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Impact of cooking on the phenolic, flavonoids content and the antioxidant activity of *Corchorus Olitorius* (Molokhia) and *Malava Parviflora* (Mallow) leaves

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Abstract

In this study the total phenolics content expressed as gallic acid equivalents (GAE) was higher in raw mallow leaves (459 mg GAE/100g DW) than in raw Jew's mallow leaves (339 mg GAE/100g DW). Results showed that, the concentration of total flavonoids in Jew's mallow was: 189, 163, 156 in raw, cooked and aqueous extract respectively. Whereas were in mallow as following: 188 in raw mallow, 174 in cooked mallow and 165 in aqueous extract. The antioxidant activity of raw and cooked Jew's mallow and mallow leaves and their aqueous phenolic extracts were: 2.947±0.09, 3.315±0.05, 9.4±0.04, 2.076±0.09, 2.440±0.02, 5.9±0.03, respectively. Phenolic compounds in raw and cooked Jew's mallow and mallow leaves (mg/ 100g on dry weight basis) were: 2.2376 in raw Jew's mallow, 1.9918 in cooked Jew's mallow, 9.563 in raw mallow, 6.9432 in cooked mallow.

I. INTRODUCTION

Phenolic compounds are one of the largest and diversified groups of phytochemical compounds ubiquitous in the plants, with more than 8000 phenolic compounds (Trabelsi *et al*., 2013). Phenolic compounds are considered as secondary metabolites that are synthesized by plants during their normal development and in response to stress conditions such as infection, wounding and UV- radiation. (Naczk and Shahidi, 2004). The major active nutraceutical components in plants are flavonoids (Khatiwora *et al*., 2010). They account for approximately two-third of dietary phenols, with more than 6000 flavonoid molecules identified in plants. The basic flavonoid structure (C_6- C_3- C_6) is that of diphenyl propane called the flavan nucleus. Difference in generic structure of heterocyclic C ring classify them as flavonols, flavanones, flavan-30b, isoflavone, flavanols, flavones and anthocyanidins Proestos and Komaitis, 2013.).The total flavonoids content (mg/100g fresh weight) in Jew's mallow shoot was 63.9. Leaves of the Jew's mallow contains (mg/100g dry weight) 393, 314 total of phenolics and total of flavonoids, respectively. (Yang *et al*. (2008)., Oboh *et al*. (2012)). Ozturk and Savaroglu (2012) reported that Jew's mallow leaves methanolic extract contains 42.1mg gallic acid equivalent (GAE) /g of total phenolics. Azuma *et al*. (1999) identified six phenolic compounds (chlorogenic acid, 3.5 dicaffeoylquinic acid, quercetin 3-galactoside,quercetinglucoside, quercetin 3-C6 malonylgalactoside) in the leaves of Jew's mallow.Ola *et al*. (2009) detected eight phenolics in Jew's mallow leaves. They were caffeoyl quinic derivative, chlorogenic acid, hyperoside, isoquercitrin, 1.5-dicaffeoyl quinnic acid, dicaffeolyl acid, dicaffeolys derivatives and quercetin derivatives. Daniela *et al*. (2007) identified the following phenolic compounds in common mallow leaves: quercetin, 5.7-dimethyl oxycoumarin, Kaempferol, myricelin and genistein. Afolayan *et al*. (2008) reported that the methanolic extract of mallow plant contains 2.9mg as GAE/g dry weight of total phenolics and 32.0mg as quercetin /g dry weight of total flavonoids.Antioxidants are substances which when present at equal or higher concentration compared to those of an oxidizable substrate, significantly delay or prevent the oxidation of the substrate (Halliwell and Gutteridge, 1989). Free radicals and reactive oxygen species, such as super oxide radical (O_2) hydroxyl (OH)

radical, peroxyl radical (ROO·), nitric oxide ($NO^{\cdot}$) and nitrogen dioxide (NO_2·) are constantly formed in the human body during normal metabolism (Cavas and Yurdakoc, 2005). However, excessive amounts of reactive oxygen species may play an important role in the development of several patHhhological conditions such as lipid peroxidation, protein oxidation, DNA damage and cellular degeneration, which are related to cardiovascular diseases, diabetes, inflammatory diseases, cancer, Alzheimer and Parkinson diseases, mongolism, and ageing process (Halliwell, 1991). Plant antioxidants such as α-tocopherol, ascorbic acid, carotenoids, and polyphenols could scavenge reactive oxygen species, thereby, minimize oxidative damage (Thomas, 1995). Ozturk and Savaroglu,(2011). Reported that percentages of inhibition of methanol, aceylacetate and water extracts of Jew's mallow leaves estimated by means of antiradical activity (DPPH) were 25.6, 34.9 and 26.8% at 1.6g/ml, respectively.

