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Total Phenolic Content, Total Flavonoid and Antioxidant Activity of Methanolic and Ethanolic Extract of the Flowers of a Fruit Tree *Cydoniaoblonga*: Quince from Eastern Algeria

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ABSTRACT

Cydonia oblonga is a fruit tree of the Rosaceae family. The objective of this study is the evaluation by spectrophotometric assay, of polyphenols and flavonoids as well as the antioxidant activity by DPPH scavenging tests, of the flowers of *Cydonia oblonga*from eastern Algeria. The determination of the total phenolic contents, shows that the highest content was measured for the methanolic extract, with a value of 135.50±1.21 mg GAE/g, followed by the ethanolic extracts, with a value of 75.25±0.9 mg GAE/g. The determination of the total flavonoids shows that the highest content was measured for the methanolic extract, with a value of 209.69 ±2.42 mg CE/g followed by the ethanolic extract with a value of 174.67± 1.81 mg CE/g. In antioxidant measurement the methanol extract have ,the higher percentage of DPPH inhibition with the IC_{50} equal to 1.70± 0.26 mg/ml, followed by ethanolic extract with value 2.50±0.38 mg/ml.The results showed that the methanolic extract had a highest amount, of total phenol and total flavonoid which by consequently gives it the highest antioxidant activity.

Keywords: *Cydonia oblonga*, flavonoids, polyphenols, antioxtdant activities

Introduction

The quince tree, *Cydonia oblonga* is a fruit tree, from the Rosaceae family in Pomaceae tribe. Our study focuses on the evaluation of phenolics content and flavonoids content by spectrophotometric assay, as well as the antioxidant activity by the DPPH method of methanolic and ethanolic extracts of flowers of *Cydonia oblonga* from eastern Algeria. Quince is native to southwestern Europe and Asia Minor [1], produces golden yellow pome fruits when ripe. Quince is a rich source of pectins and containing higher amounts of these compounds than apples [2].
Quince is also a rich source of polyphenolic compounds, where Quince pulp contains a much lower amount of phenolic compounds than quince skin [3].
The polyphenols present in the fruits of the quince can have preventive or protective effects against several diseases, in particular antiulcer effects [4], chronic inflammation [5], atopic

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dermatitis [6]. Alesiani et al. (2010) identified several compounds with antiproliferative effects in quince peels although [7], however the study of Carvalho et al. (2010) could not show the inhibitory effects of quince on human kidney and colon cancer cells [8]. Moreover, the leaves and fruits of the quince are used for the medicinal treatment of various ailments, including cariovascular diseases, hemorrhoids, bronchial asthma and cough [9].
The present study was carried out to characterize the total phenolic content, total flavonoid content and the antioxidant activities of the flowers of the eastern Algerian quince.

1. Materials and methods

1.1 Chemicals and reagents

Methanol, Ethanol, Folin-Ciocalteau reagent, Sodium Carbonate, Gallic acid, Aluminium chloride, Catechin, 1,1-diphényl-2picrylhydrazyle (DPPH).

1.2 Plant material

The flowers of *Cydonia oblonga* was collected in April 2019, during the flowering period in the region of Annaba and identified by Dr. Hamel Tarek. Department of Biology University Badji Mokhtar Annaba. The flowers were put to dry at room temperature protected from light, then reduced to powder.

Figure 1. Flower for *Cydonia oblonga*.

1.3 Preparation of extracts

Our extracts were prepared from 10g of the powder of the flowers of *Cydonia oblonga*, the powder was put in an ultrasonic bath, with a gradient of increasing temperature going from 25°C to 60°C. using two different solvents, methanol (100%) and ethanol (100%), for 10 min at a temperature of 25°C, after a rest of 10 min, this same solution was put back for 10 min at a temperature of 40°C, and after another rest of 10 min this same solution was put back for another 10 min at a temperature of 60°C. The extracts obtained were filtered and evaporated, under reduced pressure and at a temperature between 40 and 60°C to obtain dry extracts.

