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Polyphenols with antioxidant activity

Research article

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Abstract
Polyphenols from natural fruits were extracted in ethanol and determined using Folin-Ciocalteu method.

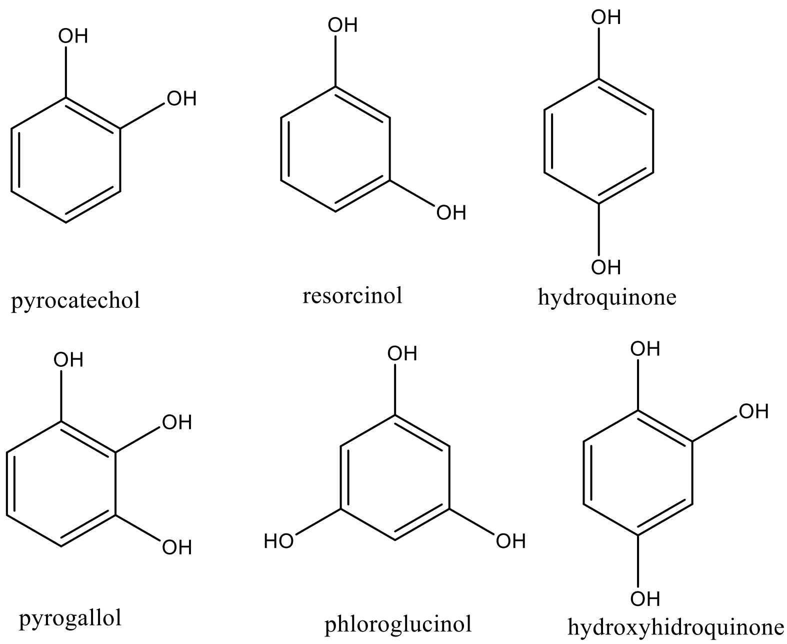
Keywords: polyphenols, ethanol extraction, Folin-Ciocalteu method, UV-Vis spectrometry

1. INTRODUCTION

Natural antioxidants are present in plants. Major sources are fruits (apples, citrus fruits, red and purple hued fruits), legumes (yellow and green peas, chickpea, lentils, common beans, fava beans, beach bean, and yellow and black soybeans), vegetables (garlic, tomatoes, potato, cabbage, yellow and red onion), olive, nuts, oilseeds, whole grains, green tea, black tea, coffee, wine, beer, herbs and spices [1]. C and E vitamins, carotenoids and phenolic compounds are typical antioxidants.

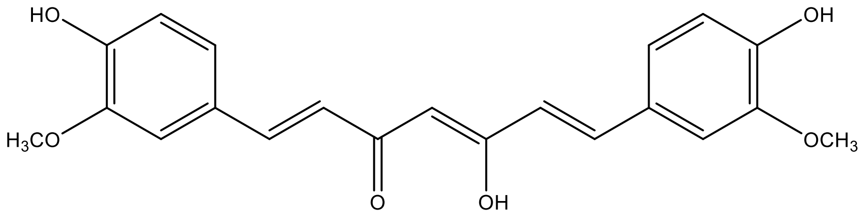
Structurally, polyphenols contain two or more phenolic groups. The simplest representatives of the series are pyrocatechol, resorcinol, hydroquinone, pyrogallol, phloroglucinol, hydroxihydroquinone (Scheme 1).

The main phenolic compounds are diferuloylmethane (curcumin), stilbenes (trans-resveratrol), flavonoids (flavonols, flavones, isoflavones, flavanones, anthocyanidins, flavanols), phenolic acids (caffeic, ferulic and coumaric acids) and tannins (gallotannins and ellagitannins) [2].



