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Full Length Article

Characterization of different Varieties of *Olea europaea* Using Metabolomics Combined with Multivariate Data Analysis

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Abstract

The content of secondary metabolites varies among different cultivars or varieties of *Olea europaea*. There is ever high increase of olive cultivation and utilization of its oil, fruit, and even leaves in various products for human consumption. There is an increase concern about characterization of different varietie and traceability of various parts of olive, as being used in food processing. The objective of this study was to assess the metabolomic profiling and antioxidant activity of drupes in five varieties of *Olea europaea* (Napali, K18, improved Napali, Galat and Kalamata) growing under the same environmental conditions in Aljouf region of Saudi Arabia. Spectrophotometer and HPLC combined with multivariate data as analytical and data processing strategy for this study, where four distinct groups were observed in principal component analysis based on metabolic discrimination among various varieties. Galat was clearly discriminated from others due to higher contents of total secondary metabolites (phenols, flavonoids, flavonols, tannins, and anthocyanins), high total antioxidant capacity (TAC), and high amounts of individual polyphenols, such as 2-hydroxybenzoic acid and ferulic acid. Further, K18 was separated into another group, by having least contents of all total metabolites, quercetin, and 2-hydroxybenzoic acid, but with higher contents of luteolin, luteolin-7-glucoside, syringic acid, and oleuropein. Napali and Kalamata were separated together into a third group, characterized by moderate contents of the total and individual secondary metabolites, as well as moderate TAC, but with a higher content of quercetin. The fourth group included the Improved Napali variety, with moderate contents of all metabolites and a higher content of caffeic acid. The antioxidant activity is discussed based on the polyphenol contents of each variety. The present study provides new insights into the factors controlling the selection of five olive varieties for either oil production or the pickling industry based on polyphenol distribution and total antioxidant activity.

Keywords: Bioactive metabolites; DPPH and antioxidant activity; Galat and K18; PCA; Polyphenols

Introduction

Olive drupes are mainly used for the production of olive oil, one of the most important ingredients of our daily diet, particularly in the Mediterranean region (Gouvinhas *et al.* 2017). Most *Olea europaea* cultivation (98%) is concentrated in the Mediterranean and Middle Eastern countries (Tabera *et al.* 2004; Ghanbari *et al.* 2012). The annual world production of olive oil is 2.2 million tons. The European Union produces around 75% of that amount (1.875 million tons). Spain, Italy, and Greece are the three most dominant producers of olive oil (Aragüés *et al.* 2004).

In Saudi Arabia, most of the cultivation of olive trees is concentrated in the Al-Jouf district (more than 15,000,000 trees), producing 68% of Saudi Arabia's olive oil. Olive drupes have different arrays of secondary metabolites. Plant secondary metabolites not only affected their nutritional and pharmaceutical values but also associated with the physiological properties and tolerance to different biotic and abiotic stresses which consequently affecting the quality of plants (Guodong *et al.* 2017; Yeshi *et al.* 2022). Secondary metabolites such as polyphenols are a crucial component of the defense system in plants and are considered the most important defensive barriers against biotic and abiotic stresses to plant growth (Latouche *et al.* 2013; Gómez-Caravaca *et al.* 2014; Ahanger *et al.* 2020; Šamec *et al.* 2021). Moreover, polyphenols are well documented as antioxidant and anticancer agents (Pereira *et al.* 2007; Abaza *et al.* 2015; Vogel *et al.* 2015). The role of the rich polyphenol extract

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of some plant fruits in the protection against oxidative stress and the reduction of heavy metal toxicity such as mercury in human red blood cells (RBC) has been reported (Tortora *et al.* 2019).

The knowledge of the content of metabolites, especially concerning particular metabolites such as polyphenols, is very crucial not only for variety classification but also, to understanding the varieties gained value in terms of the agronomic traits and nutraceutical potentiality (Olmo-García *et al.* 2018; Esposito *et al.* 2021). The olive fruits were reported to have high amounts of polyphenols including phenolic alcohols, phenolic acids, flavonoids and secoiridoids including oleuropein, ligstroside, demethyloleuropein, hydroxytyrosol, tyrosol, chlorogenic acid, luteolin-7-O-glucoside, luteolin, quercetin-3-O-rhamnoside, verbascoside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside and apigenin-7-O-glucoside (Kulak and Cetinkaya 2018). Many of these metabolites are responsible for the nutraceutical value of the drupes and the oils extracted from the drupes (Esposito *et al.* 2021). Olive fruits have many pharmaceutical features and can be used for many diseases, such as coronary artery disease and atherosclerosis, by helping to stop platelet aggregation (Carluccio *et al.* 2003). The use of olive oil for nutritional and medicinal purposes is mainly attributed to its high content of antioxidant polyphenols (Gordon *et al.*, 2001). Some polyphenols such as oleuropein and hydroxyl tyrosol have radical scavenging activities (Paiva-Martins *et al.* 2003).

Different metabolomic approaches were used to assess the distribution of secondary metabolites in different cultivars and varieties as well as different parts of *O. europaea*. NMR-based metabolomics combined with multivariate data analysis (MVDA) was used to differentiate between different cultivars of *O. europaea* (Piccinonna *et al.* 2016; Esposito *et al.* 2021). LC/MS and GC/MS-based metabolomics were also used to evaluate the metabolites content in different organs of the olive tree (Olmo-García *et al.* 2018). Different varieties of *O. europaea* were also evaluated using LC/MS based metabolomics combined with MVDA (Difonzo *et al.* 2022).

