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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT EFFICACY OF THREE PARTS OF THE PUMPKIN *CUCURBITA MOSCHATA* AND THE EFFECT OF THE DRYING METHOD ON THEM

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ABSTRACT

This study aims to determine the total phenolic content and antioxidant activity for three parts of the pumpkins *Cucurbita moschata* which are the peel, leaves, and the pulp dried under sun and determine the Antioxidant activity of these parts using five different solvents: distilled water at room temperature, boiled distilled water,50% ethanol in water, 5% hydrochloric acid in water & 1% hydrochloric acid in water. Folin-Ciocolateau method used for assessment of phenolic compounds & synthetic free radical inhibition DPPH(2,2-diphenyl-1-picrylhydrazyl radical) for determination of oxidant activity. Statistical analysis showed that phenolic content depends on the part of the plant and type of the solvent used in the extraction process in that acid solvents, 5%HCL & 1% HCL were better than 50% ethanol, cold & boiled distilled water in both phenolic compound extraction & assessment of antioxidant activity. Results also showed that types of the solvent & plant parts are also important in determination of the phenolic content of the pumpkin & its antioxidant activity.

KEYWORD: *Cucurbita moschata*, Total phenolic content, Antioxidant activity.

INTRODUCTION

Pumpkin *Cucurbita moschata* belongs to the cucurbitaceae family, whose plants are annual, long-standing, climber, or shrub. The shrubs and their fruits may be soft-grained. They may be dry or loose. The seeds are often oil-rich and are spread in the tropics, sub-tropical, temperate and semi-desert regions.

It is a family of many known and used medicinal plants such as *Cucurbita pepo*, *Cucurbita maxima*, *Cucumis melo*, *Citrulls lanatus*, *Cucumis sativus*, wild species such as *Citrullus colocynthis*, *Bryonia multiflora*, *Ecballium elaterium* and other plants (AL-Musawi, 1987).

Pumpkin is an important source of complex carbohydrates and contains a high percentage of Fiber, Vitamins (A, C and E), Carotenoids, Potassium, Zinc and and also contains Linoleic acid, vegetable estrogen, Selenium, Calcium, Amino acids and Cucurbitan E compound, a compound used to get rid of worms and ringworms (Maheshwari *et al*, 2015). The fruits of the Pumpkin are rich in beta carotene and the color of the orange fruit is due to the abundance of this substance (Norshazila *et al*, 2012). The fruit of the pumpkin *C.moschata* is used as food and medicine in traditional medicine and has the potential to be preserved for long period. The most common use for its seeds is as a dietary supplement or as a kind of nuts. Pumpkin seeds are high in unsaturated fats, proteins, amino acids, magnesium, zinc, phytosterol, cytosterol (Bahramsultany *et al*, 2017). It also contains the fatty acids palmitic, steraic, oleic, linoleic (Al-Shahwani *et al*, 2008). It is a food used in diet or weight loss and acts as an anti-fat (Lee et al, 2012).

Objectives of the study

1- Determination of total phenolic content for three parts of the Pumpkin plant *C. moschata* Duchesne ex Poir, which is cultivated in different parts of Iraq.
2- Evaluation of Anti-oxidative activity by estimating the efficacy of 2-2 diphenyl-1-picrylhydrazyl DPPH for extracts prepared with five different solvents.

MATERIALS AND METHODS

Study Samples

Pumpkin plant *C.Moschata* was chosen for the experiment and it was collected from farms in three cities (Qaratepah, Jalawla, & Khanaqin) during the period of January 8- 11th of 2017 and plant parts (peel, leaves and the pulp) used in the experiment collected and washed by tap water to remove dust then samples were dried under the sun, then grinded to powder by electric grinder and stored in dark container, wrapped in aluminum paper to prevent oxidation& refrigerated till the day of experiment.

Plant Extracts Preparation

Five different solvents used for preparation of plant extracts.

Distilled water at room temperature: forty milliliter of distilled water added to 400 mg of plant powders used to measure total phenolic content (TPC)in a concentration of 10 mg/ml. The same method of preparation was used For free radical determination concentrations used 10mg/ml . Boiling Distilled Water: Same steps in 1 were used but using boiling DW instead of DW at room temperature. Ethanol alcohol(96%) diluted to 50% in Distilled water at room temperature: 40 ml of 50% ethanol added to 400 mg of plant powder for determination of TPC and 40ml of it added to 400mg of the plant powder in free radical experiment. Hydrochloric acid HCL(36%) diluted in water (5%): HCL diluted in DW to 5% at room temperature by addition of 5 ml of HCL to 95 ml of DW and used in determination of TPC by addition of 40 ml to 400mg of the powder in concentration of 10mg/ml and in free radical experiment 40 ml of diluted HCL added to 400 mg of plant powder to get a concentration of 10 mg/ml. . Hydrochloric acid(36%) diluted in water (1%): HCL diluted in DW to 1% at room temperature by addition of 1 ml of HCL to 99 ml of DW and used in determination of TPC by addition of 40 ml to 400mg of the powder in a concentration of 10mg/ml and in free radical experiment 40 ml of diluted HCL added to 400 mg of plant powder to get a concentration of 10 mg/ml.. Extracts were left for 2 days at room temperature then centrifuged at 3000 rpm for 10 minutes, then filtered by filter paper & used in the experiment.