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1.4 Qualitative study

The existence of coumarins, flavonoids, alkaloids, and phenolic compounds was qualitatively evaluated in powder and extracts of *Cydonia oblonga*.The qualitative phytochemical screening tests were performed by the following qualitative tests:

1.4.1 Test for alkaloids

The tests are carried out by precipitation reactions with Dragendorff's reagent. Place 10 g of dry vegetable powder in an Erlenmeyer flask, to which 50 ml of H_2SO_4 diluted 1/10 with distilled water is added. This mixture was stirred and macerated for 24 h. Then, in 1 ml of the filtrate, 5 drops of Dragendorff's reagent are added. The appearance of an orange precipitate reveals the presence of alkaloids [9].

1.4.2 Test for flavonoids

Flavonoids have been investigated by the reaction to cyaniding, 2 ml of each extract were evaporated, and the residue was taken up in 5 ml of hydrochloric alcohol diluted twice. By adding 2 to 3 magnesium shavings, there is a release of heat, then a pink-orange or purplish color. The addition of 3 drops of isoamyl alcohol intensified this coloration which confirmed the presence of flavonoids [9]

1.4.3Test for phenolic compounds

2 ml of test solution and iodine solution in the test tube is applied. The presence of phenol was suggested after 2-4 minutes of red color formation [10]

1.4.4 Test for coumarinic compound

In a capsule, 5 ml of etherextract is evaporated, and then 2 ml of hot water is added to the residue.The solution is shared between 2 test tubes.To the contents of one of the tubes, 0.5 ml of NH_4OH is added at 25%. Fluorescence is observed under U.V at 366 nm. Intense fluorescence in the tube where it was added ammonia indicates the presence of coumarins[11].

1.5 Quantitative analysis

1.5.1 Total phenolic content

By the method of folinciocalteu with some modifications (Slinkard and Singleton, 1977) [12].We determined the content of total polyphenols (TPC) contained in the extracts of flowers of *Cydonia oblonga*. Firstly, we prepare a mixture of three solutions with 1.5 ml of distilled water, 500 µl of 7% sodium carbonate (Na_2CO_3) and 200 µl of the extract. After 3 min, 1 ml of folinciocalteu reagent was added. Then the blank is prepared by mixing 1 ml of folinciocalteu reagent, 500 µl of methanol, 3.8 ml of distilled water and 2 ml of Na_2CO_3 in a test tube. All the preparations are left for 2 hours at room temperature in the obscurity, after the termination of the oxidation of phenolic compounds, the color change from yellow to blue is observed. This can be read by a spectrophotometer against a white at 760 nm.The standard curve was constructed using gallic acid, in the range of Parallel a gallic acid standard is prepared at different concentrations (100, 50, 25, 12.5 mg/ml).The TPC was calculated from the standard curve, y= 0.0044x+0.0195, R^2=0.9986 and the TPC was expressed as (mg of GAE / g of extra).The result was presented as mean ± standard deviation (Figure 2).

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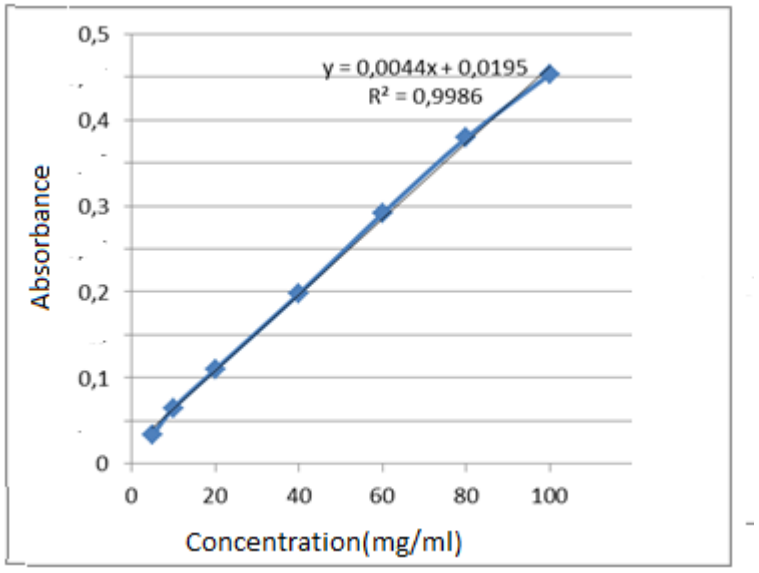