I. MATERIALS AND METHODS

Materials:

Fresh Jew's mallow plant (*Corchorus olitorius* L.) balady variety was collected at the maturity (50 kilograms) from local farm in Abeese village, Alexandria, Egypt. Fresh mallow plant (*Malva parviflora*) was gathered (50 kilograms) at the time of its most frequently consumption stage from local farm in Abeese, Alexandria, Egypt.

Chemicals and reagents

Twelve phenolic compound standards (chlorogenic acid, gallic acid, protochatchuic acid, gentisic acid, vanillic acid, syrngic acid, caffeic acid, ferulic acid, sinapic acid, rosmarinic acid, coumarin and catechine) were purchased from Sigma (St. Louis, Mo, USA). HPLC grade methanol and acetonitrile were purchased from Aldrish Chemical Company (Milweukee, WI). 2.2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrish Co. (St. Louis, Mo, USA).

Preparation of sample and cooking

All samples were destalked to separate the edible portions (mostly the leaves). The edible portions were thoroughly washed with tap water followed by distilled water, allowed to drain and the excess water was removed using paper towel. The edible portions were well mixed using a blender (National model- MX- 291N) for 2 minutes, and divided into two portions. The first portion was freeze-dried (Alpha 1-4 LSCplus, Chirst). The second portion of each of the blended leaves was added to distilled water (1: 1, w/v) that just reached to boil, in a covered stainless steel pan, and cooked for 10 minutes, then rapidly cooled in an ice bath before being freeze- dried (Alpha 1-4 LSCplus, Chirst). The lyophilized raw and cooked samples were ground using a food grinder (Model M X 491N, National) into powder, 40 mesh screen, the powders were transferred to plastic containers with screw caps and kept frozen at −20 Cº until using for analysis.

The third portion of each leaves was cooked and cooled as mentioned above, then centrifuged at 10000 rpm for 15 minutes (Beckman, ModelJ-21C). The cooking solution was collected while the solid was discarded. The cooking solution was filtered with mucilen cloth and the filtrate was packed in plastic bottles, kept at −20 Cº for subsequent analysis and utilization.

Total phenolics and total flavonoids determination

One gram of lyophilized samples was mixed separately with 100 ml methanol and homogenized (Moulinex blenderLM2221BA, France). The homogenates were kept at 4 °C overnight and then centrifuged at 10,000 rpm for 20 min and used for total phenolics and total flavonoids determination as follows:

Total phenolics were determined by the Folin-Ciocalteu (Blainski *et al*., 2013). 500 µl of methanolic extract was transferred into a test tube and oxidized by the addition of 250 µl of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na_2CO_3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was calculated using a calibration curve prepared with gallic acid, and the result was expressed as µg of gallic acid equivalent (GAE) per g of sample (DW).

Total flavonoids content was determined according to the method outlined by Lin and Tang (2007). Briefly, 500 µl of extract was mixed with 250 µl of 5% sodium nitrate solution. After 6 min, 205 ml of 10% Aluminum chloride solution was added and kept for 7 minutes. Thereafter, 1.25 ml of 1 M sodium hydroxide was added and the mixture was centrifuged at 5000 *g* (Hermle, 2320) for 10 min. The absorbance of the supernatant was measured at 510 nm against the blank. The total flavonoids content was expressed as µg of catechin equivalent (CE) per ml of sample (DW).

Phenolic compounds analysis by HPLC

Phenolic compounds were extracted according to the method of (Dewanto *et al*. 2002). Briefly, one gram of sample was mixed with 20 ml of 2M sodium hydroxide, flushed with nitrogen gas and the flask was stoppered. The pH of the sample was shacked for 4 h at ambient temperature. The pH was adjusted to 2 by using 6 M HCl, then the content was centrifuged at 5000 rpm for 10 mins. The phenolic compounds were extracted twice with 50 ml ethyl ether: ethyl acetate (1:1, V/V). Hexane layer was separated and evaporated by rotary evaporator (Model RE52A, China) at 45°C, then the residue was dissolved in 2ml methanol and filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI), then used for

analysis by HPLC using Agilent Technologies 1100 series liquid chromatography, equipped with an auto sampler and a diode-array detector. The injection volume was 50 µl and The analytical column was a Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient program. The peaks were monitored simultaneously at 280 and 320 nm for benzoic and cinnamic acid derivatives, respectively. Phenolic compounds were identified and quantitified by congruent retention times and UV spectra and compared with those of the standards (Salawu *et al*., 2009).