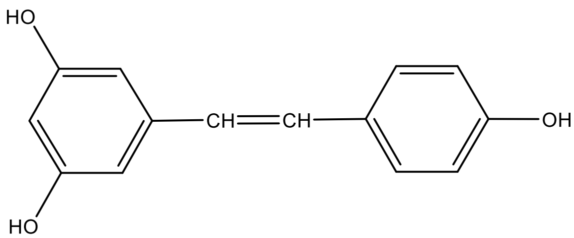
Scheme 1. Structure of some polyphenols

Curcumin named 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (Scheme 2) is found in turmeric and have antioxidant, anti-inflammatory, antimutagenic, antimicrobial and anticancer activity [3].



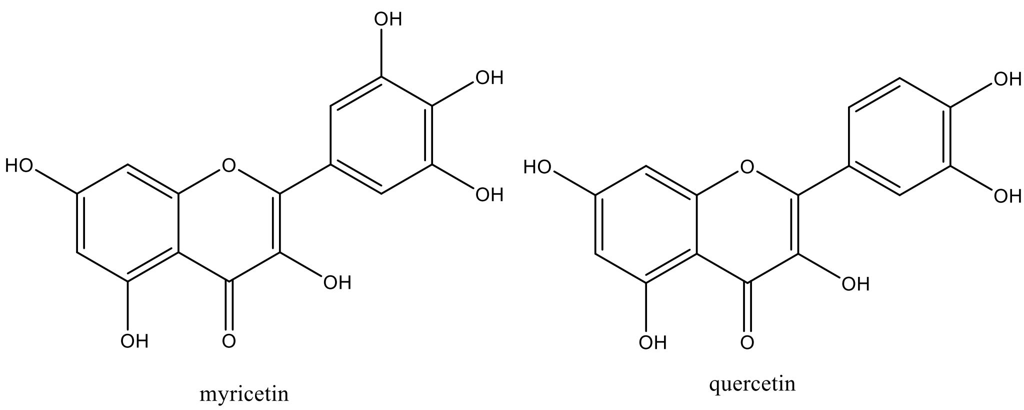
Scheme 2. Structure of curcumin

Resveratrol (3,5,4′-trihidroxistilbene) (Scheme 3) is found in red wine in 1-3 mg/L [4].



Scheme 3. Structure of resveratrol

Myricetin, fisetin, quercetin and kaempferol are the most important flavonols. Myricetin (Scheme 4) is found in black grapes peels, but it is not found in white grapes [5]. Quercetin (Scheme 4) is a flavonoid present in kales, onions, berries, apples, red grapes, broccoli, cherries and red wine [6].



Scheme 4. Structure of myricetin and quercetin

It is the purpose of this research to extracte the polyphenols from some fruits in ethanol and to determine using Folin-Ciocalteu method.

2. MATERIALS AND METHODS

2.1. Materials

Ethanol, sodium carbonate, Folin-Ciocalteu reagent were purchased from Fluka or Aldrich. All the chemicals were analytical grade and used directly without any further purification. All sample solutions were prepared with deionized water.

2.2. Apparatus

UV-Vis spectra of the analyzed extract were recorded from 200-800 nm by using an UV-Vis Varian Cary 50 Bio spectrophotometer.

2.3. Methods

2.3.1. Polyphenols extraction

1 g of analyzed fruit was crushed and finely ground with 10 mL ethanol. It was filtered and the obtained filtrate was used for the total soluble phenols dosage.

2.3.2. Calibration curve drawing

A stock solution of gallic acid (0.4 mg·mL^{-1}) was diluted in order to obtain 6 solutions with concentrations between 25 and 400 μg·mL^{-1}, as shown in Table 1. 0,1 mL of each solution, 1 mL of 10-fold diluted Folin-Ciocalteu and 1 mL of 0.4M Na_2CO_3 solutions have been added. After 15 minutes, the absorbances have been read at 765 nm.

Table 1. The absorbances of gallic acid solutions

A_{765}/nm	Concentration/μg·mL^{-1}
0.15	25
0.29	50
0.41	100
0.79	200
1.08	300
1.56	400

The calibration curve (Figure 2) has been obtained.

