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Quantification of phenols and antioxidant activity for Iraqi walnut husk

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ABSTRACT

Plant waste exporter of active compounds, it have a variety biological activities. The aim of research was to getting a new source, walnut husk was the choice were extracted then chemical methods were used to determine the content of total phenols, flavonoids and proanthocyanidin which amounted to 80.35%, 35.7%, 25.5%, respectively, antioxidant activity of walnut husk extract(WHE) was evaluated by chelating ability and reducing power, it was found the extract reducing power was 116% better than ascorbic acid 88.53% at the same concentration(10mg/ml),reducing power was determined compared with BHT as reference,the latest gave reducing power 30.65% to 135.56% at concentration 10 mg/ml and 50 mg/ml while extract was more than it 85.6% to 144.4% at the same conc. The chemical composition of extract was 1.04%, 3.045%, 73%, 22.34%, for protein, fat, fiber and carbohydrate. These result gave evidence of the high content of phenols, flavoniods and proanthcyanidin relevant effective antioxidant pointing to husk of walnut as promising source to obtain active compounds can be used in various applications.

KEY WORDS
Walnut husk, phenols, bioactive compounds,Chelating ability, Reducing power.

INTRODUCTION

Etymologically, the word Walnut derives from the Germanic walnut and old English wealth rutu, literally "foreign nut" wealth meaning foreign [1]. The two most common major species of Walnuts are grown for their seeds. The persain a English Walnut and the Black Walnut. The English Walnut (Juglone regia) originated in Persian and the Black Walnut (J. regia) is native to eastern North American [2] A Walnut is an edible seed of any tree of the genus, Juglone especially the Persian as English Walnut, J. regia [3]. Walnuts have been listed one the 38 substances to prepare Bach flower remedies [4], akind of alternative medicine promoted for its effect on health. Walnut seed shell inside its green husk [3]. The Walnut fruit is enclosed in a green, leathery, fleshy husk. The husk is in edible, After harvested, the removal of husk reveals the wrinkly Walnut shell, which is in two halves. The shell is hard and encloses the kernel [5]

The husks of Walnut contain a Juice that will realily stain anything it comes into contact with. It has been used as a dye for edloth [3]. The general photochemical composition of nut and their potential mechanisms of action that suggest their contribution to health pormation and the prevention of chronic disease [6]. Nuts also contain potewtially beneficial phytochemical compounds, as besides well as mutrienls [7] The tannins present in Walnuts act as a mordant, aiding in the dyeing process [8,9] and are usable as a dark ink or weed stain. Proanthocyanidin flavonoid oligomers, are comprised principally of (+) catechin and (-) epicatechin linkages [10]. (Pros) are also known under several others names, among the most common :OPC, pycon. Genols and leuko cyanidins, proanthocyanidins is a name for a class of bioflavanoids. In 193 this bioflavinoids are classified and referred to as vitamin P although didn't gain official vitamin category status [11]. PAS have attracted considerable attention due essentially to their potential beneficial health effects [12], and their physical, chemical and biological activities depend largely on their chemical structure and particularly on their

degree of polymerization [13,14]. Proanthocyanidins are found in high concentrations from such sources as cranberries, grape skins, grape seeds, pine bark, lemon tree bark and hazelnut tree leaves [11]. A walnut in an edible seed of any tree of the genus juglone, especially the Persian is English Walnut, J. regia. Broken nutmeat of the eastern blak Walnut from the tree, J. nigra are alse commercially available in small quantities, as are foods prepared with butler nut meats from J. cinerea [3]. The aim this study to known the chemical structure of Iraqi Walnut husk and its bioactive compounds, phenols, flavoniods and proanthocyanidins as natural antioxidants.

MATERIAL AND METHODS

The local Walnut (husk) has been collacted during autum season of 2012 Al-Hawaman - seliamania (the west-north of Iraq). The fruit were manually collected, then removal husk from it handling and drying naturally, at shade at room temperature. Sample was grounded into powder.

2.1. Extraction:

Applied method according to method described by [15] to 25 gm sample added 250 ml mixture of acetone and water (7:3) in a glass flask (scraw cab) at room temperature for 1 hr with stirring. the step of adding mixture of solvent repeat 3 times to complete extraction and then the mixture was filterated, then concentrated by rotary evaporator under reduced pressure at 38˚C until the volume become 150 ml, them,the obtained extract was put in separator funnel and adding 150 ml Hexane for there line with the same volume of solvent in order to remove oils, and them added 150 ml of Petrolum ether in stead of dichloromethane to extract (3 time also) and then dried in an oven at 40˚C and the extract was preserved in sterile dark bottles in a cool enviroment (4˚C)until further use.

