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Total phenolic contents and antioxidant activity of the pomaces of two tomato (*Phoenix dactylifera*) cultivars cultivated in Iraq

[1]Munther Radhi, [1]Abdul-Lateef Molan and [2]Doaa Abdulwahab

[1]Assistant Professor, Department of Biotechnology, College of Sciences, Diyala University, Baquba, Diyala, Iraq,
[1]Professor, Department of Biotechnology, College of Sciences, Diyala University, Baquba, Diyala, Iraq,
[2]Assistant Lecturer, Department of Biology, College of Sciences, Diyala University, Baquba, Diyala, Iraq,

Address For Correspondence:
Professor Abdul-Lateef Molan (B.Sc., M.Sc., PhD, FRSB), Diyala University, Department of Biotechnology, College of Sciences, PO Box: 268, Baquba, Diyala, Iraq.
Phone: +964 7723 487 549; E-mail: prof.molan@sciences.uodiyala.edu.iq / molanal99@gmail.com

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ABSTRACT

BACKGROUND: Tomato (*Phoenix dactylifera*) fruits are rich sources of nutrients and secondary compounds which are important for the human health. In addition, they are important sources for minerals, vitamins C and E, lycopene, flavonoids, organic acids, phenols and chlorophyll. Free radicals are the major cause of various chronic and degenerative diseases such as aging, coronary heart diseases, stroke, diabetes mellitus and cancer. **OBJECTIVE:** The main objective of the present study was to determine the total phenolic content (TPC) and antioxidant activities of the extracts prepared from tomato by-products (pomaces, skins and seeds) of two tomato cultivars (Gehan and Definis) cultivated in Iraq. The correlation between TPC and antioxidant activity was also assessed. **RESULTS:** The TPC was measured using Folin–Ciocalteau method; while the antioxidant activity was measured using DPPH-radical scavenging and Ferric reducing antioxidant activity (FRAP) assays. Two solvents (distilled containing 1% and 5% of hydrochloric acid) have been used for the first time for preparation of the extracts from the pomaces and their ingredients and these solvents showed superiority in terms of the quantity of the phenolic compounds and the antioxidant activity over most of the other solvents. The extracts prepared from pomace and its ingredients (skins and seeds) of Defnis cultivar had significantly higher ($P< 0.5$) TPC and free radical scavenging activity than their counterparts from Defnis cultivar. In both cultivars, positive correlation was found between the TPC and the antioxidant/antiradical activity which may indicate that the phenolic compounds are the main ingredients contributing to the antioxidant activity. **CONCLUSION:** The results of the present study showed that the determination of phenolic compounds and antioxidant activities was dependent on the extracting solvent used and the cultivar of the tomato and that the tomato by-products (generated from the extraction process of tomato syrup/paste) could be considered as a potential source of antioxidants and may be used as alternatives for the synthetic antioxidants in pharmaceutical and food formulations. The addition of very low concentrations of hydrochloric acid to the distilled water significantly increased the efficiency of water as extracting solvent for the phenolic compounds. Further studies are needed to determine the nature of the phenolic compounds found in the tomato pomaces and their ingredients.

INTRODUCTION

Free radicals are the major cause of various chronic and degenerative diseases such as aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Cheng *et al*., 2003) and it has been suggested that the phytochemical content of vegetables and fruits, such as polyphenols, vitamins and carotenoids that have shown potent antioxidant/free radical scavenging activities contribute to their protective effect against these

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diseases (Record *et al*., 2001; Schotsman *et al*., 2008; Molan, 2013, 2017; Molan *et al*., 2009, 2012, 2016a,b, 2017).

As a vegetable, tomato (*Solanum Lycopersicum* L.) is considered one of the major vegetable with a global production of about 161.8 million tons (FAOSTAT, 2012**)** and when tomatoes are processed into products, 10–30% of their weight becomes waste or pomace (King and Zeidler, 2004). Tomatoes are excellent sources of many nutrients and secondary metabolites that are important for human health such as minerals, vitamins C and E, carotene, lycopene, flavonoids, organic acids, phenolics and chlorophyll (Giovanelli and Paradise, 2002; Kalogeropoulos *et al*., 2012).

