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Chemical properties of Olive (*Olea europaea* L.), date palm *(Phoenix dactylifera* L.), grape (*Vitis vinifera* L) and pomegranate (*Punica granatum*)

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Abstract

This study is aiming to screen the phytochemical constituents of four selected plants and determination of quantitative and qualitative analysis of secondary metabolizes as well as physiochemical properties of tested plant oils. Date palm seed showed high concentration of sterols, triterpens and moderate concentration of alkaloids and flavonoids, while tannins, saponin and flavonone/flavonol were not present. Grape and pomegranates showed presence of only the two secondary metabolites alkaloids and saponin. Olive showed only presence of flavinoids. Date palm showed high concentrations of sterol, triterpen and phenol (480mg/100g), with moderate concentration of alkaloids (0.2%). The Grape seed powder gave the highest phenol concentration (500mg/100g) and the highest flavinoids concentration (60mg/100g), with moderate concentration of alkaloids 0.4%. The highest concentration of total alkaloids was recorded for pomegranates as 0.6%, moreover this plant seeds showed moderate concentration of phenols. Olive seed powder showed absence of alkaloids and the lowest concentration of phenol (60 mg/100g).

The concentration of flavonoids as 40mg/100g was obtained by date palm, pomegranate and olive.The Free Fatty Acids (FFA) content in grape seed oil was found to be 10.7% ,while in date palm seed oil, pomegranate seed oil and olive seed oil 4.5% , 2.8% , (31.02 – 21.99)% respectively. The level of refract index in grape seed oil 1.4720, in olive oils 1.4519 – 1.4652. While in pomegranates, date palm is 1.5129, 1.4549. Iodine number of date palm seed was found 68,58 while in grape 73.66 , in pomegranate 93.98 , in olive (152.4 – 86.36).

Keywords: Chemical properties**,** Olive, date palm, grape, pomegranate**.**

Introduction

Olive, date palm ,grape and pomegranate were proved to have antimicrobial and anti oxidant activities (Ghisalberti,2008). Olive (*Olea europaea* L.) has a long history of medicinal and nutritional values. (Soler-Rivas *et al*.,2000). The health benefits of olive oil are mainly ascribed to the presence of high content of monounsaturated fatty acid (MUFAs) and functional bio actives including tocopherols, carotenoids, phospholipids and phenolics, with multiple biological activities (Covas *et al*.,2006).

The current explosion of interest in pomegranate (*Punica granatum*) as a medicinal and nutritional product is evidenced by a MedLine (year) search from 2000 to present, revealing over 150 new scientific papers pertaining to its health effects. Pomegranate mediated antioxidant activity can be considered a means of lowering the threshold for inflammation. Antioxidant activity, as well as suppression of inflammation, may contribute to chemotherapeutic and chemo preventiveutility against cancer. The phytochemical composition of grapevine (*Vitis vinifera* L) includes a great variety of known bioactive compounds such as vitamin E, flavonoids, linoleic acid and procyanidins (also known as condensed tannins, and oligomeric proanthocyanidins) the last of which are highly concentrated in grape seeds Acording to Yilmaz and Toledo (2004) resveratrol is another very important example of grape's health promoting compounds in addition to the powerful antioxidant properties

Date palm *(Phoenix dactylifera* Linn.) was the only native plant species among the four selected plant species to conduct this study. It contains digestible sugar (70%), mainly glucose,fructose,sucrose,dietary fiber and less amount of protein and fats. They also contain vitamins like riboflavin, thiamine, biotin, folic and ascorbic acids that are essential for the body (Al Farsi and Lee,2008). It is rich in iron, calcium, cobalt, copper, fluorine, magnesium, manganese, potassium, phosphorus, sodium, copper,

sulfur, boron, selenium and zinc(Al Farsi and Lee,2008) . Colegate and Molyneux (2008) bio-activity of phytochemicals from a given plant.

