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Utilization of Egyptian Tomato Waste as a Potential Source of Natural Antioxidants Using Solvents, Microwave and Ultrasound Extraction Methods

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ABSTRACT

This study was conducted to access the extraction yield of antioxidant and their activity from Egyptian tomato waste. Conventional and novel assisted extraction methods (ultrasound and microwave) using different hydrophilic and lipophilic solvents at different times and temperatures for optimizing extraction conditions were used. Total phenolics, flavonolids and lycopene were individually determined in different solvent extracts. Activity of their antioxidant compounds was performed using radical scavenging activity and β-carotene-linoleic acid bleaching methods. It was found that novel assisted extraction methods gave higher yield of antioxidants and antioxidant activity. In addition preliminary High Performance Liquid Chromatography (HPLC) was used to identify the antioxidant components. Increase in temperature and extraction time had a significant effect on antioxidant yields and their activity. Eco-friendly solvents such as water and ethyl lactate gave better results than the others. This study demonstrates the possibility of recovering high amounts of natural antioxidant compounds with higher antioxidant activity from tomato wastes using eco-friendly solvents and novel assisted extraction methods.

Key words: Tomato waste, novel assisted methods, lycopene, phenolics, flavonolids, HPLC analysis, antioxidant activity

INTRODUCTION

By-products produced from fruits and vegetables processing represent a major disposal problem for industry concerned but they are also promising sources of compounds which may be used for various purposes in food, pharmaceutical and cosmetics industries (Schieber *et al*., 2001).

It is well known that, tomato is one of the most consumed vegetable in the world and approximately 30% is consumed as processed products. Both fresh and processed tomato possesses a high nutritional value, due to its content of different types of micronutrients (carotenoids and polyphenolic compounds). The polyphenols market is currently being dominated by grape and tea seeds; however, other alternatives can be utilized in this respect.

The extraction and purification of phytochemicals and carotenoids from natural sources is desirable, since these bioactive substances are often used in the preparation of dietary supplements, nutraceuticals, functional food ingredients, food additives, pharmaceuticals and cosmetic products

(Chavan *et al.*, 2001). The purpose of the extraction of phytochemicals from their plant sources is to liberate these compounds from the vascular structures where they are found, either through rupturing the plant tissue or through a process of diffusion. One of the most important considerations in developing new processes and technology for extraction is to achieve safety for final products when used as food (Tabart *et al.*, 2007; Goli *et al.*, 2005).

A critical and demanding step is the selection of proper safe solvent. Water, aqueous mixtures of ethanol, methanol and acetone are commonly used as solvents (Sun and Ho, 2005). However, ethyl lactate has been recently examined as safe extraction solvent with promising results. The chosen extraction method should enable complete extraction of the compounds of interest and avoid their chemical transformation (Zuo *et al.*, 2002).

The ability of several methods, such as enzymes, pulsed electric field or ultrasound, to assist in the extraction of natural antioxidants from tomato has been evaluated by different researchers (Luengo *et al.*, 2014; Papaioannou and Karabelas, 2012).

The application of ultrasound as a laboratory-based technique for assisting extraction from plant material is widely published (Vilkhu *et al.*, 2008). Among the several types of sonicator systems currently available, bath and probe-type sonicators are used. The homogenization is also a process which is commonly used for assisting extraction (Castillo-Munoz *et al.*, 2009).

By now, it is known that ultrasound and microwave radiation could accelerate the extracting process and this may improve bioactive compound extraction (Stanisavljevic *et al.*, 2007; Hoang *et al.*, 2007; Wang and Weller, 2006). The interest on applying sonochemistry to natural product extraction has increased because of its advantage (e.g., reduction in extraction time, saving in energy, increased yield, etc.) (Rodrigues *et al.*, 2008). Microwave assisted extraction heats the extracts quickly and accelerates the extraction process for adsorption and desorption of the targeted compounds from matrix, while its disadvantage is in homogeneous heating (Hemwimon *et al.*, 2007; Proestos and Komaitis, 2008).

The objective of this study was to obtain optimum conditions suitable for maximum extraction of natural antioxidants from tomato waste particularly polyphenolics compounds. Conventional and novel assisted extraction methods, namely, ultrasound and microwave with other extraction parameters were used. Moreover, different hydrophilic and lipophilic extraction solvents including eco-friendly solvents were used. The total yield, phenolics, flavonoids and carotenoids (expressed as lycopene) and antioxidant activity were spectrophotometrically evaluated. Preliminary HPLC analysis of selected extracts was carried out to detect some antioxidant components as trial for future detailed investigation.

MATERIALS AND METHODS

Chemicals: All solvents are analytical grade, were purchased from Elnasr Pharmaceutical Chemicals Co. (ADWIC), Egypt. (S)-(-)-ethyl lactate were purchased from Merek Schuchardt OHG, Germany. Folin-Ciocalteu reagent were purchased from Sisco Research Laboratories Chemicals, India. DPPH from Sigma-Aldrich (St Louis, MO, USA). β-carotene from Acmatic, Egypt and linoleic acid from MP biomedicals, France.