Measurement of total phenolic content (TPC)

Folin-ciocalteu method used for determination of total phenolic content in the prepared extracts with some modifications (Molan,2009) were 10 microliter of the extract with 200 microliter of 2% diluted sodium bicarbonate added to micro plate wells of Enzyme Linked Immunosorbent Assay(ELISA) the mixture left for 5 minutes to react then 10 µl of Folin detector diluted 50% in water & the micro plate left for 30 minutes to react. Time was carefully considered in the experiment because of its importance and to ensure time compatibility in the experiment. After 30 minutes optical density measured by ELISA at wave length 490nm.

Standardization was done by aqueous solution of Gallic acid in different concentration(0.11,200,400,600,700,,800,1000,1200) µg/ml & the following equation used Y=0.0008X+0.0596 .

Y= is the absorbance, X =is the required value.

Free radical scavengers

To determine the Antioxidant activity of the extracts used in this study,modified DPPH method used(Molan *et al*,2009) were 20 µl of the extract mixed with 200 µl of DPPH prepared in absolute ethanol alcohol(96%) and the mixture put in ELISA micro plate wells and incubated at 37^0C for 30 minutes and then the absorbance measured at wavelength of 490 nm and the following equation is used A-B/A-100.

A = the absorbance of control group (D.W and DPPH only)

B = the absorbance of the mixture of extract and the solvent

Statistical analysis

Statistical analysis by SPSSv.22 for numerical variables described in means and standard error (Mean ±SE) and t-test to compare two groups & ANOVA test to compare more than two groups.

Averages were compared by Duncan test and correlation coefficient(R2) used to find the possible relations between variables & in this study all tests were considered significant at 0.05 & the results represent two different experiments & three replicas for each experiment.

RESULTS AND DISCUSSION

The current study agree with other researches and studies that determined the total phenolic contents of different plants and its antioxidant capacity and considering these plants an important source for natural antioxidants as its antioxidant capacity was linked to its content of phenolic and other active compounds whether in medicinal plants or in food like fruits and vegetables(Molan *et a*l., 2012; Adamkova *et al*, 2013; Siti-Mahirah *et al*, 2014; Nordin *et al*, 2017; Rathi and Turki, 2018; Rathi and Abdulhay, 2019).

Although most studies depend on alkaline solvents as a good solvent for phenols, this study showed that 5% HCL and 1% HCL were more effective than 50% ethanol in distilled water, distilled water at room temperature & boiled distilled water were more in its phenolic contents and its capacity to inhibit DPPH and this may be due to ability of HCL to destroy plant cell wall more than other solvents and release more phenolic compounds(Sani, 2012; Yang *et al*., 2013; Rathi *et al*., 2018).

Kim et al(2011) showed that active compounds in Cucurbitaceae varies according to plant type, plant part and environmental conditions in addition to genetic factors and capacity to produce secondary metabolites(Cosmulescu *et al.*, 2017).

And the substitutes on the aromatic ring (Balasundram *et al*, 2005) and the reduction in antioxidant efficacy sometimes may be due to two causes whiach are destruction of antioxidant compounds or formation of Prooxidants with antioxidants (Al- Rekabi, 2007). and Zhou eta al (2017) mentioned that Pumpkin *C.moschata* has more phenolic content and more inhibitory effect on DPPH than other types *C.pepo* and *C. maxima*.

This study also agree with Bernard(2014) that studied effect of drying method on the phenolic content of cinnamon plant (*Cinnamomun zeylanieum*), he mentioned that that the best way is drying by oven at 50C were it showed more phenolic content and more DPPH inhibition higher than those dried by sun, dried at room temperature and dried by freezing at -45C and showed exposure to extreme sunlight with high intensity and long periods leads to destruction of enzymes and chemical compounds in plant parts and high phenolic content in oven samples may be caused by ripening activation of phenol deactivation enzymes and their also proved by(Lopez *et al.*, 2013) in gold breey plant and he showed that air drying decreases phenolic content while the revers was seen using oven in different temperatures (50,60,70, 80 and 90C) although the differences was not significant for degrees from 50-80C but the highest total phenolic content was at 90C and this may be due ability to produce phenol precursors during non enzymatic transformation of phenolic compounds and the highest antioxidant activity was at highest temperature (90C) due to high phenolic content while at 50C was less inhibitory for DPPH because of exposure to temperature for long period during drying in addition to other factors affecting antioxidant activity like nature of the plant part used and its response to drying process, components of plant cell wall and position of glucosides in the cells and phenolic compound all are related to drying conditions.