Assay methods vary depending on what bioactivity is target and these may include antimicrobial as well as anticancer activity

The objectives:

1. This study is aiming to screen and explore the phytochemical constituents of four selected plants
2. Determination of quantitative and qualitative analysis of secondary metabolizes of four the tested plant species.
3. Determination of physiochemical properties of selected plant seeds oils.

Methodology

Plant material used: Seeds of olive (*Olea europea* L .),date palm (*Phoenix dactylifera* L.),pomegranate (*Punica granatum*) and grape (*Vitis vinifera* L) were used in this study. They were collected and developed for use in the phytomedicine programme (Eloff ,1998).

Phytochemical assessment of secondary metabolites :

- Qualitative analysis include: Test for flavonoids , saponins , sterols and/or triterpens had been carried out according to (Harborne,1998). Test for tannins and alkaloids and/or nitrogen bases According to (Balbaa,1974).
- Quantitative analysis include:

1. Determination of total flavonoids according to (Zhuange et al.,1992).
2. Determination of total phenolic compounds : The colorimetric method of Folin Denis as described by Swain and Hillis (1959).
3. Determination of total alkaloids according to Sabri et al., (1973).

Physiochemical analysis of oils : This include; Iodine Number, Free Fatty Acids, Acid Value and Refractive Index were carried according to AOCS (1993).

TLC-separated components: Separation and isolation of oil extract was done by using the procedure of Stahel (1964).

The developing TLC solvent system: Hexane /Diethyl ether /formic acid (80:20:2).Ferric Chloride ($FeCl_3.6H_2O$) reagent.

Results and Discussion

Qualitative analysis of seeds powder: Results shown in Table (1) displayed high variation in the chemical constituents of seeds of the four selected plant species thes component mainly are alkaloids, flavonoids, tannins, sterols, triterpens, saponins , flavonon and flavonol . Date palm seed extract showed high concentration of sterols, triterpens and moderate concentration of alkaloids and flavonoids, meanwhile tannins, saponin and flavonone/flavonol were not present. These results were in agreement with Al-Farsi and Lee (2008). Whereas, grape showed moderate concentration of alkaloids and saponin, while pomegranates showed high saponin concentrations meanwhile it was moderate in alkaloid . In addition to that, olive showed moderate concentration of flavonoids. This result are not in a line with Kennedy (2008) who reported absence of flavonoids in grape this could be attributed to different climatic conditions. Both grape and pomegranate showed absence of other secondary metabolizes such as sterol and steroids. These results were also not in a line with Tsuyuki *et al*. (1981) who found sterol and steroids in pomegranates. Olive showed only presence of flavinoids at moderate concentration unlike results reported by Ghanbari *et al*.(2012) who found phytosterols, carotenoids, chlorophyll and squalen in olive. Other metabolizes such as tannin, flavonon and flavonol were absent in all the samples tested. Nevertheless, Yilmaz and Toledo(2004) found high concentration of tannins in grape. Differences among studies with respect to presence and absence of the second arymetabolizes might be attribute to differences in biological and environmental factors such as chemical and physical properties of plants, type of soil, availability of nutrients, temperature and relative humidity (Gargouri *et al*.,2006).

The importance of the bioactive natural products come from the fact that they are chemical compounds produced by living organism and exert biological effect on other organism. Some include therapeutic activity for diseases of human and animal (Colegate and Molyneux, 2008). In this regard, it was believed that natural products researches remain one of the main means of discovering bioactive compounds (Ghisalberti, 2008).

Table (1): Qualitative analysis of secondary metabolites in seed extract of date palm ,grape ,olive and pomegranate .

Secondary Metabolites	Tested plants			
	Date palm	Grape	Olive	**Pomegranates**
Tannins	-	-	-	**-**
Sterols and / or Triterpens	++	-	-	**-**
Alkaloids	+	+	-	+
Flavonoids	+	-	+	**-**
Saponins	-	+	-	++
Flavonon / Flavonol	-	-	-	**-**

The symbol ++ , + , - indicate a compound present in high , moderate and absent level respectivley.