Tomato pomace is one of the agricultural and industrial wastes that cause a big problem for the industries due to the pollution they cause to the environment (Devesa-Rey *et al*., 2011). Tomato pomace can be defined as the mixture of tomato skin, pulp, and crushed seeds that remain after the juicing processes for preparing ketchup, juice, soup, and other products, and it is an inexpensive by-product of tomato manufacturing (King and Zeidler, 2004)). The dried tomato pomace contains 22.6 - 24.7 % protein, 14.5 – 15.7% fat and 20.8 – 23.5% fiber and this by-product is a good source of vitamin B1, B2 and A (Aghajanzadeh *et al*., 2010). In addition, tomato skin (peel) contains high levels of lycopene compared to the pulp and seeds (Sharma and Le Maguer, 1996).

The objectives of the present study were to compare the phenolic content, antioxidant capacity and free radical scavenging in the pomaces and their ingredients of two tomato cultivars grown in Iraq. The correlation between total phenolic contents and antioxidant/ free radical scavenging activities was also evaluated.

MATERIALS AND METHODS

Preparation of plant extract:

The tomato pomace was prepared as described previously (Molan *et al*., 2016). Briefly, 10 kg of fresh ripe tomato fruits that have been purchased from the local market in Baquba City, Diyala Province, Iraq were crushed using house mixer and then the mixture was strained through double layers of cheesecloth and squeezed until no liquid was left. The squeezed pomace was dried using an electrical oven at 50 ^{0}C until complete dryness. One third of the dried pomace was left as is and labeled as the entire pomace. The leftover was used to get the seeds and peels which were manually separated. The dried pomace, skins, and seeds were powdered using coffee grinder.

Forty milligrams of powdered pomace, seeds and peels of each cultivar have been weighed and put in 50-ml plastic centrifuge tubes and then 40 milliliters of the selected solvents [distilled water at room temperature, boiling distilled water, hydroethanolic solution (1: 1 v/v) and diluted hydrochloric acid (concentration: 36%) solutions (1-5% HCL with cold distilled water/ actual concentrations are 0.36 - 1.79%)] have been poured into each tube, mixed by vortexing for 5 minutes and then left overnight (stock solution; 10 mg per ml) at room temperature. After 16-20 hours, the tubes have been centrifuged at 1000 rpm for 5 minutes and the supernatant was used in the experiments.

Determination of total phenolic content (TPC):

The total phenolic content (TPC) in the extracts was determined according to the Folin-Ciocalteu procedure as used by Molan *et al*. (2009) with some modifications. Briefly, an aliquot of 12 µL of water-soluble extract was mixed with 200 µL of 2% sodium carbonate solution in 96-well microplates and allowed to react for 5 min at room temperature. Then, 12 µL of Folin-Ciocalteu phenol reagent (50% with water) was added and allowed to stand for 30 min at room temperature before the absorbance of the reaction mixture was read at 650 nm using a plate reader. Calibration was achieved with an aqueous gallic acid solution (0.1-1.0 mg/ml). The TPC of the extract was expressed as mg gallic acid equivalent (GAE) per gram of peels and seeds on a dry basis.

Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals:

This assay determines the ability of the tested compound to scavenge free radicals via scavenging the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical. This assay was performed as described previously (Molan *et al*., 2009). Briefly, 20 µL of each extract was allowed to react with 200 µL of 0.2 mM DPPH in 96% ethanol in a 96-well microplate. The plate was then incubated at 37 ^{0}C for 30 min after which the absorbance was measured at 550 nm using a microplate reader. The antiradical activity was calculated as a percentage of DPPH discoloration relative to a negative control using the following equation:

Antiradical activity (%) = absorbance of control incubation - absorbance of the extract / absorbance of control incubation X 100.

