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Main pomegranate phytochemicals and their contribution to the antioxidant potencies of pomegranate juice

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Abstract. This research aims to screen the main phytochemicals and their antioxidant capacities from juice of some pomegranate cultivars from southern Tunisian. Total polyphenols, flavonoids, anthocyanins and hydrolysable tannins were determined by using classical spectrophotometric methods. Free radical scavenging activities were determined by DPPH and ABTS tests. Antioxidant capacities were determined by FRAP and ORAC methods. Results showed that the antioxidant potency of pomegranate extracts was correlated with their phenolic contents. In particular the highest correlations were reported between DPPH (as IC₅₀) and respectively total flavonoids ($r = -0.960$), anthocyanins ($r = -0.866$) and total polyphenols ($r = -0.788$). Therefore both total flavonoids and total anthocyanins showed high correlation to FRAP and ORAC assays. All of these findings confirm the differential contributions of pomegranate phytochemicals to the antioxidant potencies of pomegranate juice. The richness of pomegranate juice with antioxidants might encourage for the development of antioxidant function dietary food.

Keyword. *Punica granatum* L. – Juice phytochemicals – Antioxidant capacities – Correlation.

I – Introduction

Pomegranate has an abundance of antioxidants gaining a lot of attention in the past few years as a super fruit for the heart. The consumption of pomegranate fruit has been associated with a reduced risk of chronic human illness such as certain types of cancers, inflammation, and cardiovascular and neurodegenerative diseases (Aviram *et al.*, 2002). Recent studies on pomegranate antioxidants bring attention to the richness of pomegranate tree and particularly peel, juice and seed oil with natural antioxidants. Therefore, it can explain the interest of traditional medicine practitioners on pomegranate fruit and why they considered pomegranate as a medicinal plant (Seeram *et al.*, 2006).

A natural source of phenolic compounds, pomegranate is loaded with antioxidants such as tannin, polyphenol, flavonoid and vitamins C. Other antioxidants in pomegranate include tocopherols and anthocyanins were demonstrated to have protective and therapeutic qualities. The bioactivities of this phytochemicals are mainly due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and also may have a metallic chelating potential (Seeram *et al.*, 2006).

The aim of this research was to quantify Main phytochemicals in pomegranate juice and to compare the efficiency of ABTS, DPPH, FRAP, and ORAC assays to estimate antioxidant activities of pomegranate juice and their correlations with the contents of total polyphenols, flavonoids, anthocyanins and hydrolysable tannins.

II – Materials and methods

1. Plant material

Six Pomegranate cultivars were used in this work: Chetoui (CH), Gabsi2 (GB2), Gabsi3 (GB3), Garsi (GR), Mezzi (MZ) and Zehri (ZH). All cultivars were grown in the same collection at Gabès oasis (south-East of Tunisia). Fully mature fruits were randomly picked from each tree and stored at room temperature for a few days until used.

2. Screening of natural phenolic compounds

Pomegranate juice extract was prepared following the method described previously by Elfalleh *et al.* (2011). The total phenolic compounds, flavonoids, anthocyanins and hydrolysable tannins were measured. Total polyphenols were estimated using the Folin-Ciocalteu method (Elfalleh *et al.* 2009). Total flavonoids were measured spectrophotometrically, based on the formation of a flavonoid–aluminium complex with maximum absorbance at 430 nm (Elfalleh *et al.*, 2009). Total anthocyanin content of pomegranate juice was determined by a pH differential method (Çam *et al.*, 2009). Hydrolyzable tannins were determined by modified method of Çam and Hişil, (2010).

3. Determination of antioxidant activities

The free radical scavenging activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)) assays (Elfalleh *et al.*, 2009). Antioxidant activity in the samples was determined using Ferric Reducing Antioxidant Power (FRAP) and Oxygen radical absorbance capacity (ORAC) reported by (Elfalleh *et al.*, 2011). This four methods were used to evaluate the antioxidant potencies of pomegranate juice. Results from ABTS, FRAP, and ORAC tests were expressed as Trolox equivalent antioxidant capacity (TEAC) and The DPPH value was expressed as IC₅₀ values.