2.2. Determination of total phenolic content:

The method was used as described by [16]. To a 0.5 ml (extract) 1mg/ml added 2.5 ml of a ten-fold diluted folin-Ciocalten's reagent and 2ml of 7.5% sodium carbonate solution were added before the reaction allowed standing for 30 min at room temperature. The absorbance was red recorded at 760 nm spectrophotometrically. The total phenolic compounds were determined according to gallic acid standard curve Fig (1).

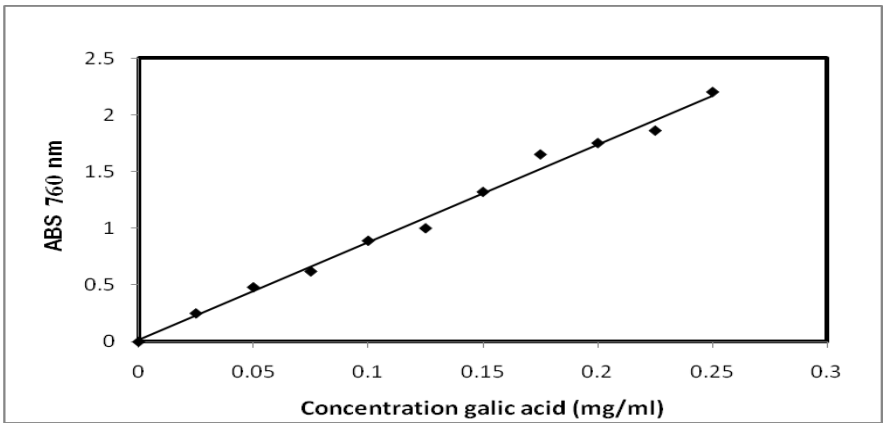

Fig. 1: standard curve of gallic acid

2.3. Determination of total flavonoid content:

The flavonoids were determined by using Catechin standard curve Fig (2) and the absorbance were recorded at 510 nm according to a previously described method [17]. 1ml extract solution (1mg/ml) was placed in 10ml volumetric flask. 5ml of distilled water and 0.3ml of 5% $NaNO_2$ solution were added. After 5 min 0.6ml of 10% $AlCl_3$ was added 2ml of 1M NaOH solution was added after another 5 min, and the volume was made up to 10ml with distilled water. The mixture was mixed thoroughly then absorbance was measured.

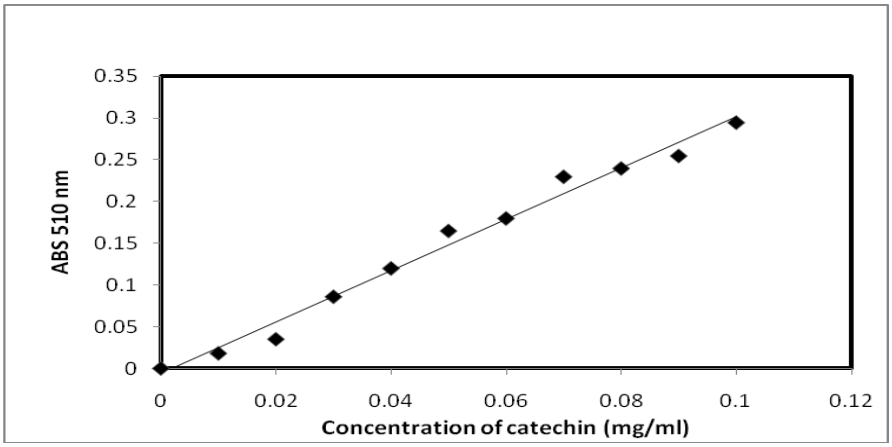

Fig. 2: standard curve of catechin

2.4. Determination of proanthocyanidin(OPCs):

The content was determined according to described method [18]. 1mg (dried extract) in 1ml methyl alcohol, and added to it 9ml vanillin reagent with mixing by vortex for 10 sec, then incubated at [19-21] C˚ for after 15 min. The absorbance was measured at 500 nm by return to standard curve of proanthocyanidin Fig (3) determination proanthocyanidin in extract.