Evaluation of antioxidant activity as measured by the ferric reducing antioxidant power (FRAP):

The antioxidant capacity of the extracts was determined using the ferric reducing antioxidant power (FRAP) assay, a colorimetric assay that measures the ability of the tested sample to reduce the intense blue ferric tripyridyltriazine complex to its ferrous form, thereby changing its absorbance (Benzie and Strain, 1996). The working FRAP reagent was prepared by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 1 volume of 10 mmol/L TPTZ (2,4,6-tripyridyls-triazine) in 40 mmol/L hydrochloric acid and 1 volume of 20 mmol/L ferric chloride. Briefly, an aliquot of 8.5 µl of extract was added to 275 µl of diluted FRAP reagent using a microplate and the plates were incubated at 37 oC for 30 minutes before measuring the absorbance at 395 nm using a plate reader. A standard curve was prepared using different concentrations (200-2000 µmol/L) of FeSO4.7H2O. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as micromole FeSO4 equivalents per litre of aqueous extracts. All solutions were used on the day of preparation and all determinations were performed in duplicate.

Statistical analysis:

The data were analyzed by using one way ANOVA followed by Tukey's test was performed to determine the difference in larval and pupal mortality between different treatments.

RESULTS AND DISCUSSION

The principal finding of the present study is that the total phenolic content (TPC) and the antioxidant activity of the extracts prepared from the entire pomaces of two tomato cultivars and their ingredients (skins and seeds) are affected significantly ($P< 0.05$) by the solvent used in the extraction, the selected part of the by-products and the cultivar of tomatoes (Figures 1 and 2). Khalaf *et al*. (2014) exposed tomato pomace samples to gamma radiation at dose levels of 0, 1, 3 and 5 kGy and then they assessed the total phenolic content (TPC), total flavonoids content (TFC) and antioxidant activity properties of both non-irradiated and irradiated samples extracted in acetone 70%, methanol and chloroform: ethyl acetate (1:1). The authors found that the solvents used in the extraction affected the TPC, TFC and the antioxidant activity of both irradiated and non-irradiated samples of tomato pomace and concluded that tomato pomace is a very promising source of bioactive compounds and it is a very potential natural source of antioxidant compounds. Recently, Gaafar *et al*. (2015) evaluated the efficiency of different organic solvents such as, water, ethanol and acetone for extraction of phenolic, flavonoid and tannin compounds from the powdered tomato pomace and their antiviral, antimicrobial and antioxidant activities and reported that the solvent play a vital role in the extraction of the plant constituents and tomato pomace is a very promising source of bioactive compounds and can be used or its extracts as antiviral, antimicrobial and antioxidant agent. More recently, Molan *et al*. (2017) assessed the total phenolic content (TPC) and antioxidant activities of the extracts prepared from the date pomaces and their ingredients (flesh and seeds) of two Iraqi date cultivars using five different solvents and reported that the type of the solvent used in the extraction plays an important role in determining the TPC and the antioxidant activity of the pomaces and their ingredients of dates. The authors concluded that the determination of TPC and antioxidant activities was dependent on the extracting solvent used and the cultivar of the date and that the date pomaces could be considered as a potential source of antioxidants and may be used as alternatives for the synthetic antioxidants in pharmaceutical and food formulations. Moreover, it has been shown that the polarity and solubility of the solvent affect significantly the phenolic components extraction from plant materials (Wieland *et al*., 2006).

Two solvents (distilled water containing 1% and 5% of hydrochloric acid) have been used for the first time for preparation of the extracts from the pomaces and their ingredients and these solvents showed superiority in terms of the quantity of the phenolic compounds and the antioxidant activity over the other solvents (distilled water, 50% ethanol, and sometimes boiling water). Yang *et al*. (2013) assessed the effect of acid and alkali hydrolysis on the extraction of phenolic compounds and found that acid hydrolysis was suitable for the extraction of phenols from *Geranium sibiricum* and hydrochloric acid (HCL) hydrolysis was more efficient than alkali hydrolysis and reported that the quantity of the phenolic compounds extracted from the plant cells increases with increasing the concentrations of HCL. The authors concluded that HCL hydrolysis provides an efficient and rapid approach for the natural products extraction. In a recent study, Molan *et al*. (2016) determined the TPC in the extracts prepared from the peels and seeds of Iraqi oranges using different solvents and found that the addition of hydrochloric acid at 1-5% to the distilled water resulted in a significant increment in the quantity of phenolic compounds in comparison with other solvents such as 50% ethanol, boiling water and distilled water only. More recently, Molan *et al*. (2017) have used these two solvents in the preparation of the extracts from the date pomaces and their ingredients and these solvents showed superiority in terms of the quantity of the phenolic compounds and the antioxidant activity over the other solvents. The mechanism by which acidification of cold distilled water with HCL (1-5%) facilitates the extraction of phenolic compounds from tomato pomaces and their ingredients has not been studied in the current study and needs to be investigated.

