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CEREAL GRAIN AND BRAN EXTRACTS AS VALUABLE SOURCES FOR PHENOLIC COMPOUNDS AND ANTIOXIDANTS

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ABSTRACT

Cereal grains are reported to be a good source of phytochemicals such as polyphenols (phenolic acids and their derivatives). Three wheat varieties (giza 168, seds 1 and beni suif 1) and barley (giza 2000) were tested for their health value for their flour and waste bran. The flour of each variety of wheat, barley grain and bran were evaluated for their phenolic compounds, antioxidative activity and other tests not mention in this paper.

Acetone, ethanol, hexane and methanol extracts had been applied for wheat, barley grain and bran flours. Wheat giza grain hexane extract was found to posses higher phenolic content (more than 140 mg gallic acid equivalent/ gram dry grain) than giza bran. Antioxidative measurements were detected using reducing power activity and DPPH scavenging methods. Acetone extract of wheat giza bran has the highest reducing power activity (more than 20 μmol L-ascorbic acid equivalent/gram dry matter) and that was correlated with the total phenolic compounds.

On the other hand, wheat giza bran has higher DPPH reduction values than that for flour. Wheat seds defatted bran samples exceeded than 90% reduction ratio followed by wheat giza ethanol, acetone bran extracts. Gallic acid and L-ascorbic acid were used as standard antioxidants for measurements. Research gives importance for waste grain parts as valuable antioxidant source for future use in food industry.

INTRODUCTION

Cereal, wheat (*Triticum aestivum* L.) in particular is an important crop for economy. In 2007-2008, more than 60 million acres of wheat were planted yielding a projected 2 billion bushels of wheat in the US (**Vocke and Allen, 2007**). Several food products are made from different varieties of wheat. On the other hand, barley (*Hardeum vulgare*) is a widely consumed cereal, because it was one of the first agricultural domesticates together with wheat, pea, lentils dating from about 10,000 years ago (**Smith, 1998**). However, barley has not been perceived as such an important grain in human diet.

Okarter *et al.* (2010) reported that free phenolic content ranged from 255 to 499 μmol gallic acid equivalents/100 g DW. While, the bound fraction contributed 53.8-69.7% of the total phenolic content of the wheat varieties analyzed. Whole grain phytochemicals have antioxidant activity, the ability to scavenge free radicals that may oxidize biologically relevant molecules (**Liu, 2007**). Antioxidants are believed to contribute to the beneficial effects of grains, fruits, and vegetables through several possible mechanisms, such as directly reacting with - and quenching - free radicals, chelating transition metals, reducing peroxides, and simulating the antioxidative defense enzyme activities (**Yu *et al.*, 2002a & Yu, 2001**). Significant levels of antioxidant activities were detected in wheat and wheat-based food products (**Yu *et al.*, 2002a & 2002b**), suggesting that wheat may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion.

The demand for natural antioxidants for use in foods has increased recently because of questions about the long-term safety of synthetic antioxidants such as butylated hydroxytoluene (BHT). In addition to their long-term safety and capacity to improve food quality and stability, these natural antioxidants can also act as nutraceuticals to terminate free radical chain reactions in biological systems and thus may provide additional health benefits to consumers. Aim of this work is to investigate the value of grain waste as source for phenolic and antioxidant compounds for prospective use in food industry.

MATERIALS AND METHODS

Three varieties from wheat (**W)** (*Triticum aestivum* L.); giza 168, seds 1 and beni suif 1, and Barley (**Br)** (*Hordeum vulgare* L.);

giza 2000 were grown on 2008 in *Agricultural Research Center*, Com-ompo, Aswan Governorate, Egypt. Cereal varieties were tested to reveal their biochemical constituents for phenolic compounds, and antioxidant activity.

Wheat and barley grains varieties have been brought from *Agricultural Research Center*, Aswan in the winter of 2008. Three wheat varieties; *giza 168* (**Wg**), *seds 1*(**Ws**), *bani suif 1* (**Wb**) and barley variety *giza 2000* (**Brg**) have been chosen for the study. Grains were washed well with cold tap water and distilled water to remove debris, before dryness in the room condition. After dryness of wheat and barley grains, flour grains (**F**) were well separated from their bran (**B**). Flour grain or bran was well blended and their flours were collected.