Preparation of tomato waste samples: Tomato-processing waste, composed of skin and seeds, was supplied by Kaha for Preserved Food Factory in 2012. Moisture content was determined for fresh tomato processing waste (80%). The material was subsequently air dried for 2 days,

homogenized in a domestic blender and ultimately ground in a laboratory mill (Janke and kunkel, IKA-labortechnik). Moisture content of ground dry tomato waste amounted to be 6.55±0.195 and kept at 4°C until needed (Strati and Oreopoulou, 2011).

Extraction of antioxidant compounds: Different hydrophilic and lipophilic solvents (distilled water, ethanol, isopropanol, ethyl lactate, ethyl acetate and n-hexane) were used for obtaining different extracts from tomato waste. Tomato waste (5 g) was extracted in 50 mL of each above mentioned solvents individually at 20 and 60°C for stirring time of 10, 20, 30, 40, 50 and 60 min, however, 40 min was found to be more suitable for stirring time (Duh, 1998).

Tomato waste (5 g) was extracted in 50 mL distilled water, ethanol, isopropanol, ethyl lactate, at 20 and 60°C for 40 min by immersing the flask in the ultrasonic water bath (NEY Ultrasonik 28 H, 220 V 50/60 Hz 9A 200 W), at 20 and 60°C for 40 min (Navarro-Gonzalez *et al*., 2011). Ethyl acetate and hexane were excluded as they gave very low yield beside their toxicity.

The simple microwave-assisted extraction was performed using a microwave oven (Samsung model 9245, MB 245) with emission frequency of 2450 MHz and maximum output power 750 W. Samples of 5 g of the dried powder were extracted with 50 mL of distilled water at 30, 60 and 90 sec at water boiling point while other organic solvents were excluded to prevent risk of explosion (Zheng *et al*., 2011).

The yield of solvent extracts was determined for all extraction methods and expressed as mg/100 g dry tomato waste (DW) (Duh, 1998).

Determination of antioxidant components: Total phenolic compounds were analyzed in extracts using a modified Folin-Ciocalteu colorimetric method using a UV-visible spectrophotometer (Shimadzu, UV-spectrophotometer UV-240). Turbidity of some samples was observed, therefore, the samples were subjected to centrifugation to obtain clear solution for accurate measurements. The results were calculated as mg Gallic Acid Equivalents (GAE) per 100 g (DW) (Chang and Liu, 2008).

Total Flavonoid (TF) contents of tomato waste extracts were determined by a modified colorimetric method described previously (Navarro-Gonzalez *et al*., 2011). TF content was expressed as mg catechin equivalents per 100 g DW.

The carotenoid content (expressed as lycopene) of the extracts was measured spectrophotometrically (Shimadzu, UV-spectrophotometer UV-240) at λ_{max} (Strati and Oreopoulou, 2011).

Determination of antioxidant activity

DPPH Radical Scavenging Assay (R.S.A): The decrease of the absorption at 515 nm wavelength of the DPPH$^{\bullet}$ solution (1,1-diphenyl-2-picryl-hydrazil) after addition of the blank or sample extract was measured. The 3.9 mL of methanolic DPPH$^{\bullet}$ solution (0.0024 mg/100 mL) were mixed with 150 μL of sample extract and after 30 min the absorption was measured at 515 nm (Musa *et al*., 2011).

From plotting of different concentrations of each solvent antioxidant extracted under optimum conditions against R.S.A, EC_{50} was calculated via equation.

β-carotene-linoleic acid oxidation method (Coupled autoxidation): Antioxidant activity was also determined by β-carotene-linoleic acid bleaching method (coupled autoxidation) (Guil-Guerrero and Rebolloso-Fuentes, 2009).

HPLC analysis of tomato extract: HPLC analysis was carried out according to Talhaoui *et al.* (2014) in cooperation with research group on Faculty of Food Science, Wroclaw University of Environmental and Life science, Poland. The selected samples analyzed were ethyl lactate and water extracts using ultrasound assisted extraction method at 60°C for 40 min extraction time.

Statistical analysis: Results are presented as the Mean±Standard deviation from three replicates of each experiment. A p-value <0.05 was used to denote significant differences between mean values determined by the Analysis of Variance (ANOVA) with the assistance of Statistica 7.0 (Stat Soft Inc., Tulsa, OK) software.

RESULTS

Effect of novel assisted extraction methods on extracts yield: From the results recorded in Table 1, it was found that after 10, 20 and 30 min extraction time lower results were observed, however, after 40, 50 and 60 min extraction time nearly similar and higher results were observed. It was found that no marked difference concerning results of antioxidant component and antioxidant activity of extracts carried out at 40 and 60 min extraction time at all temperatures. Therefore, 40 min is economically suitable for conventional extraction method. Distilled water extract was more efficient than other solvents extracts in obtaining higher yield (15540 mg/100 g DW at 20°C and 19380 mg/100 g DW at 60°C at 40 min) from tomato wastes. However, other solvents (ethanol, isopropanol, ethyl lactate, ethyl acetate and n-hexane) gave lower yields (6960, 7220, 7960, 8200 and 7700 mg/100 g DW at 20°C and 10400, 9380, 8660, 9640 and 6920 mg/100 g DW at 60°C at 40 min), respectively.