This study will enable to assess which variety among the five evaluated varieties is the richest in the health-promoting polyphenols. Furthermore, we will test if the spectrophotometer and HPLC-based metabolomics combined with multivariate data analysis is a potential approach for differentiation between the evaluated varieties. In present study, the polyphenol profiles and antioxidant activities of drupes from five varieties of olive grown in the Al-Jouf region were evaluated to assess their nutritional value and to help decision-makers in selecting the appropriate varieties for oil production and or pickling based on their metabolomic analysis and antioxidant activity.

Materials and Methods

Sample collection and extraction

Drupes of five olive varieties (Napali, K18, Improved Napali, Galat and Kalamata) were collected from the Al-Qurayat district in the Aljouf region. Plants grow under the same environmental conditions and are exposed to the same abiotic stresses that dominating the arid and semiarid climate. The drupes of different varieties of olive were subjected to the same conditions in the laboratory, where they were prepared for extraction and analysis. The materials of exocarp and mesocarp together of drupes of different varieties of olive were used for the determination of total metabolites as well as extracted twice in hydro-methanol (80% MeOH). After filtration, the extracts were dried and concentrated using a rotary evaporator. The aqueous methanol extracts of the different varieties of olive were used to profile the polyphenols using HPLC and antioxidant activity.

Quantitative determination of metabolites by spectrophotometer

The anthocyanin content was determined by using acidified methanol as previously reported (Padmavati *et al.* 1997). The plant materials (100 mg) were dissolved in acidified methanol in a well closed brown tube. Samples were incubated for 24 h in a refrigerator. After centrifugation, the absorbances of the filtrates were measured at 530 nm and 657 nm. Anthocyanins content was expressed as µmole/g dry weight.

The Folin–Ciocalteau method was used to determine phenolic contents (Singleton *et al.* 1999). One mL of Folin-Ciocalteau reagent (prepared by dilution Folin Ciocalteau: distilled water (1:5) was mixed with 1 mL of each extract. Then 1 mL of 10% sodium carbonate was added and vortexed. The mixtures either samples or standard were kept for 1 h at room temperature. The absorbance was noted at 700 nm. The content of total phenolic was calculated from the standard curve as mg gallic acid equivalent/g extract. The contents of flavonoids and flavonols in plant extracts were determined by a spectrophotometer using aluminum chloride (Zhishen *et al.* 1999; Kumaran and Karunakaran 2007). For flavonoid estimation, 0.3 mL of 5% sodium nitrite solution was added to the extract. Six min later, 0.3 mL of 10% Aluminum chloride solution was mixed and the resulting mixture was allowed to stand for 6 min after vortexing. 0.4 mL of 1 *M* sodium hydroxide was added to the mixture. The mixture was allowed to settle for further 15 min and the absorbance was read at 510 nm. The content of flavonoids was calculated as mg quercetin equivalent/g extract. Furthermore, for flavonols determination, 0.25 mL of 0.2% aluminum chloride and 1.5 mL of 5% sodium acetate were added to 0.25 mL of the extract. A series of concentrations of quercetin was treated as same as samples.

After 2.5 h, the absorbances of both standard and samples were read at 440 nm. Flavonols content was expressed as mg quercetin equivalent/g extract. Saponins were quantified using vanillin based on Ebrahimzadeh and Niknam (1998), where, 2.5 mL of 2% vanillin reagent in sulfuric acid was added to 1 mL of plant extract or with saponin standard. The mixture was vortexed and incubated at 60ºC. After 1 h, samples and standards were put in an ice bath for 10 min. The absorbance was read at 473 nm. Saponins content was calculated as mg saponins equivalent/g extract. The total tannins was determined by using vanillin based on Julkunen-Tiitto (1985), where, 4% vanillin in methanol was added to 0.05 mL of plant extract. After vortexing, HCl is added to this mixture and was left to stand for 20 min. In similar way, a series of catechol standard was treated as above. The absorbance of samples and standards was noted at 550 nm. The total tannins was estimated as mg catechols equivalent/g extract.

Assessment of total antioxidant activity

The ammonium molybdate method was used to assess the antioxidant capacity as previously reported (Prieto *et al.* 1999). A reagent solution composed of sodium phosphate, sulfuric acid, and ammonium molybdate was added to the plant extract, and the solution was incubated at 90ºC for 90 min. A series of ascorbic acid concentrations was prepared as a standard and same like samples, these were left to cool at room temperature. The absorbance was recorded at 695 nm and TAC was calculated as mg ascorbic acid equivalent/g extract. Reducing power (RP) was estimated using the methods of (Oyaizu 1986; Basar *et al.* 2013). Different concentrations of plant extract were mixed with 2.5 mL of 0.2 *M* phosphate buffer at pH 6.6 and 2.5 mL of 1% potassium ferricyanide solution. The mixtures were incubated at 50°C for 20 min and 2.5 mL of 10% trichloroacetic acid was added. The mixtures were centrifuged at 1000 rpm for 10 min. From each supernatant, 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbances of these mixtures were recorded at 700 nm. DPPH was estimated as the percentage of inhibition as previously reported (Blois 1958). The methanolic extract was vigorously shaken with a mixture of acetic acid buffer solution (pH 5.5), 50% ethanol aqueous solution, and DPPH in ethanol. Different concentrations of ascorbic acid, positive and negative controls were treated as above. The absorbance was noted at 517 nm after 30 min dark incubation at room temperature. The inhibition percentage was calculated from the following equation:

$$\% \text{ DPPH Radical Scavenging Activity } = \frac{AC - AS}{Ac} \times 100$$

Ac is the absorbance of negative control and As is the absorbance of the sample.