Figure 2. Standard Curve of gallic acid

1.5.2 The total flavonoids content

The total flavonoids content was measured by spectrophotometry, at 415 nm by the method of Arvouet-Grand et al (1994) [13]. The sample solutions are prepared by mixing 1 ml of the plant extract and 1 ml of aluminum chloride 2%. The blank is prepared in parallel with 1 ml of the extract and 1 ml of the methanol. All preparations were put in the obscurity at room temperature for 10 min. The absorbance is read at 415 nm. The standard curve was constructed using catechin in the range of concentrations (100, 50, 25, 12.5 mg / ml).The TFC was calculated from the standard curve, y= 0.0022 x+0.0022, R^2=0.9996 and the TFC was expressed as (mg of GAE/g of extract).The result was presented as mean ± standard deviation (Figure 3).

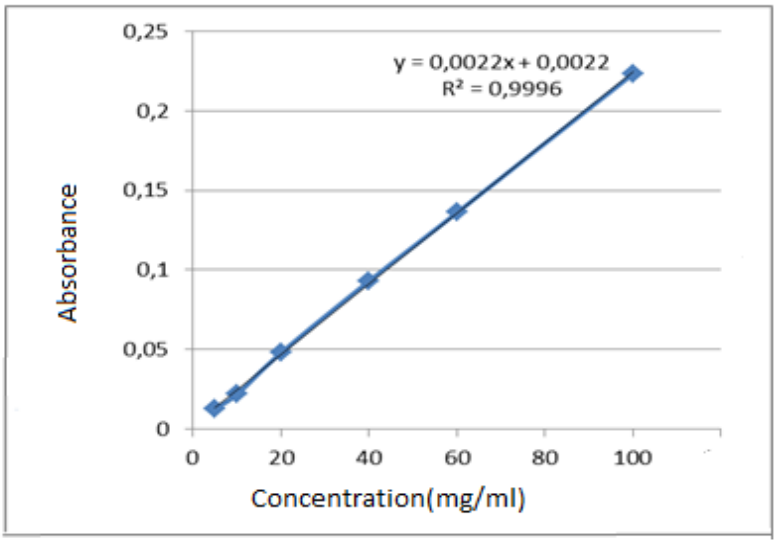

Figure 3. Standard Curve of catechin

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1.6 Antioxidant activit

1.6.1 DPPH free radical scavenging capacity

The antioxidant capacity of methanolic and ethanolic extracts was determined by the spectrophotometric method DPPH. A decrease in absorbance at 517 nm is due to the reduction of free radicals DPPH by the antioxidants present in the plant extracts which act as hydrogen donor [14].The DPPH free radical scavenging activity was tested using Sarikurkcu et al method [15] with some modification. Briefly, sample solutions were prepared with 1 ml of DPPH solution (0.4 mM), 1 ml of methanol and 1 ml of extract. While control was prepared without the addition of the extract, and the blank was methanol only. All preparations were put in the obscurity at room temperature. After 30 min the absorbance was read at 517 nm, the DPPH scavenging ratio (I%) of extract samples were calculated using the following formula :

$$I\% = 100 \times (A_0 - A_1) / A_0$$

A_0: Absorbance of control.
A_1: Absorbance of extract

The scavenging activity on DPPH was evaluated by, the determination of the half maximal inhibitory concentration (IC_{50}) value of the extract. A low IC_{50} refers to a high antioxidant capacity.