Extracts prepared from the pomace and its ingredients (skins and seeds) of Defnis cultivar had significantly higher ($P < 0.05$-0.001) free radical scavenging activity in the DPPH assay than their counterparts from Gehan cultivar in almost all the solvents used (Figure 2). Similarly, significant differences were found in antioxidant activities among the two cultivars as measured by the ferric reducing antioxidant power (FRAP). Figure 3 showed clearly that extracts from the pomace and its ingredients of Defnis cultivar showed the highest FRAP values while the pomace and its ingredients of Gehan cultivar had the lowest FRAP values and the differences were statistically significant ($P < 0.05$-0.001) between the two cultivars. Although it is difficult to compare between tomato cultivars and other plant and vegetable cultivars, some studies have shown significant differences in antioxidant/antiradical activity between different strawberry and blueberry cultivars (Meyers *et al*., 2003; Scibisz and Mitek, 2007; Molan, 2017).

The results of the current study showed the presence of a positive correlation between the TPC and the antioxidant activity, represented by the ability to scavenge the DPPH-radical (Table 1) and by the reducing power (FRAP values) as measured by the ferric reducing antioxidant power (Table 2) in both cultivars. This may indicate that the phenolic compounds are the main ingredients contributing to the antioxidant activity of the two tomato cultivars. Some studies also showed a positive correlation between the total phenolic contents and antioxidant activity in tomatoes, strawberry and blueberry cultivars (Meyers *et al*., 2003; Gaafar *et al*., 2015; Pranprawit *et al*., 2015; Uthijumnonk *et al*., 2016; Molan, 2017).

CONCLUSIONS:

The results of the present study showed that the determination of phenolic compounds and antioxidant activities was dependent on the extracting solvent used and the cultivar of the tomato and that the tomato by-products (generated from the extraction process of tomato syrup/paste) could be considered as a potential source of antioxidants and may be used as alternatives for the synthetic antioxidants in pharmaceutical and food formulations. The addition of very low concentrations of hydrochloric acid to the distilled water significantly increased the efficiency of water as extracting solvent for the phenolic compounds. Further studies are needed to determine the nature of the phenolic compounds found in the tomato pomaces and their ingredients.

Table 1: Correlation Coefficients between DPPH radical scavenging activity and total phenolic contents (TPC) in the extracts prepared from the entire pomaces and their ingredients of two tomato cultivars (Gehan and Defnis) using different solvents.

Correlation Coefficient (R^2; DPPH versus TPC)						
	Entire pomace		Skin		Seeds	
Solvents	Gehan	Defnis	Gehan	Defnis	Gehan	Defnis
Cold distilled water	0.81	0.76	0.79	0.87	0.48	0.87
Boiling distilled water	0.53	0.98	0.72	0.77	0.42	0.67
1% HCL solution	0.48	0.65	0.64	0.93	0.57	0.52
5% HCL solution	0.51	0.78	0.87	0.71	0.41	0.70
50% Ethanol	0.76	0.79	0.71	0.60	0.64	0.60

Table 2: Correlation Coefficients between antioxidant activities [FRAP values) and total phenolic contents (TPC) in the extracts prepared from the entire pomaces and their ingredients of two tomato cultivars (Gehan and Defnis) using different solvents.

Correlation Coefficient (R^2 values; FRAP versus TPC)						
	Entire pomace		Skin		Seeds	
Solvents	Gehan	Defnis	Gehan	Defnis	Gehan	Defnis
Cold distilled water	0.75	0.81	0.63	0.81	0.77	0.54
Boiling distilled water	0.83	0.60	0.71	0.70	0.77	0.71
1% HCL solution	0.93	0.77	0.91	0.73	0.94	0.68
5% HCL solution	0.84	0.67	0.89	0.78	0.72	0.75
50% Ethanol	0.92	0.70	0.91	0.85	0.73	0.77