Profiling of individual polyphenols using HPLC

The HPLC method was performed based on Kim *et al.* (2006) with slight modification using Thermo Scientific Dionex UltiMate 3000 UHPLC+ focused standard system connected to DAD-3000 diode array detector and using Chromeleon™ 7.2 Chromatography Data System for data recording. Calibration equations and other analytical parameters were placed in Table 1.

The separation and quantification of phenolic compounds were done at an ACCLAIM™ 120 C18 analytical column with the dimensions of 150 mm × 2.1 mm and 5 μm particle size (Thermo Scientific, San Jose, USA).

Acetonitrile (solvent A) and acetic acid dissolved in water (2% v/v) (solvent B) were used as mobile phases. Samples were injected after filtration through 0.45 μm PTFE Acrodisc syringe filter (Gelman Laboratory, MI). The following gradients from the mobile phases were used after injection of 10 μL filtrated samples with a 0.8 mL/min flow rate and 60 min run time: 100 B to 85% B for 30 min, , 85 B to 50% B for 20 min, 50 B to 0% for 5 min, B and 0 B to 100% B for another 5 min. Simultaneously, peaks were monitored at 280, 320 and 360 nm and by the congruent retention times and UV spectra, together by comparison with 6 polyphenolic standards (Table 1), the peaks were identified as previously reported (Kim *et al.* 2006).

Statistical analysis

Spectrophotometer and HPLC (polyphenols) data were subjected to multivariate data analysis (MVDA) such as principal component analysis (PCA) and hierarchical clustering analysis (HCA) using SIMCA-P software (version 14.1). One-way analysis of variance was used to assess the significant difference among the metabolite content detected by spectrophotometer and HPLC using Minitab (version 12.21) (the difference at $P < 0.05$ was considered to be statistically significant). Pearson's correlation test was used to assess the correlation between the determined metabolites and total antioxidant activity (TAC, RP, and DPPH) in the five varieties and on the level of resulting groups from MVDA.

Results

Polyphenol profiling and multivariate data analysis of drupes from five varieties of *O. europaea*

In present study, the olive varieties of the Al-Jouf region are characterized by high contents of polyphenols of diverse classes, *e.g.*, anthocyanins, phenolics, flavonoids, flavonols, and tannins. The contents of total phenolics, flavonoids, flavonols, anthocyanins, and condensed tannins were very low in K18 and very high in Galat. Principal component analysis (PCA) was performed to discriminate the five

Table 1: Analytical parameters and recovery of the method of HPLC

Metabolite	Calibration range (μg mL^{-1})	Calibration Equations	R	LOD (μg mL^{-1})	LOQ (μgmL^{-1})	Recovery %	RSD%
Caffeic acid	0.5-160	Y = 0.7141x-1.0982	0.9992	0.1804	0.6014	99.349-100.254	0.901-0.968
Syringic acid	0.5-120	Y = 0.331x-0.3395	0.9994	0.0801	0.2669	99.134-99.248	0.311-1.044
Ferulic acid	0.5-140	Y = 0.9873x-1.5977	0.9991	0.2050	0.6833	99.927-100.319	0.421-1.181
2-hydroxy benzoic acid	0.5-160	Y = 0.2105x+0.419	0.999	0.1595	0.6317	99.801-100.079	0.247-1.287
Quercetin	0.5-140	Y = 1.3867x-1.530	0.9991	0.0286	0.0952	99.792-100.647	0.301-0.465

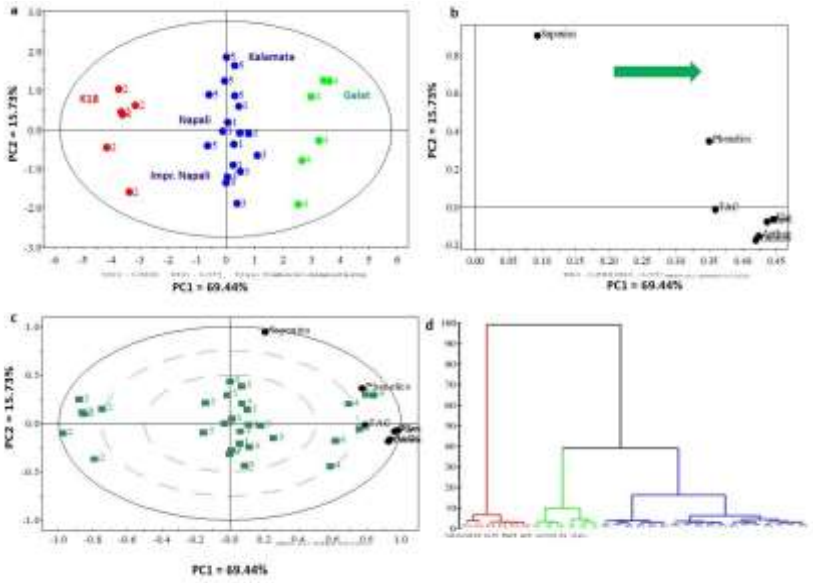

Fig. 1: Multivariate data analysis of the total secondary metabolites and antioxidant capacity of five varieties of *O. europaea* growing in Aljouf region, (**a**) PCA score scatter plot, (**b**) score loading plot, (**c**) score biplot and (**d**) the hierarchical clustering analysis. 1: Napali, 2: K18, 3: Improved Napali, 4: Galat and 5: Kalamata

