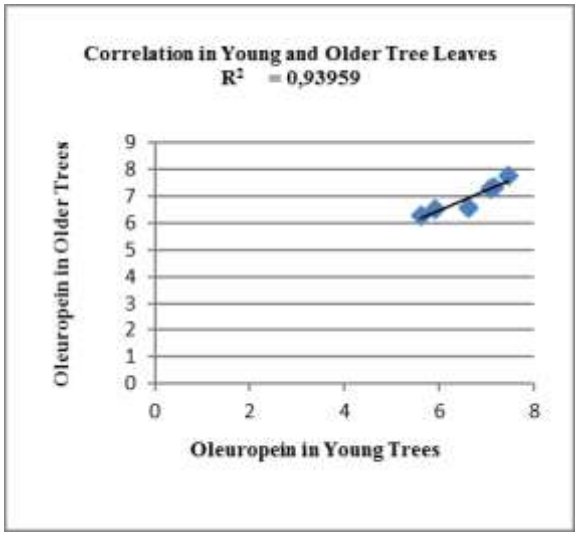

Figure 3: Correlation oleuropein content in young and older olive leaves

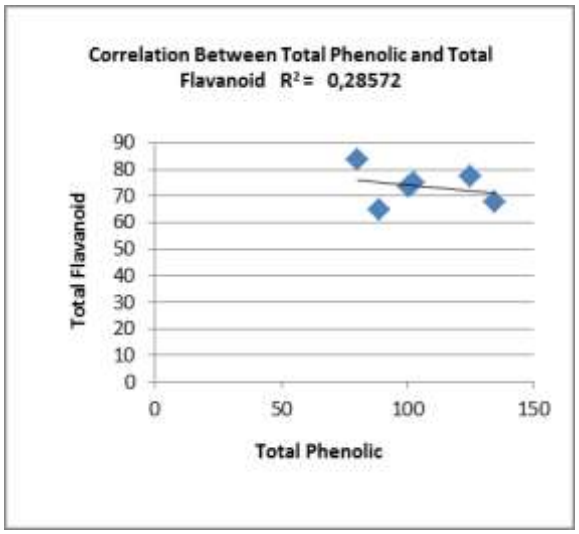

Figure 4: Correlation between total phenolic and total flavonoid content in olive leaves

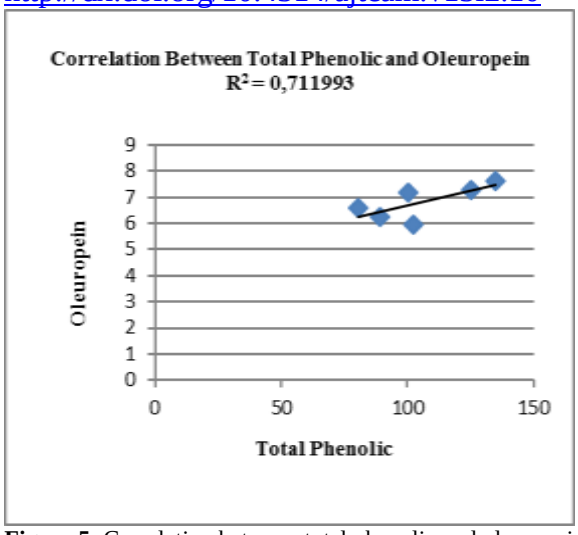

Figure 5: Correlation between total phenolic and oleuropein content in olive leaves

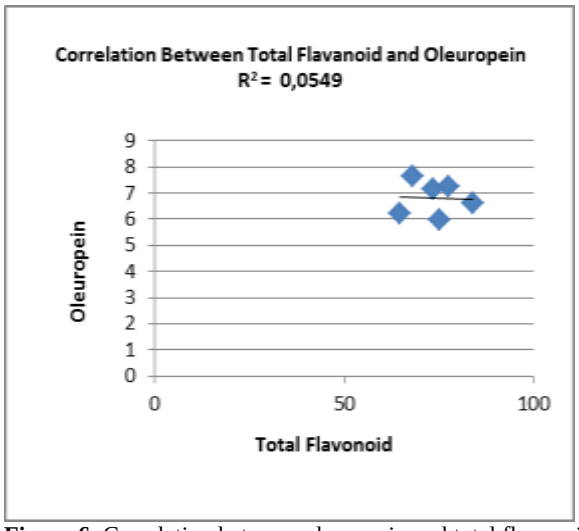

Figure 6: Correlation between oleuropein and total flavonoid content in olive leaves

Conclusion

In the current study, more phenolic compounds and oleuropein were determined in young trees of cv. Kilis Yaglık whereas the higher total amounts of flavonoids were obtained in old trees. There were positive strong-correlation in terms of total phenolic and oleuropein contents and positive-moderate correlation in relation to the total flavonoid content between old and young trees of cv. Kilis Yaglık. Also, the content of oleuropein concomitantly increased with the increases in total phenolic contents but not varied depending on the contents of total flavonoids. Hence, it can be deduced that the biologically potent metabolites may change depending on the age factors, cultivars, and ecological conditions.

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