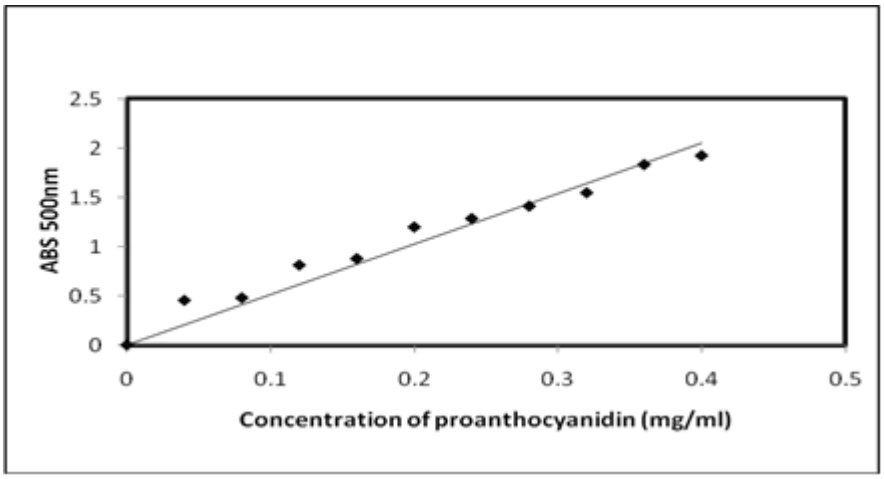

Fig. 3: standard curve of proanthocyanidin

2.5. Antioxidant activity:

The chelating ability: according to what mentioned in [19] method was described by [20] extract of walnut husk(10-50 mg/ml)was mixed with 0.2 ml ferric chloride of 2 Mm and 0.2 ml 8-hydroxyquinone (5mM),the absorbance determined after 10 min at room temperature at 562 nm. Ascorbic acid was used as reference for comparison. The reducing power: The method followed as described by [21]. mixed 1 ml of extract (2-10 mg/ml) with1% potassium ferric cyanide 2.5 ml and sodium phosphate buffer (0.2M Ph 6.6),then incubated at 50ºc for 20 min.Trichloroacetic acid (TCA) was added to mixture to stope the reaction and centrifuge for 10 min at 3000 rpm 0.5 ml of the supernatant was mixed was mixed with 1 ml of 1% ferric chloride and stand for 10 min. The absorbance was measured at 700 nm, BHT 0.02% used as reference.

2.6. Determination of main components:

According to [22] estimation of Moisture, Ash, Fat and Fiber also determination Protein by Micro-Kjeldhal as method described by [23] and the carbohydrate determiantion accroding to [24].

RESULTS AND DISCUSSION

Assessment findings in table (1) phenols, flavonoids and proanthocyanidin in Iraqi walnut husk as: 80.35%, 35.7% and 25.5% mg/ml respectively.As noted,these three phytochemicals are rich in husk of walnut,this is indication of possibility of benefiting from walnut husk as a source of phenol or proanthocyanidin to used in

some application or as supplement diet.proanthocyanidin have been sold as nutritional,therapeutic supplements in Europe for almost aquarter of a century, their introduction to the united states has been relatively recent,phenols are known for possessing the activities of the different biological activities also flavonoids and proanthocyanidin, potential benefits of OPCs like reduced the level of lipid peroxidation[11]. Some nuts can contribute to the overall flavonoids in diets when compared with fruits and vegetables such as almond in which an increase as in apricot 13mg/100 gm and apple 15mg/100 gm[25]. while we note walnut husk have 35.7% flavonoid from this experiment,it's more than apple or apricot.

Table 1: Total phenols,flavonoids and proanthocyanidin in walnut husk

Compounds	mg / 100 gm
Phenols	80.35
Flavonoids	35.7
Proanthocyanidin	25.5

Proanthocanidins are found in almonds,cashews, hazelunts, pecans, pistachios, peanuts and walnuts with concentrations varying from 9-494 mg/100g.Phtochemicals of nut have been associated with numerous bioactivities known to affect the initiation and progression of several pathogenic processes [6]. Walnut are particularly rich in total phenols with 1625 mg/100g [6]. Studies pointed to total antioxidant capacity largely dependent upon the phenolic compounds in nuts[26].

Appear from (fig.4) chelating ability of ascorbic acid 88.53% at lowest concentration test,while the extract(WHE) ability it more than ascorbic acid as it was 116% at the same conc. Also clear extract distinguished in chelating ability on ascorbic acid for all concentration testing,as well as increased activity for extract and ascorbic acid ascending with increased of concentrations.Research in vitro confirmed that proanthocyanidin as antioxidant are 50times more effective than vitamin E and 20 times more powerful than vitamin C [11]. Observation from results of present study high percentage of total phenols, flavonoids and proanthocyanidin in walnut husk extract, proanthocyanidin considered a composite phenols also called tannin,single phenplic compounds have antioxidant activity less than poly phenols[27]. Proanthocyanidin has effectiveness of antioxidant with variety mechanisms, radical scavenging, link metals, enzymes inhibition, lipid oxidation and pull of single oxygen[28]. So we can explain the high chelating ability of extract more than ascorbic acid, this agree marking of searchers in the same area on natural antioxidants extracted from plant content of phenols, and the relation of concentrations of these compounds with increasing efficiency as antioxidants[29,30].

