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Rootstocks effect on flavonoid content and antioxidant properties of Maltese (*Citrus sinensis* L.) ethanolic extracts

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Abstract – The antioxidative potential of flavonoids was one of the earliest functions proposed for these compounds. The grafting of the rootstock with the orange tree is an agronomical technique used to improve production and quality of the fruit. Ethanolic extracts of peel citrus fruit grafted on 8 rootstocks growing in Tunisia were investigated for their total flavonoids content and their antioxidant activity by radical DPPH method. IC_{50} (values denote the concentration of sample, which is required to scavenge50% of radical DPPH free radicals) for antioxidant activity ranged from 585.4-1095.1 µg/mL. Total flavonoids content (based on colorimetric $AlCl_3$ method) varied from 11.44- 44.34 mg Quercetin equivalent/g of dry extract. There were a correlation between the total flavonoids contents and antioxidant activity of peel extracts (r= -0.8654).

Keywords: Correlation, DPPH·, Essential oil, Flavonoids, Maltese, Rootstocks

1. Introduction

The genus *Citrus* L. of the family Rutaceae, is one of the most important fruit crops in the world. The orange peels are a co-product of the juice industry that can be valorized for their bioactive compounds and antioxidant content such as flavonoids.

Rootstock play an important in the rapid development of citrus in the world. The necessity of using rootstocks for citrus fruits is to have a profitable production against some limiting factors such as climate, bad soil conditions and diseases (Yildrim et al., 2010). Sour orange (*Citrus aurantium* L.) is still the most commonly used rootstock and is well adapted to calcareous and other common soil types and used in the Mediterranean basin. However, sour orange has the disadvantage to be highly susceptible to *Citrus tristeza virus* (CTV), the agent of Tristeza disease that strongly limits the use of this rootstock in many citrus growing countries. In our study, as a preventive measure, a rootstock trial was implemented in 2005 with the aim to compare the performance of the locally important cultivar 'Maltese half-blood' sweet orange grafted on 7 newly introduced tolerant rootstocks to CTV with that of the commonly used sour orange susceptible to this virus.

With regard to the Maltese flavonoids, there is a lack of knowledge about the influence of the rootstocks on the nature and content of these compounds within the ethanolic Maltese peels extract. In addition, previous studies have demonstrated the capacity of rootstock to synthesize and accumulate flavonoids (Winkel-Shirley, 2001a; Winkel-Shirley, 2001b). For these reasons, the goal of our study was focused on the rootstocks citrus trees effect on the flavonoid content and antioxidant activity of ethanolic Maltese peels extracts. The approved results can be used to select the best rootstock for plantations of Maltese.

2. Material and Methods

2.1. Plant material

The plant material consists of orange plants "Maltese half-blood" grafted on 8 rootstocks including: *Sour orange* **(SO)**, *Citrus macrophylla* **(CM)**, *Citrange Carrizo* **(CC),** *Citrus volkameriana* **(CV)**, *Mandarin Cleopatra* **(MCL)**, *Citrumelo swingle 4475* **(Citru)**, *Lime rangpur* **(LR)** and *Poncirus trifoliate* **(PT)**. The rootstock trial was implemented in 2005, in a field plot of the INRAT Gobba station, located in the Cap Bon region (Najar et *al.*, 2017).

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2.2. Solvent extraction process

5 g of the powdered plant samples [*C. sinensis*] were dissolved in 50 mL of ethanol and extracted at room temperature for 24 h. The extracts were filtered through a Whatmann filter paper n°1 and concentrated using a rotary evaporator at 40°C.
Extraction yield was expressed as: (Mass of extract/ Mass of dry matter) *100

2.3. Determination of total flavonoid content

The aluminum trichloride (AlCl3) method cited by Rajeshwari et *al.* (2013) is used to quantify the flavonoids in our extracts:1 ml of each extract and the standard (dissolved in methanol) with the dilutions suitable was added to an equal volume of a solution of $AlCl_3$ (2% in methanol). The mixture was vigorously stirred and the absorbance at 430 nm was read after 10 minutes of incubation. The results are expressed in micrograms of Quercetin equivalent per milligram of dry extract (mg EQ / g extract) (Siahpooshand Dehdari, 2014).