Non-polar and polar solvents such as hexane (**h**), acetone (**a**), ethanol (**e**) and methanol (**m**) were used after re-distillation. The grain flour (F) or the flour from the bran (B) (5 g) were mixed well with 50 ml solvent using magnetic stirrer at 25°C for 4 hours. Solutions were filtered, and the supernatants or the filtrates were concentrated on the room temperature until the final volume reached 5 ml. Different analysis were performed in triplicates, and were expressed on a dry matter basis. The concentrated thirty two extracted samples were stored at 2-8°C for different analysis.

Abbreviations were used in our investigation; four or five illustrated letters were used through the work for (1) wheat W or barley Br (2) species g, s and b (3) solvent h, a, e, and m or defatted sample d (4) either grain flour F or bran B.

Total phenolic compounds: A Folin-Ciocalteu method (**Slinkard and Singleton, 1977**) is commonly used only for assessment of the sum of phenolic compounds in plant extracts, using gallic acid as a standard phenolic compound. 100 µl of extract solution was added to 1 ml of Folin-Ciocalteu reagent in water (1:1) and the content mixed thoroughly. The concentration of total phenolic compounds in the extracts determined as mg of gallic acid equivalent by using the standard curve of gallic acid.

Reducing power measurement: The reducing power of wheat, barley flour and bran extracts were determined according to the method of **Oyaizu (1986).** Certain amount of the extracts (0.5 ml) in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 mol/L, pH 6.6) and potassium ferricyanide $K_3[Fe(CN)_6]$ (2.5 ml, 1%).

Data calculated as μmol L-ascorbic acid (structure 3) equivalent/gram dry matter (AAE/g d) as determined from the standard curve.

DPPH free radical method: The free radical scavenging activity of wheat, barley flour and bran extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH·) using the method of **Shimada *et al.* (1992).** Solution of DPPH· (1.8 ml, 0.1 mmol/L) in ethanol was prepared, and added to 200 μl of extracts in final volume of 2 ml. Vitamin C and gallic acid were used as common antioxidants. Control as test without using any antioxidant extracts was used for determination. Radical scavenging activity as a decrease in the absorbance of DPPH was expressed as percent radical quenching compared to that without the extracts (**Kamath *et al.*, 2004**). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Data were compared to that for gallic acid or L-ascorbic acid. The kinetics of DPPH radical inhibition in the absorbance of DPPH was calculated using the equation (**Korycinska *et al.*, 2009**):

$$\% \text{ DPPH scavenging activity} = 1 - A_t / A_0 \times 100$$

Where, A_t represents the absorbance after 60 min of measurement, and A_0 the absorbance at the beginning of measurement.

RESULTS AND DISCUSSION

Phenolic content measurement

Cereal grains are reported to be a good source of phytochemicals such as polyphenols (phenolic acids and their derivatives), flavonoids and tannins. **Vitaglione *et al.* (2008)** found total phenolic compounds in whole wheat and bran wheat were about 350-1505 and 2800-5643 mg gallic acid equivalents/kg, respectively.

As expected result, wheat giza flour extracts were found to posses higher phenolic content than wheat giza bran extracts (Fig. 1a). Moreover, it is obvious to say that phenolic content of WghF sample was the greatest amount (exceded than 140 mg gallic acid equivalent/ g dry wheat grain) among wheat giza samples. Phenolic compounds have attracted the attention of food and medical scientists because of their strong *in vitro* and *in vivo* antioxidant activities and their ability to scavenge free radicals, break radical chain reactions and scavenging metals.

As well, extracts of wheat seds flour samples (Fig. 1b) exceeded that for bran except for methanol extract where bran exceeded than flour extract. Wheat benisuif flour ethanol and acetone extracts exceeded that of bran extracts, and opposite results for methanol and hexane extracts. The high phenolic content of wheat beni suif (Fig. 2a), which used in spaghetti was in agreement with **Hirawan *et al.* (2010)**. They measured the commercial whole-wheat spaghetti, and found more vitamins, minerals, natural antioxidants and dietary fiber than regular refined grain. Whole wheat spaghetti exhibited total phenolic content 1389 µg/g and DPPH scavenging activity 1-2.3 µmol Trolox equivalents.