Ultrasonic Assisted Extractions method (UAE) at 20 and 60°C for 40 min showed that distilled water extract contained higher yield than other solvents. It was found that other solvents gave different yields but ethyl lactate was superior (at 20 and 60°C) to ethanol and isopropanol. Microwave-Assisted Extraction method (MAE) is a relatively new extraction technique and use only water as solvent extraction owing to its high dielectric constant and is a common solvent (Afoakwah *et al.*, 2012). In case of MAE, it was found that the yield of natural antioxidants increased as extraction time increased. It was concluded that water extract exhibited higher yield than the other solvents when using both conventional and novel assisted extraction methods.

Table 1: Total yields (mg/100 g DW) of tomato waste extracts using conventional and novel assisted extraction methods and different solvents

	Conventional extraction method (40 min)		Novel assisted extraction methods				
			UAE (40 min)		MAE		
Solvent	20°C	60°C	20°C	60°C	100°C, 30 sec	100°C, 60 sec	100°C, 90 sec
Water	1554±23.00	19380±24.32	15280±16.30	15460±15.52	15700±13.64	15900±15.16	16060±15.31
Ethanol	6960±13.31	10400±19.05	8460±11.22	9640±10.11	-	-	-
Isopropanol	7220±14.73	9380±17.71	5860±13.63	7960±13.09	-	-	-
Ethyl lactate	7960±11.78	8660±18.06	9340±12.24	9740±9.6	-	-	-
Ethyl acetate	8200±13.05	9640±13.06	-	-	-	-	-
Hexane	7700±14.84	6920±15.63	-	-	-	-	-

UAE: Ultrasound assisted extraction method, MAE: Microwave assisted extraction method, -: Not determined

Effect of novel assisted extraction methods on natural antioxidant content: By measuring the amounts of polyphenols extracted for different extraction times at 20 and 60°C, it was found that water extract was superior to all other solvents extracts. Using conventional extraction method maximum value of total phenolic was achieved when using water as a solvent and lower values were obtained in case of using other solvents. However, higher phenolic contents were observed at 60°C than at 20°C for all solvents extracts.

In case of novel assisted extraction methods, UAE at 20 and 60°C after 40 min extraction time showed that, water extract contained highest amounts of total phenolics. Remarkably, ethyl lactate extract at 20°C gave higher yield of phenolic compounds than ethanol and isopropanol extracts. However, at 60°C ethanol extract gave the higher yield of phenolic compounds than isopropanol and ethyl lactate extract. In case MAE only water was used as solvent for safety reasons. It was found that MAE had great affect on water extract of total phenolic, showing higher extraction capacity in case of 90 sec time of extraction, while at 30 and 60 sec of extraction gave 335.5 and 375 mg gallic acid/100 g DW, respectively. It was found that water extract gave higher phenolics content in conventional, UAE and MAE methods.

Concerning other constituents of polyphenols namely, flavonoids, it was showed clearly that in conventional extraction method after 40 min extraction time, water followed by ethanol and ethyl lactate extracts gave a higher yields of flavonoids (66.88, 61.86 and 5.33 mg catechin/100 g DW at 60°C), respectively and (42.77, 43.53 and 42.82 mg catechin/100 g DW at 20°C), respectively. On the contrary, hexane exhibited lower yield of flavonoids of 11.84 and 6.68 mg catechin/100 g DW at 60 and 20°C, respectively, while moderate amounts of flavonoids of 35.55, 51.33 and 44.91 mg catechin/100 g DW were given by to isopropanol, ethyl lactate and ethyl acetate extracts, respectively.

Higher flavonoid content when using UAE method at 60°C was obtained in water extract nearly equal to ethyl lactate extract, however at 20°C the flavonoids yield was higher in ethyl lactate extract. In case of MAE method, it was found that the total flavonoids in water extract increased as extraction time increased.

Polyphenols of tomato waste extract (phenolics and flavonoids) are potential source of powerful antioxidants and can be used as an easily accessible source of antioxidants. Moreover, superiority of flavonoids fraction over phenolics, particularly in modulating lipid peroxidation in certain diseases and can be expressed by the ratio (TFC/TPC) (Jia *et al*., 1999; Savatovic *et al*., 2010).

Concerning the carotenoids content lycopene has attracted the greatest attention for its potential health benefits (Zuorro *et al*., 2014). Extraction of lycopene at 20 and 60°C using conventional extraction method with different solvents at different stirring times, showed that the best results were observed after 40 min extraction time for all solvents extracts. Ethyl acetate and hexane extracts gave the higher lycopene content than other solvents while water extract gave the lowest values.

In case of UAE method, it was found that at 60°C water, ethanol, isopropanol and ethyl lactate extracts gave 0.9, 2.87, 2.64 and 4.02 mg/100 g DW, respectively. Meanwhile, at 20°C the corresponding values of lycopene were 0.79, 2.86, 1.7 and 4.29 mg/100 g DW. From these results, it was found that ethyl lactate extract, as eco-friendly solvent, generally is an efficient solvent for the extraction of lycopene at 20 and 60°C. MAE method gave higher content of lycopene at 90 sec amounting to 0.56 mg/100 g DW, while it amounts to 0.37 and 0.51 mg/100 g DW at 30 and 60 sec, respectively (Table 2).