4. Statistical and chemometric methods

All tests were carried out in triplicate and the results were presented as means ± SD. Differences at $p < 0.05$ were considered statistically significant. An analysis of variance (ANOVA) was used to compare cultivars.

III – Results and discussion

Total polyphenols, flavonoids, anthocyanins, and hydrolysable tannins of six pomegranates cultivars are shown in Table 1. Total polyphenols in pomegranate juice were about 10.75 ± 2.20 GAE mg/ml. The total flavonoids in juice were about 5.25 ± 0.59 mg rutin/ml. The highest anthocyanin content was in CH cultivar (48.27 ± 5.01 CGE mg/l) and the lowest value was 28.15 ± 5.12 CGE mg/l of juice in GB3 cultivar. Quantitatively our results are in agreement with those reported by Hasnaoui *et al.* (2011) and lower than those reported by Gil *et al.* (2000) (306.0 mg/l). The anthocyanin fingerprints among pomegranate cultivars were quite different and known to be affected by several parameters such as harvest maturity, storage temperature and relative humidity (Miguel *et al.*, 2004; Shin *et al.*, 2008).

In juice, hydrolyzable tannins ranged from 1.97 ± 0.48 mg TAE/ml to 3.38 ± 0.67 mg TAE/ml with an average of 2.69 ± 0.58 mg/ml. Hydrolyzable tannin content depends on solvent and extraction method (Cam and Hisil 2010). Gil *et al.* (2000) reported lower total hydrolyzable tannins in juice from fresh arils (0.539 mg/ml).

In previous studies we reported higher phenolic contents in pomegranate peel compared to juice (Elfalleh *et al.*, 2009, Elfalleh *et al.*, 2011). It implies that pomegranate peel can be subjected to phenolic compounds extraction for additional benefits. In particular, it makes commercialization of under-used cultivars after processing possible.

Table 1. Total polyphenols, flavonoids, anthocyanins and hydrolysable tannins from pomegranate juice[†]

Cultivar	Total Polyphenols	Total Flavonoids	Total Anthocyanins	Hydrolysable Tannins
CH	10.93 ± 0.59 B	5.75 ± 0.22A	48.27 ± 5.01 A	3.38 ± 0.67 A
GB2	13.70 ± 1.62 A	5.52 ± 0.48A	38.06 ± 6.72 A.B	2.60 ± 0.09 A.B
GB3	6.89 ± 1.97 C	4.11 ± 0.87B	28.15 ± 5.12 B	2.40 ± 0.21 A.B
GR	11.26 ± 0.65 B	5.63 ± 0.32A	43.29 ± 3.19 A	2.97 ± 0.16 A.B
MZ	10.59 ± 0.58 B	5.30 ± 0.29A	41.62 ± 6.53 A.B	2.82 ± 0.79 A.B
ZH	11.15 ± 0.63 B	5.21 ± 0.08A	35.79 ± 4.65 A.B	1.97 ± 0.48 B
Mean ± SD (n=6)	10.75 ± 2.20	5.25 ± 0.59	39.19 ± 7.69	2.69 ± 0.58

^{*}Different letters in the same column of cultivar respectively, indicate significant difference (P <0.05) analyzed by Duncan's multiple range test.

[†]Total Polyphenols expressed as grams of gallic acid equivalents (GAE) per L of juice. Total Flavonoids expressed as grams of rutin equivalents (RE) per L of juice. Total Anthocyanins expressed as milligrams of Cyanidin 3-glucoside equivalents (CGE) per of L juice and Hydrolysable Tannins expressed as grams of tannic acid equivalents (TAE) per L of juice.

Because of the multiple reaction characteristics and mechanisms involved in the estimation of the total antioxidants, no single method could accurately reflect all antioxidants in a mixed system due to the complex nature of phytochemicals (Chu *et al.*, 2000). In the present study, four methods were used to evaluate the antioxidant capacities of pomegranate juice (Table2).

The free radical scavenging activity determined by DPPH was expressed as IC₅₀ value which ranged from 15.98 ± 0.60 to 23.98 ± 1.60 µl of juice/ml DPPH. The ABTS values ranged from 4.67 ± 0.38 TEAC mM/l of juice in GB2 cultivar to 8.57 ± 1.16 TEAC mM/l of juice in GR, which enclose the highest TEAC activity.