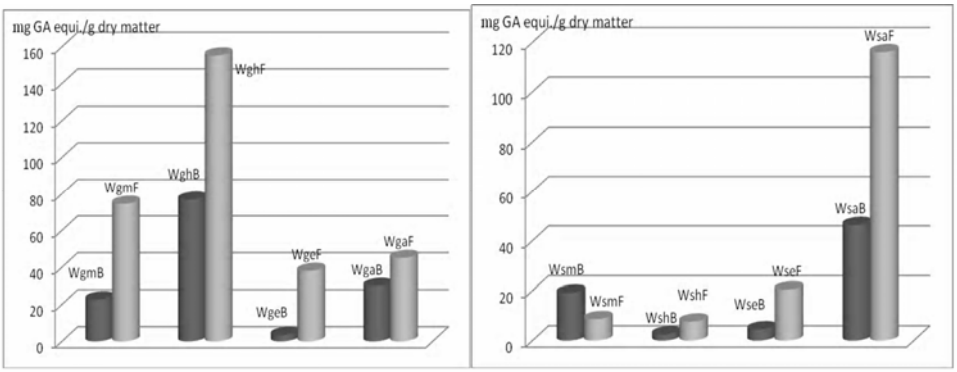

Figure 1: Gallic acid (mg) equivalent/ gram dry matter from wheat giza (a) & seds (b) extracted bran and flour samples by methanol WgmB; WgmF, hexane WghB; WghF, ethanol WgeB; WgeF and acetone WgaB; WgaF (values are the mean of three replicates).

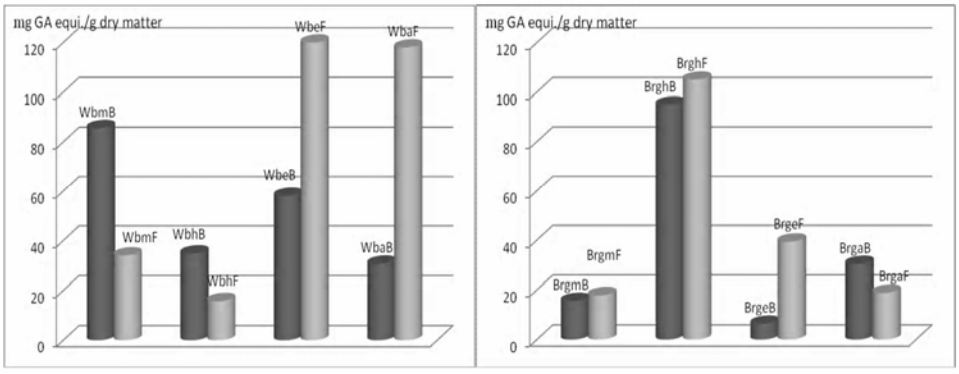

Figure 2: Gallic acid (mg) equivalent/ gram dry matter from wheat benj suif (a) & barley giza (b) extracted bran and flour samples by methanol WbmB; WbmF, hexane WbhB; WbhF, ethanol WbeB; WbeF and acetone WbaB; WbaF (values are the mean of three replicates).

Phenolic acids play an important role in competing oxidative stress in the human body by maintaining a balance between oxidants and antioxidants (**Temple, 2000**). Phenolic acids existed in cereal grains in free, soluble conjugated and insoluble bound forms. Phenolic acids in wheat grains are mostly in the bound form and exist in bran associated with cell wall materials. Except of acetone extract, barley giza flour extracts showed higher phenolic content than that for bran extracts. The abundant content of phenolic compounds in barley reveals that it may serve as an excellent dietary source of natural antioxidants with antiradical and antiproliferative potentials for disease prevention and health promotion (**Zhao *et al.*, 2008**).

Hexane was the most preferable solvent in gaining phenolic compounds for wheat giza, and barley giza varieties for both of grain and bran flour (Fig. 1a, 2b). On the other hand, acetone solvent was suitable for wheat seds garin and bran flours (Fig. 1b). While, the polar solvents were suitable for wheat beni suif flours (Fig. 2a). Generally speaking, wheat and barley flour extracts (Fig. 1, 2) have higher content of phenolics than that for bran extracts. In spite of the high content for flour extracts, bran extracts –as will show later- are having higher antioxidant activity in different determinations. **Zhou and Yu (2004)** stated that total phenolic compounds measured as gallic acid was higher in 50% acetone followed by 70% ethanol extracts, while ethanol followed by 50% acetone extracts showed the highest % DPPH remaining.