varieties based on the content of total secondary metabolites. Galat was clearly distinguished from other varieties by having the highest discrimination due to presence of all total secondary metabolites, in addition to having the highest TAC (Fig. 1a–d). Napali, Improved Napali, and Kalamata varieties were categorized into another group by moderate contents of all detected metabolites and TAC. K18 was characterized by having the lowest contents of all detected total secondary metabolites (Fig. 1a–c).

The distribution patterns of individual polyphenols were different from those of total secondary metabolites. As only six standard compounds were used in the high-performance liquid chromatography (HPLC) analysis (Table 1), it was impossible to detect all individual polyphenols present in the drupes extracts and the study was focused on the principal polyphenols present in the drupes extracts. The individual polyphenols proved to be varied significantly among the varieties subjected to this study (Table 2).

PCA was performed for only the HPLC data, and the analysis categorized the five varieties into four groups. The first group included Galat, characterized by the highest content of ferulic acid, 2-hydroxy benzoic acid, and TAC. The second group included K18, characterized by the highest content of syringic acid, luteolin, luteolin-7-glucoside, and oleuropein. The third group included Napali and Kalamata, characterized by moderate contents of individual polyphenols and the highest content of quercetin. The fourth group includes Improved Napali, characterized by moderate contents of individual polyphenols and the highest content of caffeic acid (Fig. 2a–b).

To assess the similarity and dissimilarity among the five varieties under evaluation concerning their metabolites content, HPLC and spectrophotometer data together were also subjected to multivariate data analysis (MVDA). PC1 and PC2 comprise 84.4% of the variation among the data set (Fig. 3a). PCA classified the olive varieties into four distinct groups based on the polyphenol content and antioxidant capacity of their drupe extracts (Fig. 3a–b). The first group included Galat, which is characterized by higher contents of all total determined metabolites, ferulic acid, 2-hydroxybenzoic acid, and TAC and very low contents of individual polyphenols such as luteolin, luteolin-7-glucoside, and oleuropein. The second group included K18, which is characterized by the highest content of luteolin-7-glucoside, luteolin, syringic acid, and oleuropein (Fig. 3a). The third group included Napali and Kalamata, which are

Table 2: Metabolomic profiling of drupes of five olive varieties by HPLC

Metabolites	Varieties				
	Napali	K18	Imp. Napali	Galat	Kalamata
Caffeic acid	0.06 ± 0.002^{d}	0.10 ± 0.00^{c}	0.57 ± 0.008^{a}	0.16 ± 0.006^{b}	0.10 ± 0.002^{c}
Ferulic acid	1.05 ± 0.001^{c}	0.04 ± 0.003^{d}	1.52 ± 0.019^{b}	2.81 ± 0.020^{a}	0.95 ± 0.020^{c}
Luteolin	0.33 ± 0.001^{b}	0.68 ± 0.00^{a}	0.23 ± 0.003^{c}	0.13 ± 0.002^{d}	0.22 ± 0.002^{c}
Luteolin -7- glucoside	0.48 ± 0.00^{b}	0.70 ± 0.004^{a}	0.073 ± 0.002^{d}	0.39 ± 0.001^{c}	0.44 ± 0.04^{bc}
Oleuropein	2.51 ± 0.007^{b}	2.7 ± 0.004^{a}	2.40 ± 0.002^{c}	2.02 ± 0.008^{d}	2.36 ± 0.02^{c}
Quercetin	0.13 ± 0.001^{c}	0.11 ± 0.001^{d}	0.07 ± 0.00^{e}	0.16 ± 0.002^{b}	0.21 ± 0.001^{a}
2-hydroxybenzoic acid	0.50 ± 0.013^{b}	0.46 ± 0.011^{c}	0.39 ± 0.014^{d}	0.62 ± 0.003^{a}	0.4 ± 0.011^{d}
Syringic acid	0.06 ± 0.004^{b}	0.11 ± 0.004^{a}	0.043 ± 0.00^{b}	0.10 ± 0.001^{a}	0.06 ± 0.002^{b}

Different letters in the same row indicate significant differences among varieties at $P < 0.05$ and the concentration was determined as mg g^{-1}