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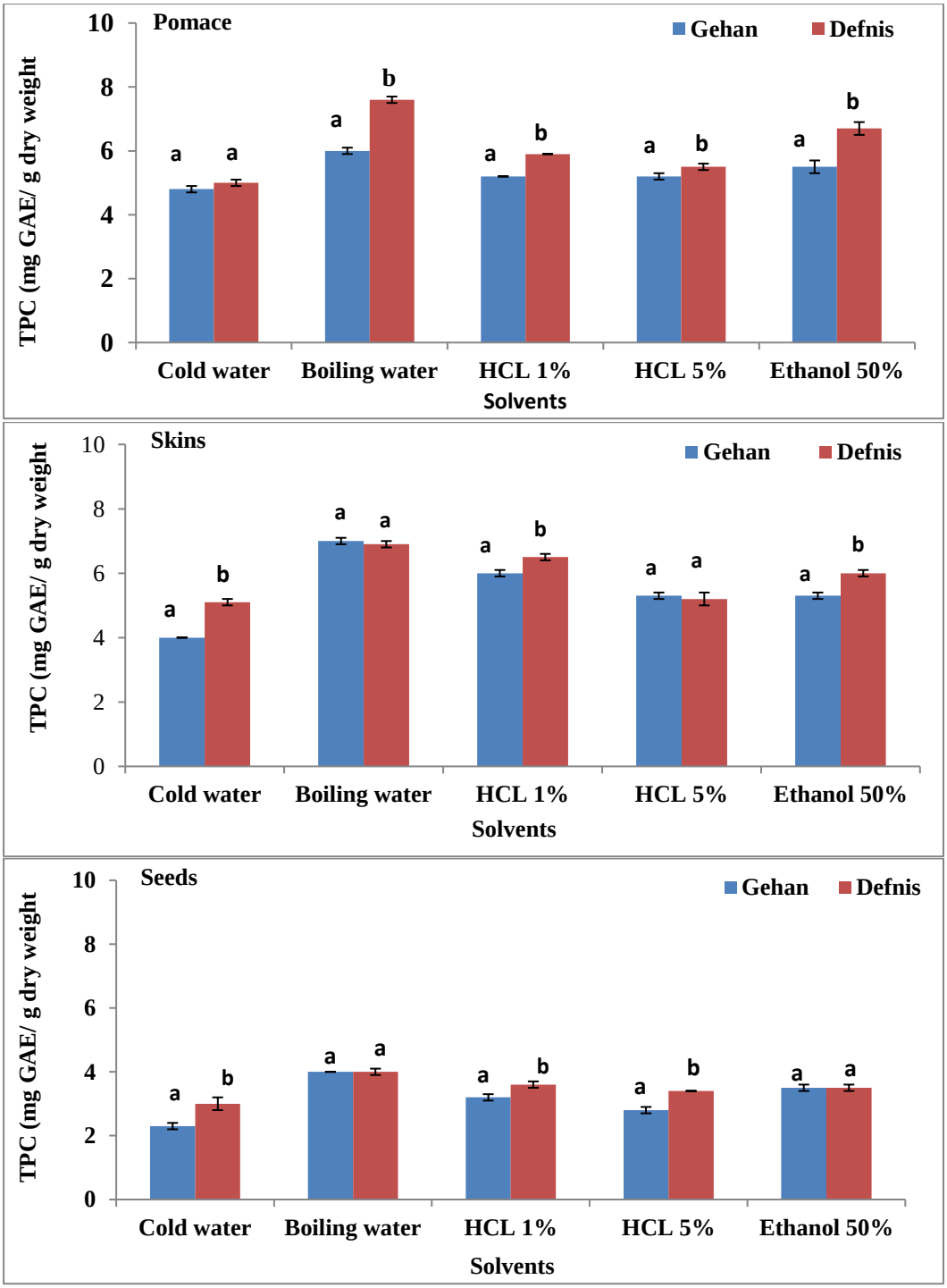

Fig. 1: Comparison between two tomato cultivars (Gehan and Defnis) concerning the total phenolic contents of extracts prepared by different solvents. Different letters on top of the bars, indicate significant differences between the two cultivars in each extracting solvent.