Effect of novel assisted extraction methods on antioxidant activity of extracts: DPPH and β-carotene-linoleic acid bleaching method were used for evaluation of the antioxidant activity

Table 2: Antioxidant content (total phenolic, total flavonoids and lycopene) of tomato waste extracts using conventional and novel assisted extraction methods and different solvents

	Conventional extraction method (40 min)		Novel assisted extraction methods				
			UAE (40 min)		MAE		
Solvent	20°C	60°C	20°C	60°C	100°C, 30 sec	100°C, 60 sec	100°C, 90 sec
Water							
LC	0.32±0.12	0.88±0.03	0.79±0.02	0.90±0.03	0.37±0.04	0.51±0.02	0.56±0.03
TPC	384.16±3.07	410.00±4.16	469.00±2.80	460.00±4.04	355.50±4.83	375.00±5.05	377.50±5.50
TFC	42.77±0.61	66.88±3.90	47.77±0.23	62.22±0.58	43.33±1.30	46.66±1.70	49.89±2.70
TFC/TPC	0.111	0.163	0.101	0.135	0.121	0.124	0.132
Ethanol							
LC	2.66±0.08	5.46±0.25	2.86±0.05	2.87±0.17	-	-	-
TPC	101.50±1.80	140.83±2.88	120.50±1.80	162.50±1.70	-	-	-
TFC	43.53±0.65	61.86±2.85	48.53±0.33	62.22±0.56	-	-	-
TFC/TPC	0.428	0.439	0.402	0.382	-	-	-
Isopropanol							
LC	2.022±0.56	4.5±0.180	1.70±0.06	2.64±0.14	-	-	-
TPC	89.000±1.85	138.83±2.44	57.70±1.00	108.00±2.07	-	-	-
TFC	23.950±0.66	35.55±3.41	26.13±0.22	31.88±0.66	-	-	-
TFC/TPC	0.269	0.256	0.452	0.295	-	-	-
Ethyl lactate							
LC	3.85±0.15	5.05±0.07	4.29±0.04	4.02±0.08	-	-	-
TPC	72.29±0.80	125.46±2.26	140.8±1.250	107.90±1.55	-	-	-
TFC	42.82±0.69	51.33±2.35	52.22±0.36	61.11±0.49	-	-	-
TFC/TPC	0.592	0.409	0.37	0.566	-	-	-
Ethyl acetate							
LC	8.88±0.14	7.52±0.28	-	-	-	-	-
TPC	55.77±0.73	69.37±1.40	-	-	-	-	-
TFC	25.55±0.70	44.91±2.14	-	-	-	-	-
TFC/TPC	0.458	0.647	-	-	-	-	-
Hexane							
LC	6.99±0.11	6.57±0.29	-	-	-	-	-
TPC	48.68±1.05	50.75±1.55	-	-	-	-	-
TFC	6.68±0.45	11.84±1.36	-	-	-	-	-
TFC/TPC	0.137	0.233	-	-	-	-	-

UAE: Ultrasound assisted extraction method, MAE: Microwave assisted extraction method, TPC: Total phenolic content (mg gallic acid/100 g DW), TFC: Total flavonoid content (mg catachen/100 g DW), LC: Lycopene content (mg/100 g DW), -: Not determined

of the solvent extracts by conventional and novel assisted extraction methods. It was found that R.S.A values obtained by DPPH in case of conventional extraction method were good for water extract, lower for hexane extract and moderate for other solvent extracts. In addition EC_{50} values indicated that at 20°C water extract give higher antioxidant potency than the other solvents extract, while at 60°C ethanol and ethyl lactate extracts gave higher values than other solvents extracts. Hexane extract gave the lowest antioxidant potency and this may be due to lower efficiency of hexane to extract completely the antioxidant compounds. Comparing conventional method with novel assisted extraction methods it was indicated that MAE gave higher antioxidant potency which increase by increasing extraction time as represented by 37.44, 34.58 and 33.92 at 30, 60 and 90 sec, respectively. In case of UAE better results were obtained in ethanol and isopropanol solvent extracts at 20 and 60°C.

Table 3: Antioxidant activity (DPPH, EC_{50} and A.O.A%) of tomato waste extracts using conventional and novel assisted extraction methods and different solvents