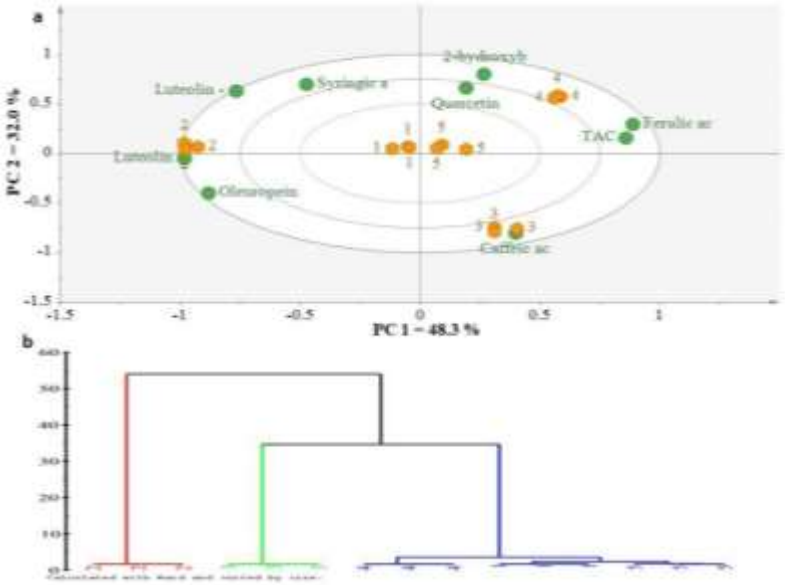

Fig. 2: PCA of polyphenolics detected by HPLC and antioxidant capacity of the drupes of five varieties of olive. (**a**) biplot of PC1 *vs.* PC2 and (**b**) hierarchical clustering analysis. Legends as described in Fig. 1

characterized by moderate contents of all detected secondary metabolites (total and individuals) except quercetin and caffeic acid. Among this group, Napali had the second-highest content of luteolin-7-glucoside, luteolin, and oleuropein after K18 and 2-hydroxybenzoic acid after Galat. The fourth group included Improved Napali, which is characterized by the moderate content of all detected metabolites and the highest content of caffeic acid (Fig. 3a). One can recognize that the Galat variety had the highest content of total polyphenols, including phenolics, flavonoids, flavonols, anthocyanins, and tannins (Fig. 1 and 3). K18 showed the lowest content of the previously mentioned total metabolites. It also showed the highest content of some important individual polyphenols such as oleuropein, luteolin, luteolin-7-glucoside, and syringic acid (Fig. 2 and 3).

The other three varieties (Napali, Improved Napali, and Kalamata) showed moderate contents of both total determined secondary metabolites and individual polyphenol compounds (Fig. 1–3). The ANOVA revealed a significant difference among the detected metabolites in the five varieties (Table 3).

Antioxidant Activity of the hydro-methanol extracts of olive drupes

Total antioxidant capacity (TAC) and reducing power (RP) showed significant differences among the five varieties as assessed by one-way analysis of variance (ANOVA) (Table 3). The data of TAC, RP and 2,2 diphenyl picrylhydrazyl (DPPH) radical scavenging activity (Fig. 4) showed that Galat and Napali had the highest TAC and the order of the varieties regarding the TAC was as follows: Galat > Napali > Kalamata > Improved Napali > K18 (Fig. 4a). The TAC of K18 was significantly different from the TAC of other varieties ($P < 0.05$), whereas no significant difference was detected among other varieties concerning TAC. The highest reducing power was detected in Kalamata, followed by Improved Napali, then Naplai, Galat, and K18 (1.4, 1.36, 1.32, 1.23 and 0.72 OD, respectively) (Fig. 4b). K18 reducing power was significantly different from the RP of other varieties ($P < 0.05$). DPPH was found at the highest percentage in K18, then Galat, Kalamata, Improved Napali, and Napali (84.5, 84.3, 82.6, 81.4 and 78.7%, respectively) (Fig. 4c).

Table 3: One way analysis of variance of the five drupes of olive varieties (F: variance and *P*: probability)

Metabolites	F-value	*P* -value
Anthocyanins	77.69	0.00
Phenolics	17.27	0.00
Flavonoids	369.4	0.00
Flavonols	536.75	0.00
Tannins	59.97	0.00
TAC	107.79	0.00
RP	1643.0	0.00
Caffeic acid	6396.3	0.00
Ferulic acid	4000.1	0.00
Luteolin-7-glucoside	496.79	0.00
Luteolin	2300.0	0.00
Oleuropein	1620.0	0.00
Quercetin	3689.0	0.00
2-hydroxybenzoic acid	183.0	0.00
Syringic acid	380.0	0.00

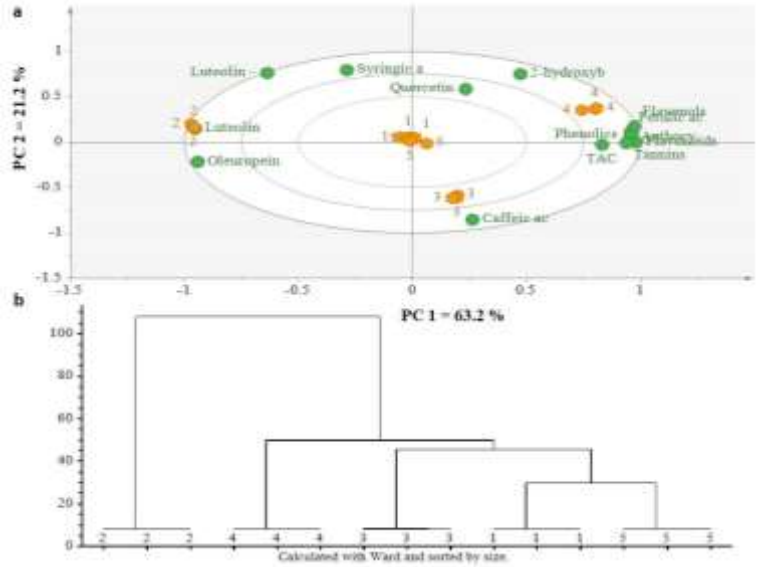

Fig. 3: PCA of polyphenolics detected by spectrophotometer and HPLC in five varieties of *O. europaea* growing in Aljouf region. (**a**) biplot of PC1 *vs*. PC2 and (**b**) hierarchical clustering analysis (HCA). Legends as described in Fig. 1

Napali DPPH significantly differed from the DPPH of other varieties ($P < 0.05$) (Fig. 4c).