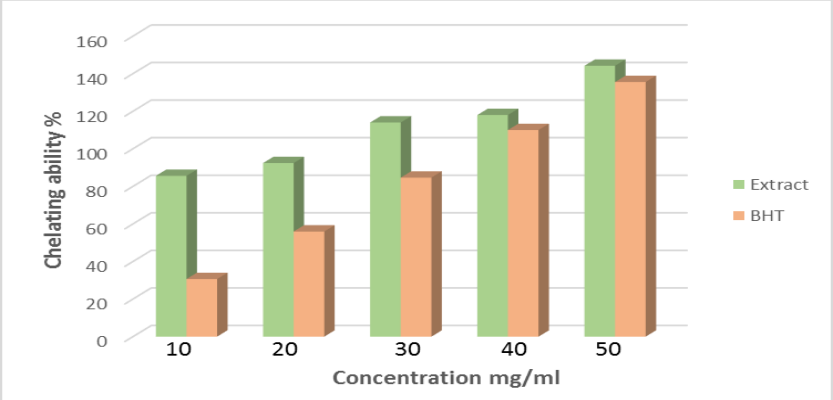

Fig. 4: Chelating ability of walnut husk extract

In assessing the effectiveness of antioxidant for extract(WHE)by reducing power method,gave activity began from 85.6% at lowest concentration(10mg/ml) arriving to 144.4% at the highest concentration while the reducing power for synthetic antioxidant BHT as a reference 30.65% at (10mg/ml) to 135.56% at(50mg/ml).Notes from fig(5) excellence activity of extract(WHE) on BHT in reducing power mechanism,and increasing activity with concentration for WHE and proanthcyanidin, later compound,which is active phenolic compound known as antioxidant may be the activity of extract due to it, studies showed that OPCs antioxidant capabilities are 50 times more potent than vitamin E and 20 times become stronger than vitamin C [31]. where its effectiveness return to the existence of B ring within composition,which it has a large property in strengthening the effectiveness of antioxidant [32]. Reducing power one indicators of antioxidant activity as well as phenolic content,this agree with other searchers about role of phenolics compounds in plant:*Capparis*

spinosa root bark and effects as natural antioxidants [33]. Total antioxidant capacity dependent upon phenolic compounds in nuts largely[26].

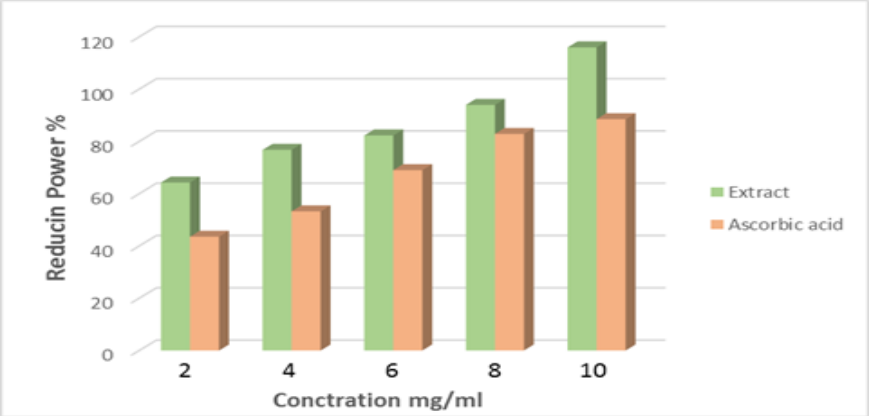

Fig. 5: Reducing power of walnut husk extract

The table (2) showed some of the major nutrient in Iraqi walnut husk including, protein 1.04%,fat 3.046%,fiber 73%,carbohydrate 22.34%,its contain the highest level of fiber Compared to other ingredients, also walnut contain the highest total level of antioxidants, including both free antioxidants and antioxidant bound to fiber [34]. In addition to other benefits of fiber which helps lower cholesterol, walnut husk is known as a natural agent, so it is applied in the food and also saved.

Table 2: Chemical composition (per 100 gm) Iraqi walnut husk

Chemical composition	%
Fat	3.046
Protein	1.04
Ash	73.0
Carbohydrate	22.34

Conclusion:

The antioxidant activity of proanthocyanidin, investigated through reducing power and chelating activity, showed that the proanthocyanidin extracted from walnut husk exhibited a higher antioxidant effect compared to ascorbic acid and BHT.The study pointed to a renewed and cheap source could be promising to get from it polyphenols compounds have a biological activities like natural antioxidants.

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