	Conventional extraction method (40 min)		Novel assisted extraction methods				
			UAE (40 min)		MAE		
Solvent	20°C	60°C	20°C	60°C	100°C, 30 sec	100°C, 60 sec	100°C, 90 sec
Water							
R.S.A%	43.50±0.59	42.67±0.42	31.29±0.45	32.31±0.33	47.35±0.51	56.64±0.42	59.39±0.49
EC_{50}	50.42±0.71	68.82±0.76	53.68±0.36	51.06±0.38	37.44±0.43	34.58±0.44	33.92±0.37
A.O.A%	-	50.43±0.53	-	77.10±0.30	55.50±0.45	59.49±0.46	66.87±0.40
Ethanol							
R.S.A%	20.41±0.46	39.23±0.40	30.05±0.35	29.26±0.39	-	-	-
EC_{50}	55.18±0.50	42.42±0.47	44.04±0.42	35.65±0.41	-	-	-
A.O.A%	-	58.68±0.42	-	99.69±0.47	-	-	-
Isopropanol							
R.S.A%	14.74±0.55	28.98±0.41	17.28±0.39	23.27±0.40	-	-	-
EC_{50}	87.93±0.66	50.62±0.50	44.88±0.47	41.31±0.43	-	-	-
A.O.A %	-	59.62±0.44	-	82.16±0.33	-	-	-
Ethyl lactate							
R.S.A%	25.78±0.32	40.40±0.47	21.05±0.26	24.87±0.45	-	-	-
EC_{50}	74.44±0.48	47.47±0.48	82.28±0.45	63.12±0.45	-	-	-
A.O.A%	-	46.26±0.45	-	52.48±0.34	-	-	-
Ethyl acetate							
R.S.A%	20.00±0.44	21.36±0.46	-	-	-	-	-
EC_{50}	67.61±0.51	56.97±0.44	-	-	-	-	-
A.O.A%	-	81.78±0.59	-	-	-	-	-
Hexane							
R.S.A%	5.87±0.39	13.66±0.43	-	-	-	-	-
EC_{50}	120.10±0.78	92.92±0.58	-	-	-	-	-
A.O.A%	-	_ve	-	-	-	-	-

UAE: Ultrasound assisted extraction method, MAE: Microwave assisted extraction method, R.S.A%: Radical scavenging activity (DPPH method), EC_{50}: Concentration of extract that causes a 50% decrease in DPPH absorbance, A.O.A%+: Antioxidant activity (value represent the percent inhibition of oxidation of the linoleic acid/β-carotene emulation), -: Not determined

From β-carotene-linoleic acid bleaching method (coupled autoxidation), the activity of solvents extracts are calculated as antioxidant activity (A.O.A%). The A.O.A% of conventional extraction method at 60°C of water, ethanol, isopropanol, ethyl lactate and ethyl acetate extracts were found to be 50.43, 58.68, 59.62, 46.25 and 81.78%, respectively, the results were supported by the data reported by Barros *et al.* (2007). The higher value of A.O.A% of ethyl acetate extract may be due to the higher original lycopene content in ethyl acetate extracts than other solvents extracts. Therefore, synergism may arise between different antioxidant components. Unexpectedly, negative value of A.O.A% shown in hexane extract may be due to interfering compounds preventing the actual absorption. It should be noteworthy that repeated test gave the same negative value and this was supported by some researchers authors dealing with similar evaluation (Wang and Weller, 2006).

In UAE method at 60°C ethanol extract gave higher value, followed by isopropanol extract, water extract and ethyl lactate extract (99.69, 82.16, 77.10 and 52.48), respectively. However, in MAE method lower values were generally obtained (Table 3).