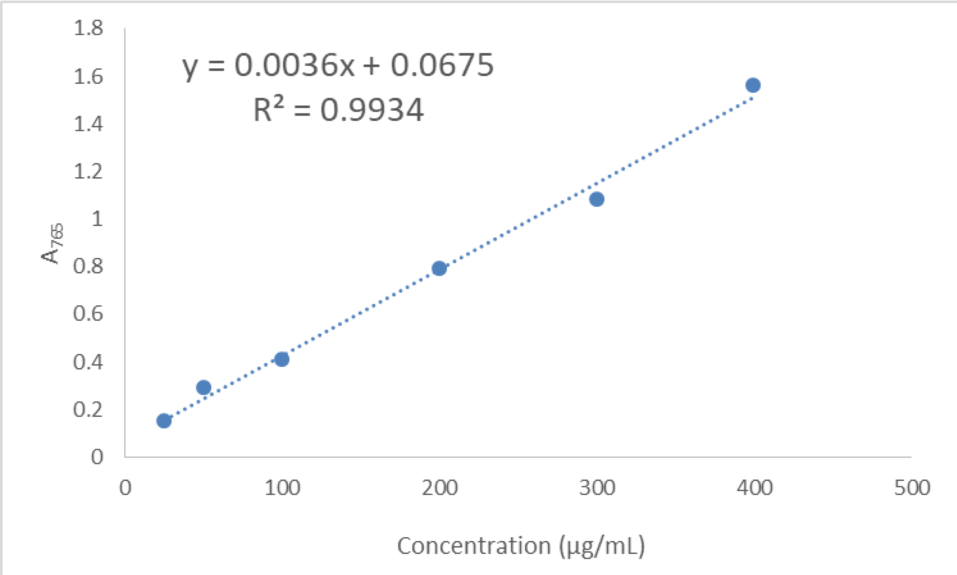


Figure 2. Gallic acid calibration curve

2.3.3. Total soluble phenols dosage

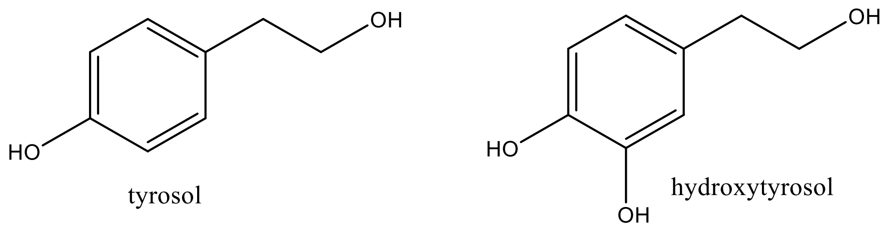
Total phenolic content from ethanolic extract was determined using Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent was diluted 1:9 with deionized water. 0.1 mL ethanolic extract, 1 mL diluted Folin-Ciocalteu reagent and 1 mL Na_2CO_3 0.4 M solution were mixed. The obtained mixture was left to rest for 15 minutes. Finally, the UV-Vis spectra were recorded and the absorbances were read at 765 nm.

3. RESULTS AND DISCUSSION

Phenolic compounds are secondary metabolites of the plants that play an important role in the defensive mechanism of the plants against diseases or harmful factors. They also have an important role in dissemination of the species. The great interest for this class of compounds is given by the antioxidant activity. This effect is assigned to the polyphenols's capacity to "catch" the free radicals using their groups of hydroxyls.

For the dosage of the soluble phenols we used the following vegetal materials: olives, grapes, blackberries, raspberries, blueberries, sea buckthorn and sour cherries.