Correlation between determined secondary metabolites and antioxidant activity in the drupes of different varieties of *O. europaea*

TAC in all varieties was positively correlated with the content of total anthocyanins, total phenolics, total flavonoids, total flavonols, tannins, and ferulic acid (Table 3) and negatively correlated with luteolin, luteolin-7-glucoside, and oleuropein. The rest of the polyphenols had no correlation with the TAC (Table 4). DPPH radical scavenging activity at 600 μg/mL was positively correlated with syringic acid. RP at 300 μg/mL was positively correlated with the content of anthocyanins, phenolics, flavonoids, flavonols, tannins, ferulic acid, and TAC. RP at 300 μg/mL was negatively correlated with luteolin, luteolin-7-glucoside, oleuropein, and syringic acid. RP at 600 μg/mL was positively correlated with the content of flavonoids, flavonols, tannins, ferulic acid, and TAC, and negatively correlated with luteolin, luteolin-7-glucoside, oleuropein, syringic acid, and DPPH at 300 μg/mL (Table 4).

On the level of each group that resulted from the PCA, in the Galat variety, TAC was positively correlated with flavonoids (r = 0.998, P = 0.037) and DPPH was positively correlated with caffeic acid (r = 0.999, P = 0.018). In Napali and Kalamata, DPPH was positively correlated with phenolics (r = 0.818, P = 0.04), and RP was positively correlated with phenolics (r = 0.942, P = 0.005), caffeic acid (r = 0.991, P = 0.00), and quercetin (r = 0.997, P = 0.00). In K18, TAC was positively correlated with flavonols (r = 0.999, P = 0.017), and RP was positively correlated with ferulic acid (r = 0.997, P = 0.014). In Improved Napali, DPPH was positively correlated with syringic acid (r = 0.998, P = 0.00).

Discussion

Quantitative variation of polyphenols was recognized among the drupes of olive varieties under investigation. The varieties varied in the maturation time and some varieties have green drupes. The color of drupes, which is linked to maturation, affects the content of polyphenol content. The ripened drupes of olives have higher contents of total phenolics and total anthocyanins than the premature drupes (Aprile *et al.* 2019). On the contrary, the drupes of some varieties or cultivars before maturation have more of some individual polyphenols such as tyrosol, hydroxyl tyrosol, and oleuropein than at the maturation stage (Youssef *et al.* 2010; Sohaimy *et al.* 2016; Tekaya *et al.* 2022).

Polyphenol contents in six Turkish varieties showed also a great variation (Ocakoglu *et al.* 2009). In Tunisian, Italian as well as in Greek varieties and cultivars, a great variation in the content of polyphenols was recognized (Youssef *et al.* 2011; Dekdouk *et al.* 2015; Mitsopoulos *et al.* 2016; Alexandros *et al.* 2019; Esposito *et al*. 2021; Difonzo *et al.* 2022). Not only do different cultivars or varieties have different contents of polyphenols, but qualitative differences may also be present as in Italian and Algerian cultivars (Dekdouk *et al.* 2015). The content of polyphenols varies based on the plant age and harvesting time (Petridis *et al.* 2012; Kulak and Cetinkaya 2018; Difonzo *et al.* 2022).

Olive varieties growing in KSA in the Al-Jouf region are characterized by high amounts of bioactive secondary metabolites, particularly polyphenols with their diverse chemical classes. Olive trees in arid and semiarid regions such as those growing in KSA are subjected to different types of abiotic stress such as high temperature and drought, so it is very important to consider these conditions when the high content of polyphenols in these varieties is interpreted, as these types of stress may induce secondary metabolites in plants, particularly polyphenols. The content of caffeic acid, ferulic acid, and oleuropein is high in Al-Jouf varieties which may be attributed to these stress factors. Water deficiency in olive cultivars was associated with the induction of high content of total phenolics and oleuropein in olive leaves (Petridis *et al.* 2012). The channeling of precursors of the metabolites in the same pathway and/or incorporation of some primary metabolites in the biosynthesis of these secondary metabolites may be a mechanism of synthesis and induction of these secondary metabolites.