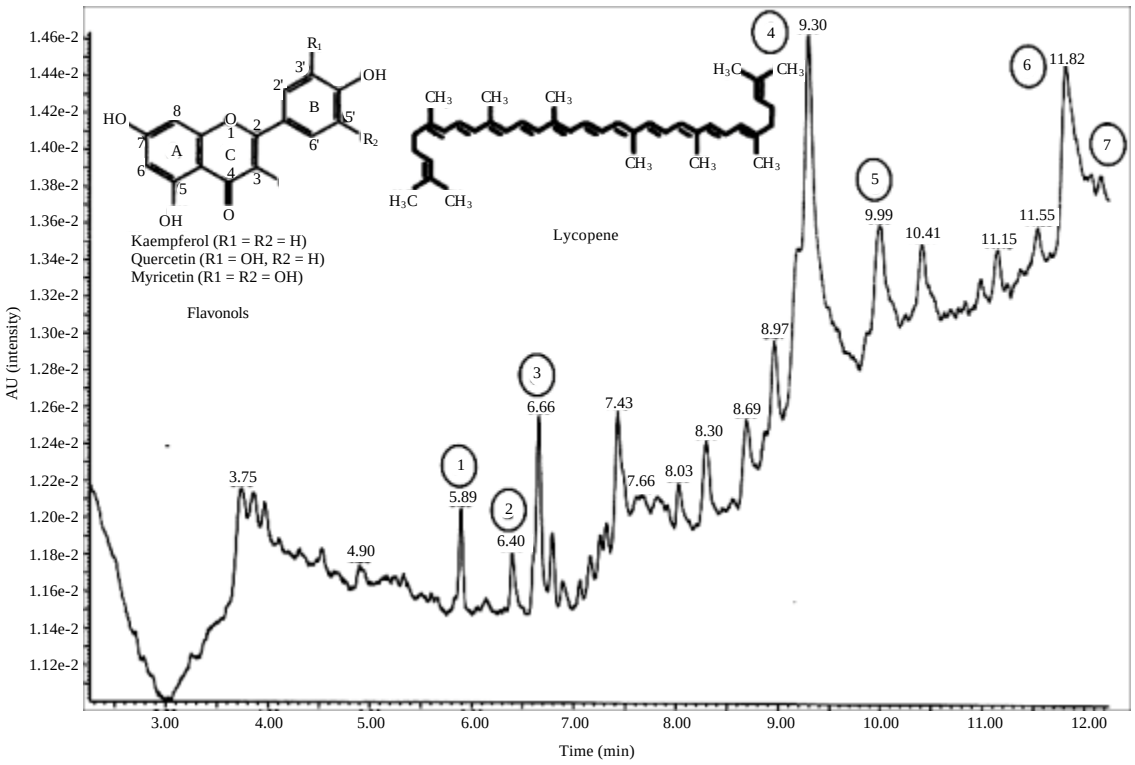

Fig. 1: Peak assignment of HPLC of some particular antioxidants mainly poly-phenols and carotenoids in ethyl lactate extract of tomato waste, using UAE method; Peaks standing for, 1: Rutine, 2: Dihydroquerecetin, 3: Lutein, 4: Lycopene, 5: Lycopene isomers, 6: **β**-carotene and 7: **β**-carotene isomers

HPLC analysis: Ethyl lactate and water extracts using UAE method at 60°C after 40 min extraction time was selected to HPLC analysis due to their high antioxidant activity.

HPLC preliminary results showed that ethyl lactate extract may stand for rutine, rutine derivatives, dihydroquercetine, lutein, lycopene, lycopene isomers, **β**-carotene and **β**-carotene isomers as shown in Fig. 1. However, water extract may give higher concentration of phenolic compounds rather than lipophilic compounds (e.g., lycopene and **β**-carotene) (Fig. 2).

DISCUSSION

By using the conventional and novel assisted extraction methods with different solvents at 20 and 60°C for different time intervals, it was found that the extraction efficiency was affected by the assisted extraction methods, solvent type, temperature and extraction time. As extraction time and temperature increased, the yield, antioxidant content (total phenolics, flavonoids and carotenoids) and antioxidant activity increased. Total phenolics including flavonoids content were found to be higher in distilled water, ethanol, isopropanol and ethyl lactate extracts (hydrophilic solvents), however, the lower values were found in ethyl acetate and hexane extracts (lipophilic solvents). In contrary lycopene content was higher in lipophilic solvents but lower in hydrophilic solvents (Xianquan *et al*., 2005).

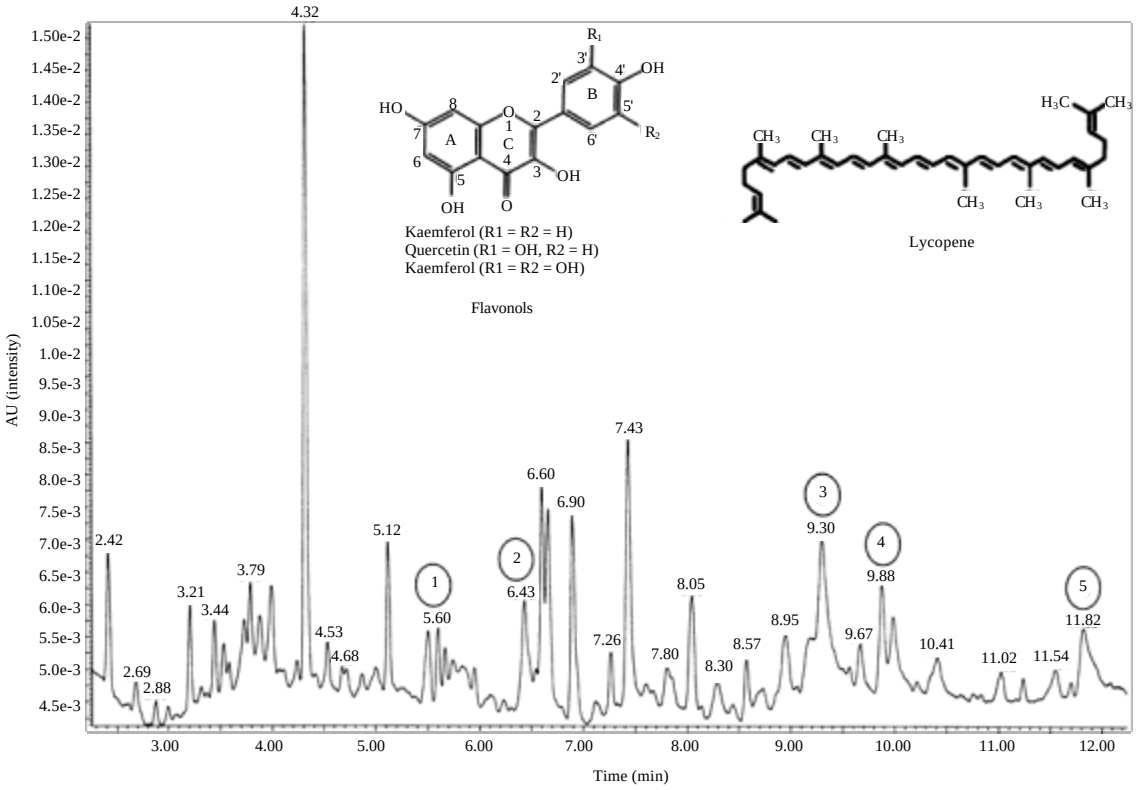

Fig. 2: Peak assignment of HPLC of some particular antioxidants mainly poly-phenols and carotenoids in water extract of tomato waste, using UAE method; Peaks standing for, 1: Rutine, 2: Dihydroquerecetin, 3: Lycopene, 4: Lycopene isomers and 5: β-carotene

It is worth to mention that ethyl lactate as extraction solvent gave high value in both total phenolics and lycopene content so it act as hydrophilic and lipophilic solvent which is used newly and widely as eco friendly solvent.