The Mediterranean diet, known for its benefit to our health, is based on olives and olive oil. The olives have a mass of poliphenols about 1-3% of the weight of the fresh fruit. The main antioxidant compounds found in olives are tyrosol and hydroxytyrosol (Scheme 5) [7]:



Scheme 5. Structures of tyrosol and hydroxytyrosol

Grapes, especially the black ones, contain the strongest antioxidant: resveratrol, which is also the pigment that gives the purple colour. Blackberries have a significant quantity of polyphenols such as: anthocyanins, ellagic acid, quercetin, gallic acid, cyanidin, pelargonidin, catechins and salicylic acid [8]. Raspberries contain strong antioxidants such as: anthocyanidins, flavonols, quercetin, α and β-carotene and C vitamin [9]. Blueberries are rich in anthocyanins, polyphenols and flavonoids with antioxidant capacities [10]. Sea-buckthorn is rich in vitamin C (twice more than rosehip and ten times more than citrus), flavonoids and β-caroten (in bigger proportion than carrots) and it has good cardioprotective activity [11].

Total phenolic content expressed in terms of gallic equivalent (mg of gallic acid equivalent/g of extract) was obtained using the following equation (1):

$$T = C \times \frac{V}{M} \quad (1)$$

T= total phenolic content ($mg \cdot g^{-1}$ extract), named EAG;
C= gallic acid concentration ($mg \cdot mL^{-1}$)
M= mass of the analyzed sample
V= sample volume

From Table 2 and Figure 3, we can see that olives represent the richest source of antioxidants. At the opposite pole, there is the sour cherry which represents a small quantity of natural antioxidants. After olives comes the frozen sea buckthorn which is known for its antioxidant properties. Also, we can observe that after they are frozen fruits lose some of their antioxidant properties. Frozen raspberry loses more than half of the concentration of antioxidants that fresh raspberry has. It is known that grapes present a huge quantity of antioxidants in peels, seeds and pulp, but in this study we used just the pulp. This is the reason why we obtain a smaller quantity of antioxidants than what is scientifically proven.

Table 2. The absorbances of analysed solutions

No.	Analysed fruits	A_{765}/ nm	Concentration/ $\mu g \cdot mL^{-1}$	Phenols amount/ $\mu g \cdot g^{-1}$
1	Grapes	0.39	91.16	9.11
2	Blackberries	0.66	165.9	16.59
3	Raspberries	0.64	161.43	16.14
4	Frozen raspberries	0.34	77.82	7.78
5	Cranberries	0.77	195.69	19.56
6	Frozen cranberries	0.59	145.39	14.53
7	Olives	1.41	371.56	37.15
8	Sour cherries	0.33	73.77	7.37
9	Frozen sea buckthorn	1.04	270.51	27.05

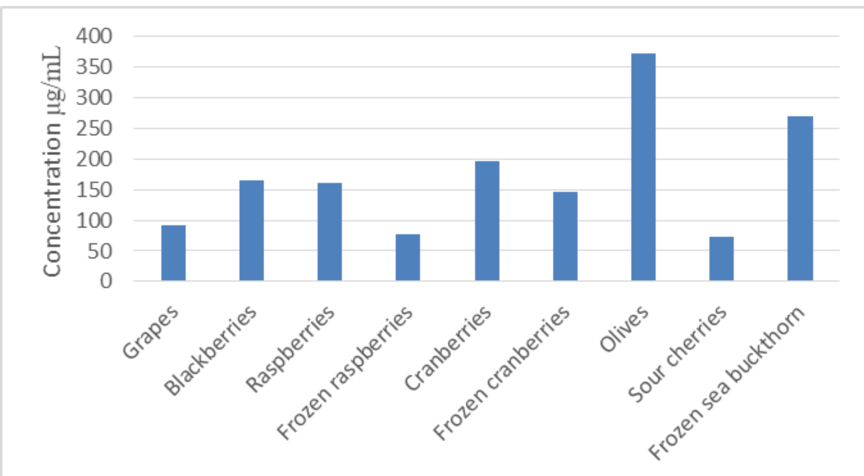


Figure 3. The polyphenol content of the analyzed samples

4. CONCLUSION

The polyphenol content of olives, grapes, blackberries, raspberries, blueberries, sea buckthorn and sour cherries was determined using Folin-Ciocalteu method. The highest amount of polyphenols is found in olives and the lowest in sour cherries. By freezing, the amount of polyphenols decreases.

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