The content of polyphenols in olive leaves, fruits, and oil is not stable and varies largely (Kulak and Cetinkaya 2018). The content of polyphenols and oil in olive drupes varied significantly based on some factors such as the cultivar or variety (Dekdouk *et al.* 2015; Mitsopoulos *et al.* 2016; Alexandros *et al.* 2019), plant individuals and population (Kulak and Cetinkaya 2018), the environmental and climatic conditions, developmental stage, harvesting stage and harvesting strategies (Vinha *et al.* 2005; Kulak and Cetinkaya 2018), the origin of sample (geographic

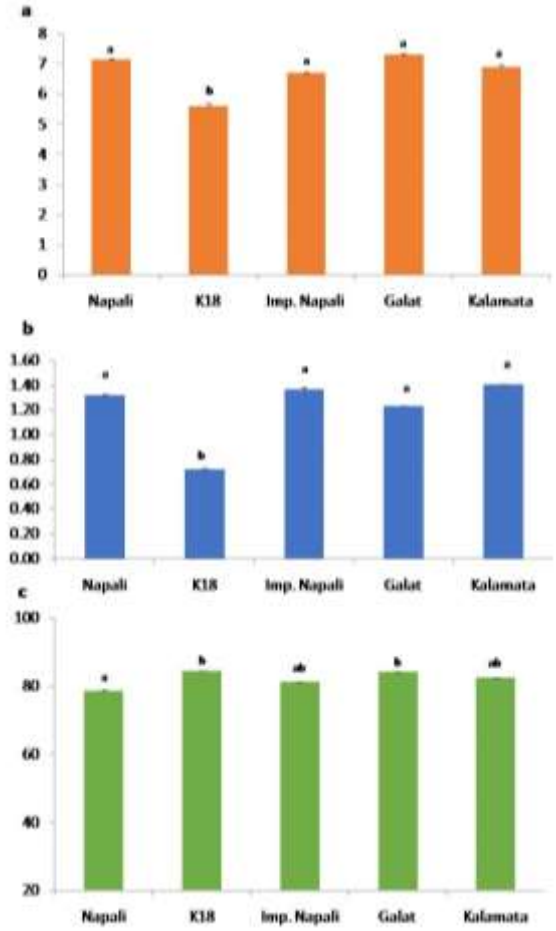

Fig. 4: Antioxidant activity of the drupes of five varieties of olive. (**a**) Total antioxidant capacity (TAC), (**b**) reducing power (at 600 μg/mL) and (**c**) DPPH radical scavenging activity (at 600 μg/mL). Different letters mean there is significant difference at $P < 0.05$

provenance and the harvesting time (Silva *et al.* 2006; Ocakoglu *et al.* 2009; Aprile *et al.* 2019: Serrano-García *et al.* 2022). Others are related to the extraction method and extraction solvents (Romani *et al.* 1999; Brahmi *et al.* 2015).

Few standard compounds were injected in HPLC and many polyphenol standards were not available, which hindered identifying all individual polyphenols in olive drupes. Although few compounds were identified and quantified, some of them showed high concentrations compared to the concentrations of these compounds in other cultivars and varieties growing in other regions (Dekdouk *et al.* 2015). Some compounds showed high concentrations in this study, including luteolin-7-glucoside, 2-hydroxybenzoic acid, and quercetin. Oleuropein, luteolin, syringic acid, and caffeic acid were found in higher concentrations in different varieties from the Al-Jouf region compared to other cultivars (Arslan and Özcan 2011; Dekdouk *et al.* 2015).

MVDA is considered as a promising tool, which, in score scatter plots, could show the similarity and dissimilarity among the varieties under investigation.

Table 4: Correlation between total antioxidant activity and determined metabolites in drupes of five olive varieties (n = 3)

P-value key color legend (*P < 0.001*) = very highly significant; *P < 0.01* = highly significant; *P < 0.05* = significant); *P > 0.05* = non-significant

When there is many data, particularly when different spectroscopic platforms are used to evaluate the antioxidant activity and polyphenols of many varieties or cultivars, it is important to use MVDA to reduce the dimensionality of the different data sets in score scatter, loading, or biplots. MVDA was previously used to discriminate between different cultivars, varieties, species, and different organs of the same species based on their metabolites content detected by different analytical techniques (Abdel-Farid *et al*. 2007, 2014; Taha *et al*. 2020; Serrano-Garcia *et al*. 2022). In the field of olive polyphenols and antioxidant activity, MVDA was successfully used to differentiate between six olive cultivars growing in the same environmental conditions in two growth stages in Spain according to their phenolics contents (Talhaoui *et al*. 2015). Principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were also used to differentiate between six Turkish cultivars grown in two successive years based on their phenolics content (Ocakoglu *et al.* 2009). NMR-based metabolomics together with PCA was used to discriminate between 19 cultivars of olive growing in South Italy (Esposito *et al.* 2021). Five Italian cultivars were evaluated using LC/MS based metabolomics combined with PCA (Difonzo *et al.* 2022).