Total phenolic content of Egyptian tomato waste extracted using UAE method and water as a solvent at 20°C reached superior value of 469 mg gallic acid/100 g DW. This result surpasses most of the previously reported data (Navarro-Gonzalez *et al*., 2011; Toor and Savage, 2005). Lycopene extracted using conventional method at 20°C for 40 min in ethyl acetate and hexane reached to markedly high values of 8.88 and 6.9 mg/100 g DW, respectively which is also surpasses most of the previously reported data (Strati and Oreopoulou, 2011; Naviglio *et al*., 2008; Vagi *et al*., 2007). This proves that the Egyptian tomato waste extract is enriched in lycopene and phenolic compounds. These results highlighted the nature of Egyptian soil as well as, cultivated variety of tomato.

The antioxidant activity (EC_{50}, DPPH and A.O.A%) also increased as extraction time and temperature increased. The highest value of antioxidant activity was obtained by water and ethanol extracts while lowest value was obtained in case of hexane extract in conventional extraction method. This means that the lipophilic solvent such as hexane is not efficient enough to extract completely the antioxidant compounds. Different solvents have selectivity for certain antioxidant compounds in dry tomato waste. Accordingly the selectivity of ethyl lactate is higher than any other solvent.

β-carotene-linoleic acid bleaching method model system, gave reasonably higher values of activity in extracts of water, ethanol, isopropanol and ethyl lactate but the highest activity value is obtained by ethyl acetate extract in conventional extraction method. This may be due to the higher original lycopene content in the sample as dominant antioxidant compound. Hexane extract gave unexpectedly negative value of the A.O.A%. This may be due to interfering compounds preventing the actual absorption. It should be noteworthy that repeated test gave the same negative value result. This was supported by some authors dealing with similar evaluation (Durmaz *et al*., 2010).

Using UAE as novel extraction method, it gave lower yield and lycopene content than conventional method but it gave higher total phenolics content than conventional and MAE method when using distilled water and ethanol as extraction solvent.

From EC_{50} it was found that UAE gave the higher antiradical scavenging activity than conventional method for distilled water, ethanol and isopropanol, while MAE gave highest value for distilled water extract in both conventional and novel extraction methods. For A.O.A% UAE gave the higher value in all cases followed by MAE and conventional method.

UAE method was found to be suitable and preferable over other methods (conventional and MAE method) due to an improvement in mass transfer and assistance of thermal treatment (Knorr *et al*., 2011).

CONCLUSION

It was concluded that safe and cheap eco-friendly solvents (water and ethyl lactate) gave both higher yield and antioxidant activity using novel assisted extraction methods. UAE and MAE were found to be more efficient than conventional extraction methods. It is generally concluded that promising results require not only the yield of solvent extract and type of solvent but also its total antioxidant activity of components were obtained. From HPLC preliminary investigation it was found that lycopene was the predominant component in lipophilic solvent extract whereas, phenolics were also the predominant components in the hydrophilic solvent extract. The results prove that the Egyptian tomato waste is enriched in lycopene and phenolic compounds and results highlighted the nature of Egyptian soil as well as, cultivated variety of tomato.

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REFERENCES

Afoakwah, A.N., J. Owusu, C. Adomako and E. Teye, 2012. Microwave Assisted Extraction (MAE) of antioxidant constituents in plant materials. Global J. Biosci. Biotechnol., 1: 132-140.

Barros, L., M.J. Ferreira, B. Queiros, I.C.F.R. Ferreira and P. Baptista, 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem., 103: 413-419.

Castillo-Munoz, N., M. Fernandez-Gonzalez, S. Gomez-Alonso, E. Garcia-Romero and I. Hermosin-Gutierrez, 2009. Red-color related phenolic composition of Garnacha Tintorera (*Vitis vinifera* L.) grapes and red wines. J. Agric. Food Chem., 57: 7883-7891.

Chang, C.H. and Y.C. Liu, 2008. Evaluation of antioxidative performance of tomato extracts obtained by different methods. J. Sci. Food Agric., 88: 612-618.

Chavan, U.D., F. Shahidi and M. Naczk, 2001. Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. Food Chem., 75: 509-512.

Duh, P.D., 1998. Antioxidant activity of burdock (*Arctium lappa* Linne): Its scavenging effect on free-radical and active oxygen. J. Am. Oil Chem. Soc., 75: 455-461.

Durmaz, G., I. Karabulut, A. Topcu, M. Asilturk and T. Kutlu, 2010. Roasting-related changes in oxidative stability and antioxidant capacity of apricot kernel oil. J. Am. Oil Chem. Soc., 87: 401-409.

Goli, A.H., M. Barzegar and M.A. Sahari, 2005. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chem., 92: 521-525.