2. Results and discussion

2.1 Preliminary phytochemical analysis

The methanolic and ethanolic extract of the *Cydonia oblonga* flowers, reveal the presence of the coumarins, the flavonoids, the phenolic compounds and the absence of alkaloids .**Table 1** shows the result of the preliminary phytochemical analysis of the flowers of *Cydonia oblonga.*

Table 1. Preliminary phytochemical analysis of methanolic and ethanolic extract of the flowers of *Cydonia oblonga*

Compound	Extract	Result
Polyphenols	Methanol extract	+++
	Ethanol extract	+++
Flavonoids	Methanol extract	++

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	Ethanol extract	+++
Coumarins	Methanol extract	++
	Ethanol extract	+
Alkaloids	Methanol extract	-
	Ethanol extract	-

+ : weak presence, ++ : Average presence , +++: strong presence, -: Absence

2.2 Total phenolic and flavonoid contents

The results obtained by this work show, that the methanolic and ethanolic extracts of the flowers of *Cydonia oblonga* are rich in TPC and TFC. For the methanolic extract, the content of the TPC is 135.50 ± 1.21 mg GAE/g followed by the ethanolic extract which is 75.25 ± 0.9 mg GAE/g and for the TFC, the content for the methanolic extract is 209.67 ± 2.43 mg CE /g followed by the ethanolic extract which is 174.67 ± 1.81 this was affirmed by the results obtained from the fruit of *Cydonia oblonga* Mill, by (Papp et al 2013)[16] And from *Cydonia vulgaris pers*, which is rich in TPT with a value of 147.47 ±1.05 by (Kiselova et al 2004) [17]

Table 2. Total phenolic and total flavonoid content, of two extracts obtained from flowers for *Cydonia oblonga.*

Extract	TPC(mg GAE/g)	TFC(mg CE/g)
Methanol extract	135.50 ±1.21	209.67± 2.43
Ethanol extract	75.25± 0.9	174.67±1.81

All values are expressed mean ±SD.
GAE: Gallic acid equivalents.
CE : Catechinequivalents.
TPC : Total phenolic content.
TFC: Total flavonoid content.

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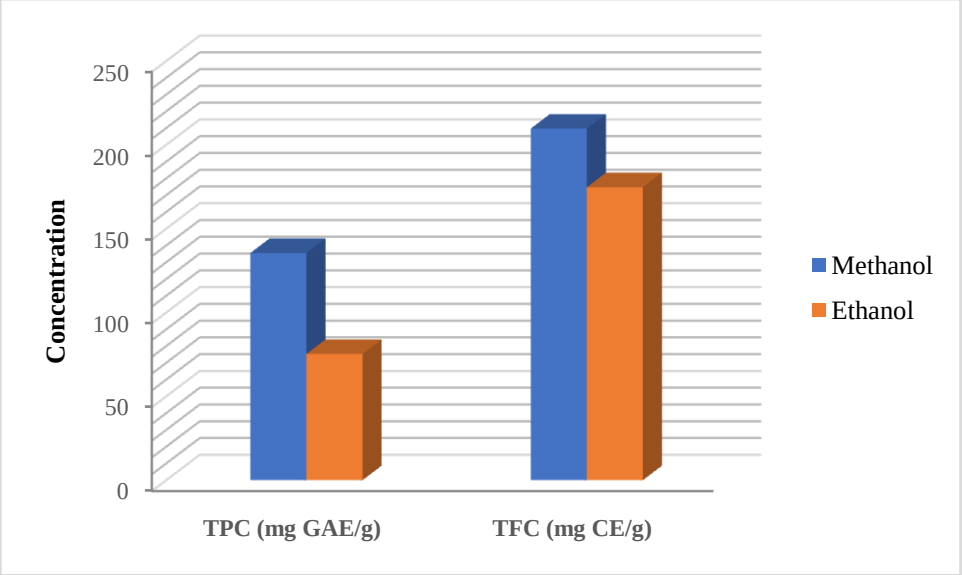