The nutritional value and physiological properties of olive drupes are linked to the qualitative and quantitative evaluation of bioactive secondary metabolites, particularly polyphenols (Mitsopoulos *et al.* 2016). The antioxidant capacity of the drupes of Al-Jouf varieties is positively correlated with all classes of polyphenols such as phenolics, flavonoids, flavonols, anthocyanins, and tannins in addition to some individual metabolites such as ferulic acid. Several reports have studied the relation between polyphenols represented by phenolics and flavonoids and the antioxidant capacity of different cultivars and varieties from different regions, for example, Tunisia, Algeria, Spain, Greece, and Italy (Vinha *et al.* 2005; Ocakoglu *et al.* 2009; Talhaoui *et al.* 2015). This is the first report that considers the relation between polyphenols and antioxidant capacity in cultivated varieties in the Al-Jouf district in KSA. In the Galat variety, the total antioxidant capacity and DPPH were strongly positively correlated with flavonoids and caffeic acid. This may mean that the highest DPPH radical scavenging activity is associated with Galat and K18. Moreover, Galat is characterized by its high content of bioactive polyphenols such as phenolics, flavonols, tannins, and anthocyanins in addition to some individual polyphenols such as 2-hydroxybenzoic acid and ferulic acid, which may work individually or synergistically. The significant strong correlation between these polyphenols and TAC in all olive varieties and particularly in Galat demonstrates the roles of these bioactive metabolites in the antioxidant capacity of olive drupes. The negative correlation between antioxidant capacity and some other important polyphenols such as oleuropein, luteolin, and luteolin-7-glucoside does not reflect that these metabolites do not contribute to the antioxidant activity of the drupes rather, they have significant roles in the antioxidant capacity of one or more of these varieties that appeared in the level of discriminated groups resulting from MVDA. TAC and DPPH of K18 were positively correlated with flavonols and ferulic acid contents, and K18 was accompanied by a high content of oleuropein, luteolin, and luteolin-7-glucoside in the score biplot of MVDA, especially as K18 and Galat have high DPPH, which negatively affects the IC_{50} of their extracts, particularly

in Galat. In Napali and Kalamata, which showed moderate contents of all detected secondary metabolites, their TAC was positively correlated with 2-hydroxybenzoic acid, and their RP was positively correlated with many bioactive metabolites, such as phenolics, saponins, caffeic acid, and quercetin. This may explain the highest RP in Napali and Kalamata.

In our study, even though the IC_{50} of the DPPH was not determined, it was reported that there is a negative correlation between the DPPH with IC_{50}, and it was indirectly reported that the highest phenolics and other bioactive polyphenols such as anthocyanins, flavonoids, flavonols, and tannins are associated with the highest DPPH, TAC, and RP. For example, in our study, among the evaluated varieties, Galat had the highest content of total phenolics, flavonoids, flavonols, anthocyanins, and tannins, so it is expected to have the lowest IC_{50} compared to the other varieties. This reveals the importance of not only phenolics but also flavonoids, flavonols, anthocyanins, and tannins in the antioxidant properties of Al-Jouf olive fruits. In a report concerning the relation between antioxidant activity and total phenolics content in Iranian olive cultivars, a negative correlation was reported between IC_{50} and total phenolics (Ghasemi *et al.* 2018). In many previous reports, it was confirmed that the lowest IC_{50} of drupe extracts is associated with the highest content of phenolics and flavonoids (Ocakoglu *et al.* 2009; Mitsopoulos *et al.* 2016). The same is recognized with K18, which is characterized by the highest content of individual polyphenols and consequently is expected to have lower IC_{50} than Galat, which shows the importance of these individual polyphenols and their negative correlation with the IC_{50} of their extracts. The high correlation between the antioxidant activity and the content of metabolites in the evaluated cultivars was due to the high content of these polyphenolics in the drupes of olive which were documented that having a strong antioxidant activity (Mitsopoulos *et al.* 2016).

Diverse classes of polyphenols from olives are reported as antioxidants and as antihypertension, hypoglycemian (Vogel *et al.* 2015; Ahanger *et al.* 2020), anticancer (Goulas *et al.* 2009), and antimicrobial agents (Pereira *et al.* 2007; Zhao *et al.* 2009). Anthocyanins contribute to the radical scavenging activity of olive fruits with other polyphenols, which in turn, have roles as antioxidant, antitumor, and anti-inflammatory roles (Scholz *et al.* 1999; Silva and Pogačnik 2020). Individual polyphenols such as caffeic acid, oleuropein, and luteolin-7-O-glucoside have antimicrobial and antioxidant activities (Scholz *et al.* 1999; McDonald *et al.* 2001; Pereira *et al.* 2007; Omar 2010). The characterization of some Al-Jouf varieties with high content of polyphenols among their diverse classes may be the reason for the good reputation of olive oil from the Al-Jouf region in local and regional markets. These features may also be exploited in pharmaceutical industries as well as in the production of high-quality olive oil.

Conclusion

The high contents of secondary metabolites in Al-Jouf olive varieties may be attributed to the abiotic stresses such as high temperature and drought in this region as a natural climate. These abiotic stresses may enhance the accumulation of secondary metabolites, particularly polyphenols, in these varieties. Quantitative characterization of each variety will help decision-makers to select the appropriate variety for oil extraction and/or pickling. This will also determine the variety (varieties) which will be cultivated extensively in the future in KSA. As it is important to fully describe the metabolomic composition of olives, different analytical platforms combined with MVDA should be used with many varieties of olives to explore the complete picture of the olive metabolome. On the level of our small-scale study, it was clarified some varieties have major bioactive polyphenols, whereas others have moderate contents of the determined secondary metabolites under the current growth conditions.

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Author Contributions

IBA conceptualization, experimental design, writing and revising the first draft of the manuscript. MJ Data collection, performing data analysis by multivariate data analysis and interpretation and revising the manuscript. HMA analysis of plants extracts for individual polyphenols. MR, MA and IS collection of plant materials and preparation of extracts. AAAM conceptualization, experimental design, analysis of secondary metabolites and determination of antioxidant capacity, statistical analysis, writing and revising the first draft of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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