DPPH˙ free radical scavenging assay

The antioxidant activity of the methanolic extracts on the basis of scavenging activity of the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was determined by the method described by Brand-Williams *et al*., (1995). An aliquot of 15 - 40 µl of each extract was mixed with 100 µl of methanolic DPPH (50 µM) and the mixture was brought to total volume of 3 ml with methanol. The mixture was mixed thoroughly and allowed to stand in a dark for 60 mins. Absorbance was read at 517 nm against the blank. The radical scavenging activity was calculated according the following formola:

$$\text{Inhibition (\%)} = 100 \times (A_{control} - A_{sample})/A_{control}$$

Where:

$A_{control}$ is the absorbance of the control reaction (containing all the reagents except the test compound).

A_{sample} is the absorbance of the test compound.

The extract concentration providing 50% of radical scavenging activity (IC_{50}) was calculated from graph of percentage inhibition against ascorbic acid concentration, and expressed as mg of ascorbic acid equivalent (AAE) per g sample (DW).

II. RESULTS AND DISSCUSION

Total phenolic content in raw and cooked Jew's mallow and mallow leaves and their aqueous phenolic extracts

In addition to general nutritional properties of Green Leafy Vegetables (GLVs), they contain a variety of bioactive substances of which phenolic substances. phenolic compounds are considered as the main factors for the antioxidant capacity of plants and have also many benefits on human health, as antioxidant, anti-inflammatory, anti-carcinogenic and anti-microbial (Manach *et al*., 2005).The total phenolics content data for Jew's mallow and mallow leaves before and after cooking as well as their aqueous phenolic extracts were investigated and the results are shown in Table 1 The total phenolics content expressed as gallic acid equivalents (GAE) was higher in raw mallow leaves (459 mg GAE/100g DW) than in raw Jew's mallow leaves (339 mg GAE/100g DW). Oboh *et al*. (2012) depicted 389.3 mg GAE/100g DW for total phenolics in raw Jew's mallow leaves. The content of phenolics in plants are influenced by numerous factors, such as variety, species, region of cultivation and maturity stages (Tlili *et al*., 2014). Boiling significantly decreased the total phenolics by 5.8%in the Jew's mallow leaves while the decreasing was insignificantly for mallow leaves. Aqueous extracts of cooked leaves had significantly lower values than either raw or cooked leaves. The results clearly indicated that the most of the total phenolics were dissolved in water used for cooking of the leaves, where the total phenolics contents were 282.8 and 438.4 mg GAE/100g DW for Jew's mallow and mallow leaves, respectively. Studies have shown that total phenolics content in some vegetables were significantly reduced by cooking Mirzaei *et al*., 2014).However, consumption of 100g of cooked leaves or their extracts are within the estimated range (25mg - 1 g antioxidants /day) reported by Stalikas (2007) which is suitable to overcome an array of diseases.

Total flavonoids in raw and cooked Jew's mallow and mallow leaves and their aqueous phenolic extracts

Total flavonoids content of raw, cooked and aqueous phenolic extracts of Jew's mallow and mallow leaves is shown in Table1 The both of the raw leaves contained almost equals content of total flavonoids expressed as mg catechin equivalents (CAE), being 189.0 and 188.0 mg CAE/100g DW for Jew's mallow and mallow leaves, respectively. The total flavonoids content in Jew's mallow is lower than (314 mg quercetin equivalent/100g) that earlier reported by Oboh *et al*. (2012). Beghdad *et al*. (2014) reported that raw common mallow leaves contain 5.69 mg rutin equivalent/ 100g dried weight. Variation in total flavonoids among plants was expected and could be attributed to species, variety, maturity and growing conditions, etc. Cooking of each leaves significantly reduced ($p \leq 0.05$) total flavonoids by about 13.8% in Jew's mallow and 7.4% in mallow leaves. These results suggest that the total flavonoids probably degraded during boiling that leads to decrease of those compounds. The aqueous extracts separated from cooked leaves were significantly lower ($p \leq 0.05$) than either their raw or cooked leaves. Ismail *et al*.(2004) stated that thermal treatments of vegetables have destructive effect on flavonoid compounds as they are highly unstable compounds.

Table 1 :Total phenolics and total flavonoids of raw and cooked Jew's mallow and mallow leaves and their aqueous phenolic extracts(on dry weight basis)*.