The amount of phenolic content in fruits and vegetables depend on several factors like structure of phenolic compound, position and number of hydroxyl group(Zhou *et al*., 2005) and species degree of ripening, light exposure,after harvest and storage conditions and intrinsic changes like enzymatic activity of phenol forming and degrading enzymes and the antioxidant activity do not depend only on phenolic compound concentration only but also shared by other antioxidants like flavonoids, ascorbic acid, beta carotene and others (Sultan and Eisa, 2013).And harvest time, growth conditions, temperature,and time are also important

factors to determine phenols in plants(Nordin *et al.*, 2017). As well correlation factors of total phenolic content (drying under Sun light) and anti-radical activity of extracts prepared from three part of pumpkin*(C. moschata*) demonstrated in table 7 while, Correlation (R2 values) between the total phenolic content(drying in oven under 40^0C) and anti-radical activity of extracts prepared from three part of pumpkin(*C. moschata*) demonstrated in table 8.

Table 1: Total phenolic content of extracts prepared from three parts of *C. moschata* by using five different solvents. Each values represents the Mean ± Standard error (SE) of three replicates.

Sun light	TPC (mg GAE/g dry wight) Mean ±SE				
	Cold Distilled water	Boiling Distilled water	Ethanol50%	HCL 5%	HCL 1%
Peel	$^{c}0.0\pm0.0^{z}$	$^{a}6.4\pm0.1^{x}$	$^{d}1.53\pm0.3^{y}$	$^{c}8.3\pm.17^{w}$	$^{c}7.3\pm0.3^{w}$
Leaves	$^{b}1.8\pm0.7^{y}$	$^{d}00\pm00^{z}$	$^{a}15.2\pm0.6^{x}$	$^{a}39.1\pm0.8^{w}$	$^{a}38.4\pm0.4^{w}$
Pulp	$^{a}4.8\pm1.5^{y}$	$^{c}2.5\pm3^{z}$	$^{b}7.9\pm0.3^{x}$	$^{b}12.8\pm4.6^{w}$	$^{c}6.0\pm0.1^{x}$

Table 2: The total phenolic content of extracts prepared from olive oil residues using five different solvents, Each values represents the Mean ± Standard error (SE) of three replicates.

Oven	TPC (mg GAE/g dry wight)				
	Cold Distilled water	Boiling Distilled water	Ethanol50%	HCL 5%	HCL 1%
Peel	$^{b}11.7\pm2.6^{y}$	$^{f}0.0\pm0.0^{z}$	$^{b}9.47\pm2.54^{y}$	$^{b}36.6\pm4.00^{w}$	$^{a}26.6\pm1.7^{x}$
Leaves	$^{b}10.4\pm1.0^{z}$	$^{a}28.8\pm1.0^{x}$	$^{a}16.7\pm0.9y$	$^{a}58.6\pm1.3^{w}$	$^{b}20.9\pm1.3^{y}$
Pulp	$^{a}14.8\pm2.5^{w}$	$^{c}10.6\pm2.5^{x}$	$^{a}14.6\pm1.5^{w}$	$^{c}13.5\pm.98^{w}$	$^{c}14.1\pm1.2^{w}$

Table 3: Effect of three part of Pumpkin *C. moschata* extracts in inhibiting the free radical (DPPH) using five different solvent (10mg/ml concentrations).

Sun light	% inhibition of free radical plant activity DPPH				
	Cold Distilled water	Boiling Distilled water	Ethanol50%	HCL 5%	HCL 1%
Peel	$^{b}13.9\pm1.7^{x}$	$^{a}7.5\pm1.8^{y}$	$^{d}2.8\pm1^{z}$	$^{c}39.6\pm1.8^{w}$	$^{b}36.5\pm0.7^{w}$
Leaves	$^{a}17.4\pm2.7^{x}$	$^{a}6.2\pm4.6^{z}$	$^{a}14.9\pm2.3^{y}$	$^{b}43.8\pm1.6^{w}$	$^{a}40.9\pm0.7^{w}$
Pulp	$^{c}9.1\pm2.5^{y}$	$^{a}8.2\pm2.9^{y}$	$^{c}4.6\pm0.7^{z}$	$^{a}48.5\pm1.9^{w}$	$^{a}39.8\pm1.8^{x}$

Table 4: Effect of three part of Pumpkin *C. moschata* extracts in inhibiting the free radical (DPPH) using five different solvent.(10mg/ml concentrations).