Guil-Guerrero, J.L. and M.M. Rebolloso-Fuentes, 2009. Nutrient composition and antioxidant activity of eight tomato (*Lycopersicon esculentum*) varieties. J. Food Compos. Anal., 22: 123-129.

Hemwimon, S., P. Pavasant and A. Shotipruk, 2007. Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*. Sep. Purif. Technol., 54: 44-50.

Hoang, T.H., R. Sharma, D. Susanto, M. Di Maso and E. Kwong, 2007. Microwave-assisted extraction of active pharmaceutical ingredient from solid dosage forms. J. Chromatogr. A, 1156: 149-153.

Jia, Z., M. Tang and J. Wu, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.

Knorr, D., A. Froehling, H. Jaeger, K. Reineke, O. Schlueter and K. Schoessler, 2011. Emerging technologies in food processing. Annu. Rev. Food Sci. Technol., 2: 203-235.

Luengo, E., I. Alvarez and J. Raso, 2014. Improving carotenoid extraction from tomato waste by pulsed electric fields. Nutr. Food Sci. Technol., Vol. 1. 10.3389/fnut.2014.00012

Musa, K.H., A. Abdullah, K. Jusoh and V. Subramaniam, 2011. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): Effect of extraction techniques and solvents. Food Anal. Methods, 4: 100-107.

Navarro-Gonzalez, I., V. Garcia-Valverde, J. Garcia-Alonso and M.J. Periago, 2011. Chemical profile, functional and antioxidant properties of tomato peel fiber. Food Res. Int., 44: 1528-1535.

Naviglio, D., F. Pizzolongo, L. Ferrara, B. Naviglio, A. Aragon and A. Santini, 2008. Extraction of pure lycopene from industrial tomato waste in water using the extractor Naviglio®. Afr. J. Food Sci., 2: 37-44.

Papaioannou, E.H. and A.J. Karabelas, 2012. Lycopene recovery from tomato peel under mild conditions assisted by enzymatic pre-treatment and non-ionic surfactants. Acta Biochimica Polonica, 59: 71-74.

Proestos, C. and M. Komaitis, 2008. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. LWT-Food Sci. Technol., 41: 652-659.

Rodrigues, S., G.A.S. Pinto and F.A.N. Fernandes, 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. Ultrasonics Sonochem., 15: 95-100.

Savatovic, S.M., G.S. Cetkovic, J.M. Canadanovic-Brunet and S.M. Dilas, 2010. Utilization of tomato waste as a source of polyphenolic antioxidants. Acta Periodica Technologica, 41: 187-194.

Schieber, A., F.C. Stintzing and R. Carle, 2001. By-products of plant food processing as a source of functional compounds: Recent developments. Trends Food Sci. Technol., 12: 401-413.

Stanisavljevic, I.T., M.L. Lazic and V.B. Veljkovic, 2007. Ultrasonic extraction of oil from tobacco (*Nicotiana tabacum* L.) seeds. Ultrasonics Sonochem., 14: 646-652.

Strati, I.F. and V. Oreopoulou, 2011. Effect of extraction parameters on the carotenoid recovery from tomato waste. Int. J. Food Sci. Technol., 46: 23-29.

Sun, T. and C.T. Ho, 2005. Antioxidant activities of buckwheat extracts. Food Chem., 90: 743-749.

Tabart, J., C. Kevers, A. Sipel, J. Pincemail, J.O. Defraigne and J. Dommes, 2007. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chem., 105: 1268-1275.

Talhaoui, N., A.M. Gomez-Caravaca, L. Leon, R. De la Rosa, A. Segura-Carretero and A. Fernandez-Gutierrez, 2014. Determination of phenolic compounds of Sikitita olive leaves by HPLC-DAD-TOF-MS. Comparison with its parents Arbequina and Picual olive leaves. LWT-Food Sci. Technol., 58: 28-34.

Toor, R.K. and G.P. Savage, 2005. Antioxidant activity in different fractions of tomatoes. Food Res. Int., 38: 487-494.

Vagi, E., B. Simandi, K.P. Vasarhelyine, H. Daood, A. Kery, F. Doleschall and B. Nagy, 2007. Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. J. Supercrit. Fluids, 40: 218-226.

Vilkhu, K., R. Mawson, L. Simons and D. Bates, 2008. Applications and opportunities for ultrasound assisted extraction in the food industry: A review. Innov. Food Sci. Emerg. Technol., 9: 161-169.

Wang, L. and C.L. Weller, 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol., 17: 300-312.

Xianquan, S., J. Shi, Y. Kakuda and J. Yueming, 2005. Stability of lycopene during food processing and storage. J. Med. Food, 8: 413-422.

Zheng, X., B. Liu, L. Li and Z. Zhu, 2011. Microwave-assisted extraction and antioxidant activity of total phenolic compounds from pomegranate peel. J. Med. Plants Res., 15: 1004-1011.

Zuo, Y., H. Chen and Y. Deng, 2002. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta, 57: 307-316.

Zuorro, A., R. Lavecchia, F. Medici and L. Piga, 2014. Use of cell wall degrading enzymes for the production of high-quality functional products from tomato processing waste. Chem. Eng. Trans., 38: 355-360.