2.4. Determination of DPPH Free Radical Scavenging Activity

The scavenger effect of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) which is used to replace the free radicals produced by the cells in response to external or internal stresses was measured according to the protocol described by Elzibeth et *al.* (2008). Different concentrations (100, 250, 500, 750 and 1000 mg. L^{-1}) were used of the samples studied together with the synthetic antioxidant Vitamin C (Ascorbic acid). The DPPH˙ solution was prepared by solubilizing 2.9 mg of DPPH in 100 mL of methanol, 100 μL of each extract as well as the positive control are added to 900 mL of the DPPH˙ solution, the mixture was left in the dark for 30 min at room temperature, the absorbance is measured at 517 nm. The antioxidant activity was estimated according to equation (1):

$$\text{Scavenging activity\%} = \frac{Abs_{Control} - Ab\ _{sample}}{Abs\ control} \quad X\ 100\ (1)$$

Where $Abs_{control}$ and Abs_{sample} are absorbance of control and sample, respectively.

The capacity of free radical scavenging was expressed by IC50 (mg/mL) value, which represents the concentration required to decrease 50 % of initial DPPH radical.

2.5. Statistical analysis

Pearson correlation ($p < 0.05$) test was conducted to determine the correlations between total flavonoid content and antioxidant activity of the citrus peel extract using the software XL-stat 2014.

3. Results and Discussion

Maltese, as other citrus fruits, had nutritional importance due to its composition. Flavonoids, especially flavanones (hesperidin, narirutin and naringin) are identified in citrus peel (Mouly et *al.,* 1998; Wang et *al.,* 2008).

3.1. Extraction Yield

The extraction yield of the ethanolic citrus peel extracts were summarized in Table 1. In this experiment, the yield of extract ranged from 5.3% for CM to 12.34 % for LR. The peels of orange grafted on sour orange (SO) and lemon rough (LR) showed higher extraction yields than those of the other citrus fruits.

Table 1. %Yields (w/w) of citrus fruit extracts grafted on different rootstocks: Sour orange (SO), Citrus macrophylla (CM), Citrane carrizo (CC), Citrus volkameriana (CV), Mandarin Cleopatra (MCL), Citrumelo swingle 4475 (Citru), Lime rangpur (LR) and Poncirus trifoliate (PT)

	MCL	CV	CM	Citru	LR	SO	CC	PT
Yields (%) (w/w)	9,46	7,06	5,30	8,16	12,34	12,10	6,77	8,75

No previous studies discussing the rootstocks effect on the extraction yield were found in literature. However, the yield difference observed in our results can be attributed to the various characteristics of rootstocks on growth, fruiting and fruit quality of the scion cultivar (Ghanim et al., 2006).

3.2. Relationship between total flavonoids content (TFC) and IC_{50} of the DPPH˙ scavenging activity of peels extract

The effects of rootstock/scion combinations on antioxidant activity, total flavonoids content have not been studied in a systematic and comprehensive manner previously (Legua et *al.,* 2017). The results obtained indicate that the interaction "rootstock × variety" significantly affected to antioxidant activity, total flavonoids in ethanolic extracts of Maltese peels (Table 2). The highest TFC values were recorded in extracts from peel of citrus grafted on CV (44.34 mg QE/ g extract), followed by the peel of citrus grafted on (LR) (39.3 mg QE/g extract). Meanwhile, the lowest TFC was determined in citrus peel grafted on (Citru) at (11.44 mg QE/100 g extract). Considering the impact of tree/rootstocks combination on the physiological state of the trees, it's obvious that rootstocks variation has an effect on the flavonoid contents within the orange peel extracts. These results were in accordance with previous studies such as those of Gil-Izquierdo et *al.* (2004) and Legua et *al.* (2017).

The reduction of DPPH radical was followed by monitoring the decrease of absorbance sample extracts at 517nm. The extract showed weak antioxidant activities: Citrus peels of fruit grafted on CV showed the highest activity (IC_{50} = 585.4 ug/mL) and those grafted on CITRU showed the weakest one (IC_{50} = 1095.1 ug/mL). The IC_{50} value for Ascorbic acid was 3ug/mL. Results summarized in Table 2.

Table 2. Total flavonoids content and IC_{50} of the power of the DPPH scavenging activity of peels extract.