Antioxidative determination

Reducing power activity

The reductive capabilities of wheat and bran samples were compared to that of vitamin C and gallic acid. Reducing power method was dependent on the measurements of the reductive ability up to Fe^{3+} – Fe^{2+} transformation in the presence of cereal samples (**Oktay *et al.*, 2003**). The reducing capacity of a standard compound or cereal sample may serve as a significant indicator of its potential antioxidant activity (**Meir *et al.*, 1995**). Gallic acid had slightly higher reducing power than that for vitamin C. Generally, wheat Giza bran samples had higher reducing power activity than that of wheat flour. Sample WgaB showed the higest reducing power activity among the four bran solvent extracts has a resonable amount of total phenolic compounds (Fig. 3a,b).

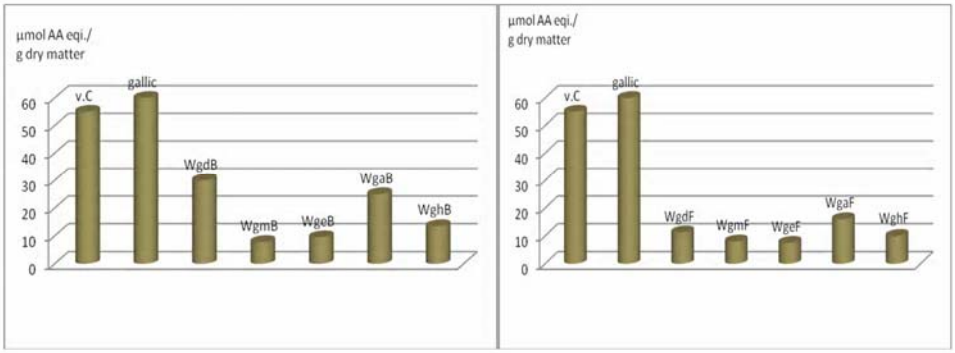

Figure 3: µmol L-ascorbic acid equivalent/ gram dry matter from whole wheat giza (a)& bran (b) defatted WgdB and extracted bran samples by methanol WgmB, hexane WghB, ethanol WgeB and acetone WgaB (values are the mean of three replicates).

In understanding structural view, gallic acid followed by vitamin C has the highest activity in general view comparing to the other wheat and barley samples. As the antioxidant activity explaination, the reducing power of cereal samples was increased with increasing the amount and valuable potent compounds.

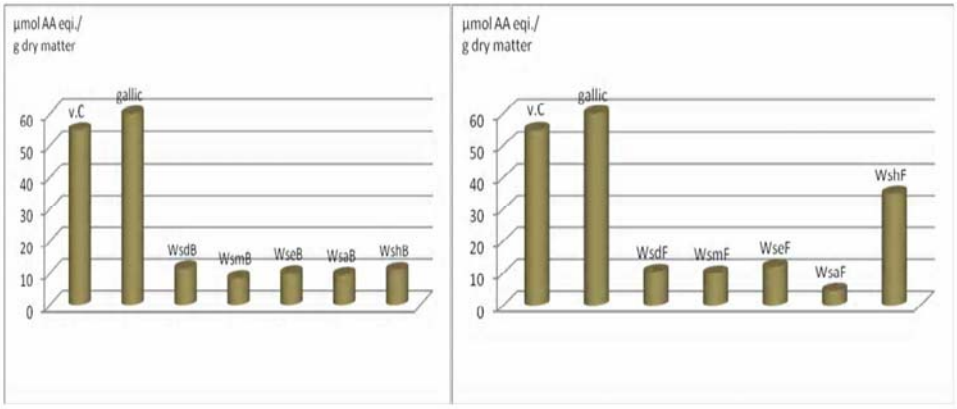

Figure 4: µmol L-ascorbic acid equivalent/ gram dry matter from whole wheat seds (a)& bran (b) defatted WsdB and extracted bran samples by methanol WsmB, hexane WshB, ethanol WseB and acetone WsaB (values are the mean of three replicates).

In wheat samples, there are three samples proved to have reducing power more than 50% comparing to that of gallic acid. These values are WbhB, followed by WshF and then WgdB (Fig. 3, 4, 5). It

has been noticed that ethanol extract had low reducing power activity. That agreed to **Oktay *et al.* (2003)** who mentioned that ethanol extract of fennel seed is the lowest potential in the reducing power determination.