4.3. Quantitative analysis of seed extracts

The values of total alkaloids, phenolics and flavonoids content varied among samples of the four species, as presented in Table (2) However, the alkaloids exist in large proportions in the seeds and roots of pants and often in combination with vegetable acids (Madziga *et al.*, 2010). The highest amount of alkaloids was found in seed of pomegranates (0.6%) followed by grape (0.4%) and date palm (0.2%). Olive showed absence of alkaloids.

With respect to phenol content, grape gave the highest concentration of phenols of 500 mg/100g followed by date palm (480 mg/100g), pomegranates (330mg/100g) and olive (60 mg/100g). Results were comparable to those of Al- Turki (2008) who found polyphenolic content of 507.03to 225.02 mg as gallic acid equivalent and antioxidant activity ranged from1400.00 to 228.06 of date palm. The interest on phenolic compounds is related with their antioxidant activity and promotion of health benefits (Ryan *et al*., 2003). Andrew *et al*. (2010) found similar results of 500mg/100g in grape. Whereas, Al-farsi and Lee (2008) and Li He *et al*. (2012) found higher concentrations of phenols of 3942 mg/100g and 651.7 to

4854.7 mg/100 g in date palms and pomegranate, respectively.

Table (2): Determination of total alkaloids, total phenolics and total flavonoids

Secondary Metabolites	Tested plants			
	Date palm	Grape	Olive	**Pomegranates**
Total Alkaloids %	0.2	0.4	-	**0.6**
Total Phenols mg/100g	480	500	60	**330**
Total Flavonoids mg/100g	40	60	40	**40**

Physiochemical properties of tested plant seeds oils

Different physical and chemical parameters of edible oil were used to monitor the compositional quality of oils (Mousavi *et al.*, 2012). Table (3) and Figure (1) are represented physiochemical properties of four type of seeds oils including free fatty acids, acid value, iodine number and refractive index. The free fatty acid ratio is an important quality characteristic. The lower it is, the better the storage and shelf life of the respective oil or fat will be. FFA are a source of flavors and aromas. Free fatty acid (FFA) content, in date palm seed oil was found to be 4.5% while 2.4% was reported by (Mirghani *et al*.,2009) .In this study the FFA in grape seed oil was 10.7% while Xueli and Yoichiro (2003) reported the unsaturated fatty acids make up about 70% in the oil on the basis

of free fatty acids.(we cannot compare FFA with unsaturated FF) FFA in pomegranate oil 2.8 and low free fatty acid content (0.65% as punicic acid) (Khoddami *et al*.,2014).

In olive oil if the FFA is high, e.g. over 0.8%, it indicates that there has been fruit damage (frost, bruising), delays between harvest and processing or harvesting of over-ripe fruit.

Setting a FFA level lower than 0.8% can provide a useful standard to ensure that growers provide high Iodine number shows the saturation value of oils, therefore by increase of iodine number, the number of double bonds in oil decreased. Previous studied have reported lower or higher content of Iodine number as compared with our finding depending on seed origin, variety and method of oil extraction. Which conceder higher than reported by Mirghani *et al* (2009). The iodine value of palm oil seed was found to be 68.58 which means that this oil is containing saturated fatty acids and this is agreed by Thomas (2002) who reported 51.6–54.8 for kernel oil of date palm. has been shown higher value in grape seed oil 124 – 143, also FAO (2001) reported the limit of acceptable of iodine number in grape seed oils is 128 – 150 and lower value in olive oil 80 – 88. This result indicate that olive oil is non-drying, highly unsaturated and it suggests that it contain high levels of oleic and linoleic acids (Elleuch *et al*., 2007). While results reported by Habibnia *et al*., (2012) showed higher value in pomegranate 225–229.As refract index shows the quality of oil, the value of this index will increase of compounds with higher molecular weight in oil and oxidation reaction. During oxidation process some initial compounds produce such as peroxide, aldehydes, ketones, and alcohols. These compounds can combine with each other and produce some compounds with high molecular weight which caused to increased refract index and density. The results of this study that the level of refract index in grape seed oil 1.4720. it was according to international standard 1.467 – 1.477 (FAO, 2001). The refractive index of pomegranate seed oil has fluctuated between 1.502 and 1.519. According to Turkish Food Codex Plant oils mentioned by name (2012), the refractive index of palm seed oil is 1.1448 – 1.4520. pomegranate varieties have higher refractive index other than other plant oils. This is one of the pomegranate seed oils characteristic specifications (Karatas and Ozdogan, 2014). The refractive index of olive oils in our study 1.4519 – 1.4652 while Borchani *et al* (2010) reported the refractive index of olive oils 1.4710.