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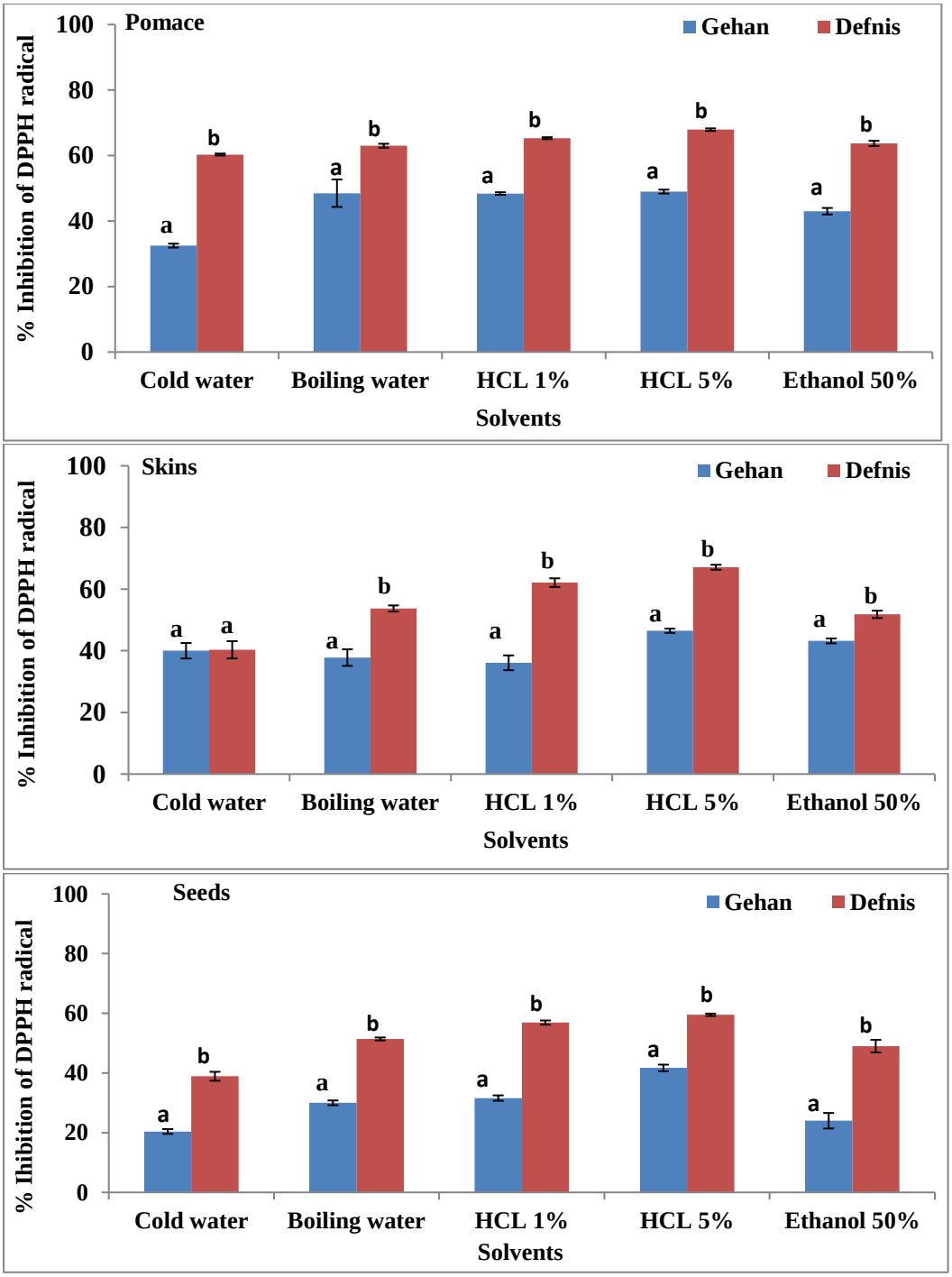

Fig. 2: DPPH radical scavenging activity of extracts prepared from the pomaces of two tomato cultivars (Gehan and Defnis) and their ingredients (skins and seeds). Data were presented as the percentage of DPPH radical scavenging and based on triplicate determinations in two separate experiments. Different letters on top of the bars indicate significant differences between the two cultivars in each extracting solvent.