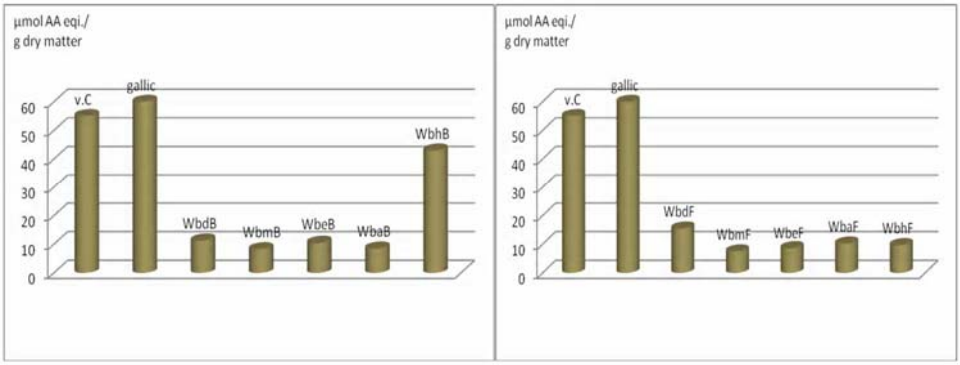

Figure 5: µmol L-ascorbic acid equivalent/ gram dry matter from whole wheat Beni suif (a)& its bran (b) defatted WbdB and extracted bran samples by methanol WbmB, hexane WbhB, ethanol WbeB and acetone WbaB (values are the mean of three replicates).

Bran defatted wheat (giza and seds) showed higher reducing power than their flour (Fig. 3, 4). As shown, hexane wheat bran extraction found to have the highest activity comparing to the other solvents. Extraction with hexane for wheat seds flour and Beni suif bran showed obvious activity (Fig. 4, 5). One of the antioxidative agents is phytic acid which was in high amount in barley giza bran, and showed high L-ascorbic acid equivalent reached about 30 micro mol per gram dry matter (Fig. 6).

Comparing to gallic acid reducing power, bran defatted barley (giza) showed higher reducing power than their flour. Methanolic extraction for barley giza bran and flour were slightly higher activity comparing to the other samples (Fig. 6). In spite of, extraction wheat flour or bran with hexane proved to have obvious reducing power activity, extraction with methanol and ethanol posses higher activity for extraction barley flour and bran. Acetone extract availability for having a potent antioxidant agreed with that of (**Liu and Yao, 2007**). As well, **Kamath *et al.* (2004)** suggested that methanol and acetone / methanol posses strong antioxidant activity.

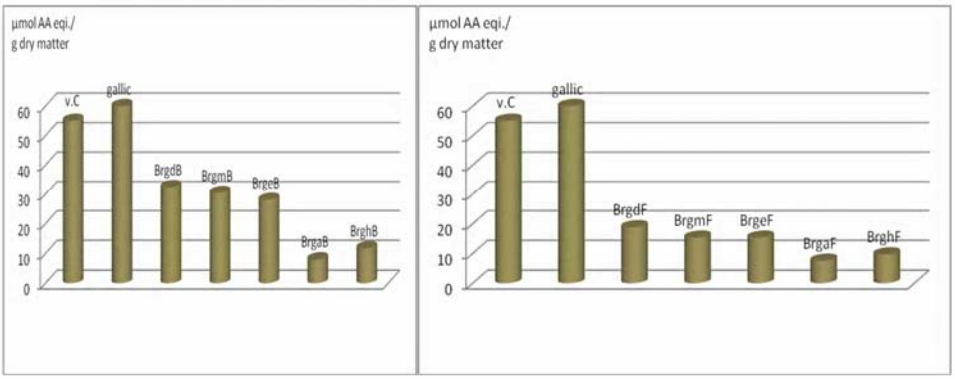

Figure 6: µmol L-ascorbic acid equivalent/ gram dry matter from whole Barley giza (a)& bran (b) defatted BrgdB and extracted bran samples by methanol BrgmB, hexane BrghB, ethanol BrgeB and acetone BrgaB (values are the mean of three replicates).

DPPH scavenging activity

Individual compounds are responsible for the reducing power, or radical capture mechanisms. DPPH radical reduction rate was used to determine the antiradical activity of the extracts from wheat, barley grain powder and bran powder products. The reduction of DPPH free radical was tested for the wheat, barley extracts and the de-fatted samples. The determination was done during a 0, 30 and 60 min experiment.