Sample	Total phenolics **mg GAE/100g	Total flavonoids ***mg CAE/100g
Jew's mallow		
Raw	339.0± 0.29[a]	189±0.39 [a]
Cooked	319.5±0.25[b]	163±0.35[b]
Aqueous extract	282.8±0.34[c]	156±0.36[c]
Mallow		
Raw	459.0±0.39[a]	188±0.39[a]
Cooked	456.1±0.37[a]	174±0.42[b]
Aqueous extract	438.4±0.35[b]	165±0.28[c]

*Means (n=3)± standard deviation
Means with different subscript in the same column separated by shaded row are significantly different at (P≥0.05)
**Expressed as mg gallic acid equivalents/100g dry weight
*** Expressed as mg catechinequivalents/100g dry weight

Table 2:Antioxidant activity of raw and cooked Jew's mallow and mallow leaves and their aqueous phenolic extracts*.

Sample	Antioxidant activity IC_{50} mg/ml
Jew's mallow	
Raw	2.947±0.09[c]
Cooked	3.315±0.05[b]
Aqueous extract	9.4±0.04[a]
Mallow	
Raw	2.076±0.09[c]
Cooked	2.440±0.02[b]
Aqueous extract	5.9±0.03[a]

*Means (n=3) ± standard deviation
Means with different subscript in the same column separated by shaded row are significantly different at (P≥0.05)

Antioxidant activity of raw and cooked Jew's mallow and mallow leaves and their aqueous phenolic extracts

DPPH radical scavenging is one of the most widely used methods for screening the antioxidant activity of plants.Analysis of the free radical scavenging activities of raw, cooked and aqueous phenolic extracts of Jew's mallow and mallow leaves on 2,2-diphenyl-1-picryihydrazyl (DPPH) radical are showed in table 2 showed that raw Jew's mallow leaves had IC_{50} (the concentration of the extract of which 50% of initial DPPH are decreased) of 2.947mg/ml and the corresponding value for raw mallow leaves was 2.076 mg/ml, these results of the antioxidant activity, being higher in raw mallow leaves than in raw Jew's mallow leaves, as the lower the value of the mg/ml at IC_{50} indicates higher free radical scavenging activity. Farhan *et al*. (2012b).

Phenolic compounds composition of raw and cooked Jew's mallow leaves

Results of this study (Table 3) show that the total individual phenolic compound in raw Jew's mallow leaves was 31.3098mg/100g DW. The simple phenolic acids were consisted of two fractions, hydroxybenzoic acids and hydroxy cinnamic acids. The first fraction had higher content of individual phenolics (2.2376 mg/100g DW) compared to the second fraction (1.1359 mg/100g DW). Vanillic acid was dominate (1.2209 mg/100g DW) followed by protochatchuic acid (0.5446 mg/100g). Gentistic and syrngic acids were not detected. The corresponding values for hydroxy cinnamic acids were sinapic acid (0.6237 mg/100g DW) followed by ferulic acid (0.5122 mg/100g DW). Caffeic acid was not detected. Chlorogenic acid (a phenolic ester) was dominate ones (13.2345 mg/100g DW) in raw Jew's mallow leaves followed by catechine (a flavonoid compound) at 11.4105 mg/100g DW. Rosmarinic acid and coumarin were also detected in raw Jew's mallow leaves at concentration of 2.8193 and 0.472 mg/100g DW, respectively.

Table 3: Phenolic compounds in raw and cooked Jew's mallow and mallow leaves(on dry weight basis).

Compound mg/100g	Sample			
	Jew's mallow		Mallow	
	Raw	Cooked	Raw	Cooked
Hydroxy benzoic acid derivatives				
Gallic acid	0.4721	ND	1.6473	0.822
Protochatchuic acid	0.5446	0.4162	1.376	2.1438
Gentisic acid	ND	ND	ND	ND
Vanillic acid	1.2209	1.5756	6.5397	3.9774
Syrngic acid	ND	ND	0.2293	0.3076
Total	**2.2376**	**1.9918**	**9.563**	**6.9432**
Hydroxy cinnamic acid derivatives				
Caffeic acid	ND	ND	ND	ND
Ferulic acid	0.5122	2.0293	29.6119	30.7566
Sinapic acid	0.6237	0.4162	6.7068	11.8909
Total	**1.1359**	**2.4455**	**36.3187**	**42.6475**
Phenolic esters				
Chlorgenic acid	13.2345	12.8183	6.4037	7.8579
Rosmarinic cid	2.8193	1.6838	3.1678	ND
Total	**16.0538**	**14.5021**	**9.5715**	**7.8579**
Coumarin	0.472	0.2491	0.9787	0.4127
Catechine	11.4105	9.6795	10.0633	8.5589
Total individual phenolic compounds	**31.3098**	**28.868**	**66.4952**	**66.4202**

*ND= Not detected

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