Figure 4. Total phenolic, total flavonoid content, of two extracts obtained from flowers for *Cydonia oblonga*

2.3 Antioxidant activity

The scavenging activity of DPPH free radical was expressed in terms of IC_{50} .In the present research, the two extracts of *Cydonia oblonga*have an excellent scavenging capacity on DPPH free radicals. Methanolic extract of *Cydonia oblonga*has a higher antioxidant capacity compared with the ethanolic extract with an IC_{50} (1.70 ± 0.26 mg/ml). Which is in correlation with the results obtained for the leaves of *Cydonia vulgaris*Pers, which are a source of natural antioxidants compounds by (Yildirim et al 2001) [18], and correlated with the results obtained from the Fruit (Pulp, Peel, Seed and Jam) from *Cydonia oblonga*Miller, when the IC_{50} values of organic acid fractions of quince (pulp, peel, and jam) were correlated with the ascorbic acid and citric acid contents by (BRANCA et al 2004) [19]. It is evident from the findings that the antioxidant activity,for flowers of *Cydonia oblonga* extracts is due to their high amounts in polyphenols and flavonoids contents.The results of DPPH free radical scavenging activities were showen in **Table 2.**

Table 2. The results of DPPH free radical scavenging activities of two extracts obtained from flowers for *Cydonia oblonga*

Extract	IC_{50}(mg/ml)
Methanol extract	1.70 ± 0.26
Ethanol extract	2.50 ± 0.38

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All values are expressed mean ±SD

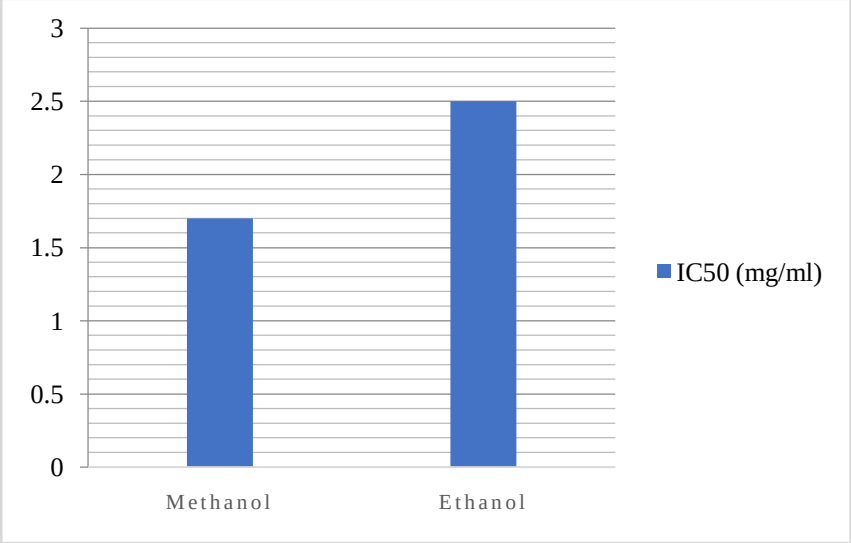

Figure 5. DPPH result of two extracts obtained from flowers for *Cydonia oblonga*

3. Conclusion

This work report the study of the Total phenolic content, total flavonoid and antioxidant activity of methanolic and ethanolic extract of the flowers of a fruit tree *Cydonia oblonga* quince, from eastern Algeria for the first time. The results obtained indicate, that the flowers of a fruit tree *Cydonia oblonga* quince are rich in phenolic and flavonoid compounds. Moreover the flowers of a fruit tree *Cydonia oblonga* quince exhibit potent antioxidant activities.

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