Oven	% Inhibition of Free Radical plant activity DPPH				
	Cold Distilled water	Boiling Distilled water	Ethanol 50%	HCL 5%	HCL 1%
Peel	$^{c}0.0\pm0.0^{y}$	$^{b}0.0\pm0.0^{y}$	$^{d}0.0\pm0.0^{y}$	$^{a}78.40\pm1.2^{w}$	$^{b}40.7\pm1.9^{x}$
Leaves	$^{c}0.0\pm0.0^{z}$	$^{a}3.50\pm2.3^{z}$	$^{b}5.47\pm1.3^{y}$	$^{a}74.63\pm2.8^{w}$	$^{a}48.0\pm1.3^{x}$
Pulp	$^{c}0.0\pm0.0^{z}$	$^{b}0.0\pm0.0^{z}$	$^{a}12.83\pm2.6^{y}$	$^{a}77.13\pm4.8^{w}$	$^{a}45.8\pm1.9^{x}$

Table 5: Effect of three part of Pumpkin *C. moschata* extracts in inhibiting the free radical (DPPH) using five different solvent.20mg/ml concentrations.

Sun light	% inhibition of free radical plant activity DPPH				
	Cold Distilled water	Boiling Distilled water	Ethanol50%	HCL 5%	HCL 1%
Peel	^{c}.00±.00^{z}	^{b}.00±.00^{z}	^{a} 16.7±1.4^{y}	^{b} 76.2±1.72^{w}	^{c} 43.7±1.5^{x}
Leaves	^{c}.00±.00^{z}	^{a} 3.47±3.4^{z}	^{a} 15.8±2.9^{y}	^{b} 76.8±1.4^{w}	^{b} 46.0±1.4^{x}
Pulp	^{a}10.3±3.9^{y}	^{a} 2.47±2.4^{z}	^{b} 10.1±1.4^{y}	^{a} 81.2±1.2^{w}	^{a} 51.6±1.5^{x}

Table 6: Effect of three part of Pumpkin *C. moschata* extracts in inhibiting the free radical (DPPH) using five different solvent.20mg/ml concentrations.

Oven.	% inhibition of free radical plant activity DPPH				
	Cold Distilled water	Distilled water	Ethanol50%	HCL 5%	HCL 1%
Peel	^{c}2.7±0.5^{z}	^{c}.00±0^{z}	^{b} 5.7±1. 1^{x}	^{a} 82.7±4.3^{w}	^{b} 55.2±.2.6^{x}
Leaves	^{a}9.4±1.8^{z}	^{a} 6.1±2.8^{z}	^{a} 31.5±0.2^{y}	^{a} 81.0±4.6^{w}	^{c} 47.6±2.5^{x}
Pulp	^{a} 11.5±1^{z}	^{b} 2.5±1.9^{z}	^{a} 30.7±0.3^{y}	^{a} 80.1±1.4^{w}	^{a} 64.6±3.3^{x}

Table 7: Correlation (R2 values) between the total phenolic content (TPC) and anti-radical activity of extracts prepared from three part of pumpkin(*C. moschata*).(Sun light samples).

Correlation coefficient (R2)			
Solvents	**Peel**	**Leaves**	**Pulp**
Cold water	**1.000**	**0.600**	**.....**
Boiling water	**1.000**	**0.773**	**1.000**
Ethanol 50%	**0.431**	**0.999**	**0.744**
HCL 5%	**0.615**	**0.436**	**0.967**
HCL 1%	**0.998**	**0.845**	**0.944**

Table 8: Correlation (R2 values) between the total phenolic content (TPC) and anti-radical activity of extracts prepared from three part of pumpkin(*C. moschata*).(oven samples at 40^{0}C).

Correlation coefficient (R2)			
Solvents	Peel	Leaves	Pulp
Cold Water	**....**	**.....**	**.....**
Boiling Water	**1.000**	**.....**	**0.912**
Ethanol50%	**0.997**	**0.888**	**0.763**
HCL 5%	**0.786**	**0.960**	**0.453**
HCL 1%	**0.984**	**0.832**	**0.412**

CONCLUSIONS

The results of the present study indicate that the total phenolic content and antioxidant activity depend on the type of solvent used in the extraction process and on the vegetable part as well as on the drying method used to dry the plant parts. The three parts of the pumpkin

plant can be considered as an important source of natural antioxidants which can be used in alternative medicine or food formulations.

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