	TFC (mg QE/dry extract)	DPPH (IC_{50}) (ug/mL)
MCL	24,18	934,03
CV	44,34	585,4
CM	18,62	946,4
Citru	11,44	1095,1
LR	39,30	782,05
SO	35,94	742,7
CC	15,64	985,4
PT	14,04	827,49

Flavonoids are probably the most important class of phenolic compounds acting as antioxidants (Bors et *al.,* 2009), which react with the free radical (DPPH.) via the phenolic hydroxyl groups giving hydrogen forming thus stable complexes (DPPH-H) that are not able of initiating oxidation reactions (Athemena et *al.,* 2010).

Interestingly, in this study, the IC_{50} was correlated with the total flavonoids content in the citrus extract of 8ethanolic extracts from citrus peel grafted on 8 rootstocks. The correlation tablewas depicted in Table 3 and figure 1. In general, samples with a high radical scavenging activity showed a high flavonoids content as well: There was a significant, high and negative correlation between DPPH values and TFC (r = –0.8645). This result was confirmed by Fadlinizal et *al.* (2010). However, some previous studies have not reported theoccurrence of this relation between them (Kamran et *al.,* 2009; Toh et *al.,*2013). This can be due to the fact thatthe antioxidant properties of flavonoids are deeply dependent on their chemical structure: Only flavonoids with a certain structure and mainly hydroxyl position inside themolecule can act as proton donating and display radical scavengingactivity (Wojdylo et *al.,* 2017; Nickavar et *al.,* 2007).

Table3. Correlation matrix (Pearson) between TFC and IC_{50}

Variables	Flavonoids	DPPH
Flavonoids	1	**0,8645**
DPPH	**-0,8645**	1

Values in bold are different from 0 with a significance level alpha=0,05

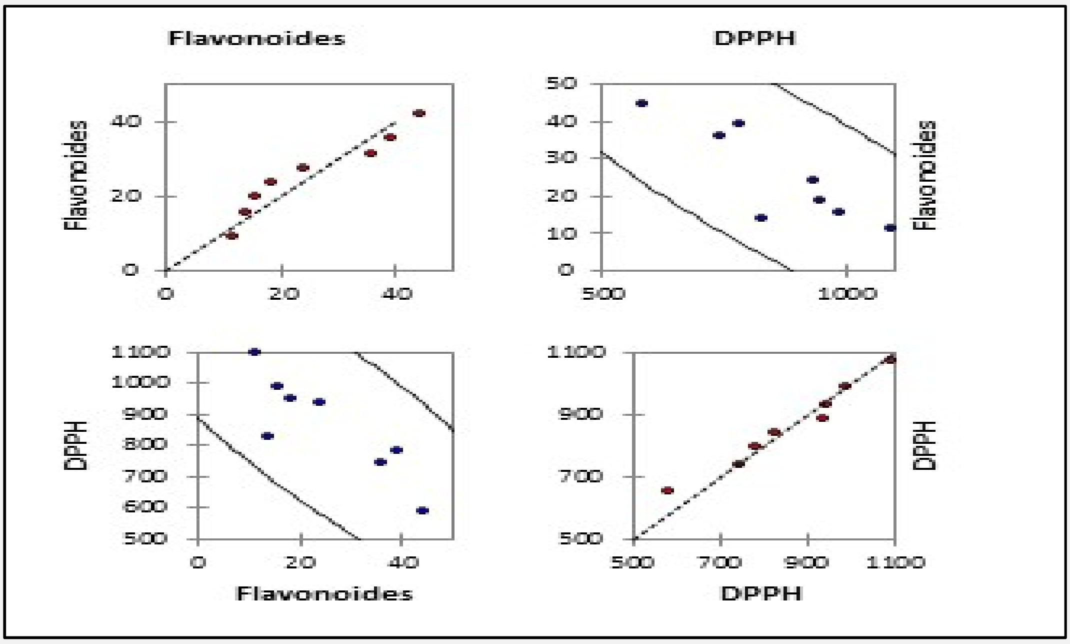

Figure1. Scatter plot relationship between TFC and IC_{50} of ethanolic Maltese peel extracts of fruit from trees grafted on 8 different rootstocks

4. Conclusion

The study revealed that rootstocks had marked effect on extraction yield, total flavonoids content and antioxidant activity. Fruits from trees budded on CV and SO rootstocks showed the highest TFC and the lowest IC_{50} in their ethanolic extracts. Ethanolic peel extracts which contained more flavonoids, had exhibited the best antioxidant activity. Statistical study shows a high correlation between DPPH value and TFC content.

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