TLC-separated components of the crude oil extracts of all species

Results of TLC separation of oil extract of all species are presented in (Figure 2).

Fig (1) : Photographs of tested plants seeds oils

Table (3): Physicochemical properties of fruits seeds oil

Tested Plants	F.F.A %*	Acid value	I No**	RI***	Oil color
Date palm	4.512	9.024	68.58	1.4549	Green
Grape R	10.716	21.432	73.66	1.4720	Light yellow
Pomegranate	5.64	11.28	93.98	1.5129	Dark orange
Olive B	31.02	62.04	152.4	1.4519	Dark green

Olive G	21.996	43.992	86.36	1.4640	Yellowish green
Local olive	1.692	3.384	63.5	1.4652	Yellowish

*Free Faty Acid ** Iodine number ****Refractive Index

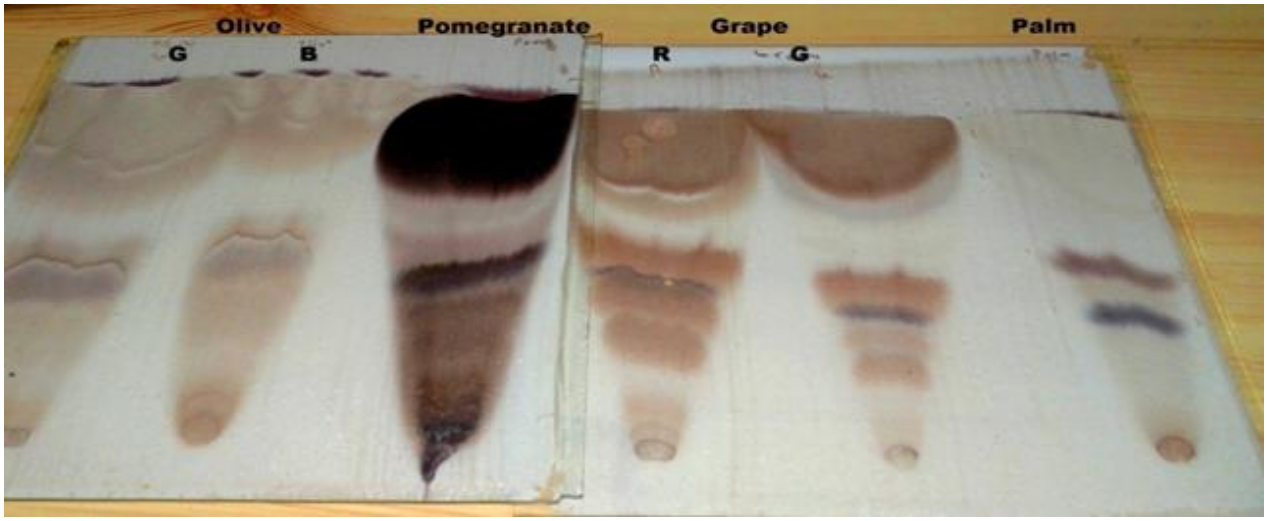

Fig (2): TLC separated Components of Seeds oils of fruits' olive , pomegranate , grape and date palm.

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