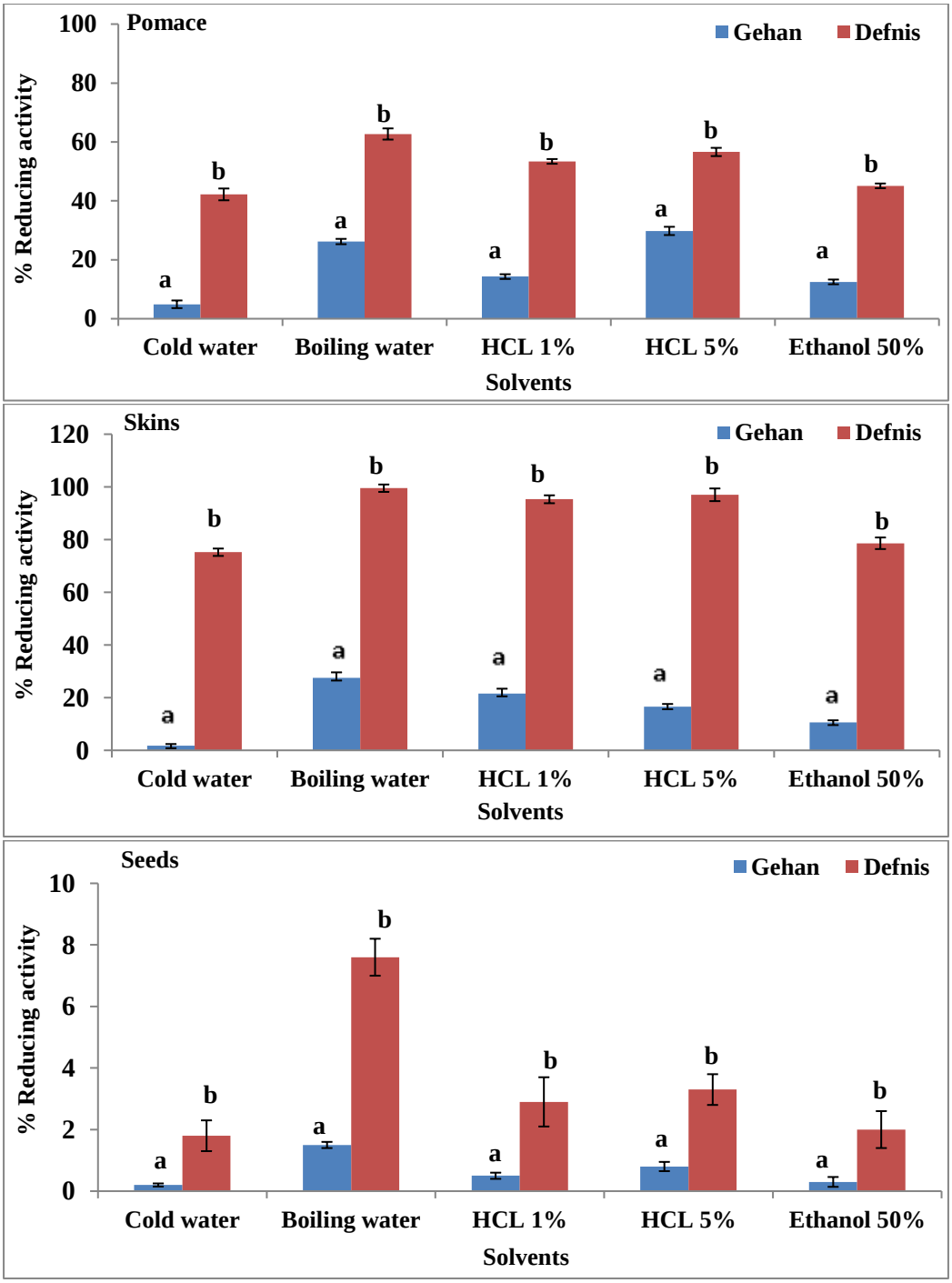

Fig. 3: Comparison between two tomato cultivars (Gehan and Defnis) concerning the reducing activity (measured by the ferric reducing antioxidant power/ FRAP assay) of extracts prepared from the pomeces and their ingredients using different solvents. Different letters, on top of the bars, indicate significant differences between the two cultivars in each extracting solvent.

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