Table 2. Total polyphenols, flavonoids, anthocyanins and hydrolysable tannins from pomegranate juice

Cultivar	DPPH (IC ₅₀ µL/ml)	ABTS (TEAC mmol/l)	FRAP (TEAC mmol/l)	ORAC (TEAC mmol/l)
CH	15.98 ± 0.60C	6.13 ± 1.10BC	7.86 ± 0.95 A	7.59 ± 2.26 A
GB2	18.08 ± 1.00B	4.67 ± 0.38C	7.31 ± 1.27 A	6.50 ± 0.14 A
GB3	23.98 ± 1.60A	4.73 ± 0.40C	6.44 ± 0.01 A	5.94 ± 1.29 A
GR	18.08 ± 0.30B	8.57 ± 1.10A	8.65 ± 1.87 A	8.18 ± 2.38 A
MZ	19.38 ± 1.20B	5.67 ± 0.80BC	6.67 ± 0.83 A	7.00 ± 2.05 A
ZH	18.08 ± 1.00B	7.07 ± 0.57B	6.36 ± 0.79 A	6.62 ± 0.09 A
Mean ± SD (n=6)	18.93 ± 2.70	6.14 ± 0.33	7.24 ± 1.24	7.00 ± 1.52

^{*}Superscript letters with different letters in the same column of cultivar respectively, indicate significant difference (P <0.05) analyzed by Duncan's multiple range test.

In pomegranate juice FRAP radical scavenging activities ranged from 6.36 mmol/l in ZH cultivar to 8.65 mmol/l in GR cultivar, with an average of 7.24 ±1.24 mmol/l. Our finding were similar to

results reported in the FRAP assay by Ozgen *et al.* (2008), in six pomegranate cultivars from southern Turkey with an average FRAP value of 7.35 ± 0.21 mmol/l.

ORAC activity ranged from 5.94 mmol/l in GB3 to 8.18 mmol/l in GR cultivar, with an average of 7.24 ± 1.24 mmol/l. In all antioxidant assays, the capacities of CH and GR cultivars were slightly higher than other cultivars. However, no significant differences between cultivars were reported with the Duncan test (Table 2).

Correlations coefficients of total polyphenols, flavonoids, anthocyanins, and hydrolysable tannins to DPPH, ABTS, FRAP and ORAC assays were shown in Table 3. DPPH highly correlated with total flavonoids ($r=-0.960$), total anthocyanins ($r=-0.866$), and total polyphenols ($r=-0.788$). Therefore both total flavonoids and total anthocyanins showed high correlation to FRAP and ORAC assays. Results clearly confirm that this phytochemicals predominantly contribute to the antioxidant potencies of pomegranate juice.

Nevertheless, not all phytochemicals showed high correlation to antioxidant assays. This fact can mainly be explained by the uncertainty surrounding the chemistry of antioxidant compounds and that is why both phenolic content and antioxidant activity information must be discussed when evaluating the antioxidant potential of extracts. The fact that there is a rather poor correlation between juice phenolic content and the FRAP and ORAC antioxidant activities may mean that other components, such as ascorbic acid need to be investigated for their contribution to the antioxidant activity.

Table 3. Correlation coefficients of phenolic compounds to antioxidant capacities of pomegranate Juice

	Total polyphenols	Total flavonoids	Total anthocyanins	Hydrolysable tannins
DPPH	-0.788*	-0.960**	-0.866**	-0.254
ABTS	0.170	0.466	0.441	0.074
FRAP	0.390	0.662*	0.693*	-0.256
ORAC	0.358	0.763*	0.850**	0.189

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

IV – Conclusion

However the studied phytochemicals contribute widely to the antioxidant potencies of juice alone they cannot explain the mechanism involved in the antioxidant activity. This phytochemicals are only one facet of the DPPH, ABTS, FRAP and ORAC antioxidant capacities of pomegranate juices. Again, this showed that the constituents of a real matrix solution did not chemically interact with selected pure antioxidants, and that the antioxidant capacities were additive.

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