As shown, wheat Giza bran has higher DPPH reduction values than that for flour. Except few samples, that was a general role in determining the antioxidant activity as DPPH radical. In wheat samples, there are three samples proved to have DPPH reduction more than 90%. These wheat values are WsdB followed by that for WgeB and WgaB.

Extracts (WgeB and WgaB) have the ability to quench the stable DPPH radical closed to that of gallic acid and vitamin C. Not usually phenolic content was the reason for the antioxidant mechanism. In unexpected data, sample WgeB showed low amount of phenolics. On the other hand, phenolic content of WghF followed by WgmF were the highest value among bran and flour extracts for wheat giza (Fig. 7a,b). Reasonable agreement for hexane extract of wheat giza flour between its phenolic content and high radical scavenging. Also, acetone extract activity in both of reducing power and radical scavenging.

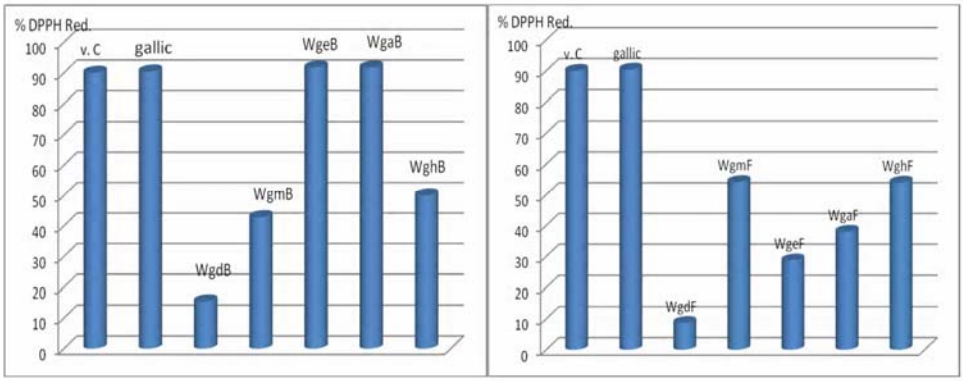

Figure 7: % Reduction of DPPH radical of whole wheat giza (a)& bran (b) defatted WgdB and extracted bran samples by methanol WgmB, hexane WghB, ethanol WgeB and acetone WgaB (values are the mean of three replicates).

In an unexpected result, wheat (seds) defatted bran showed potent antioxidant activity exceeded that for gallic and vitamin C. A diet rich in wheat or barley bran may be useful –as shown in these results- in competing diseases in which free radical production plays the key role. The scavenging activity of WsaB might be correlated with the phenolic content (Fig. 8a,b). While, WsaF which has the highest amount of phenolic content did not prove to have good scavenging DPPH radical. We can imagine, that these data might due to the phenolic special kind availability content.

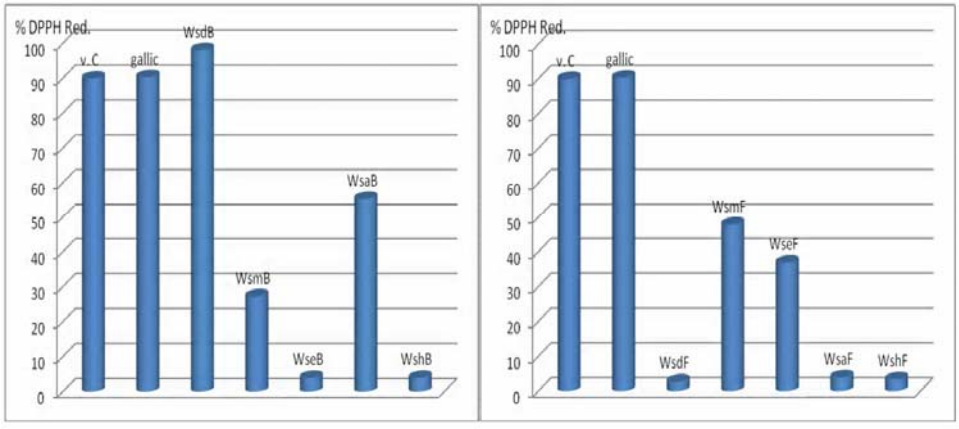

Figure 8: % Reduction of DPPH radical of whole wheat seds (a)& bran (b) defatted WsdB and extracted bran samples by methanol WsmB, hexane WshB, ethanol WseB and acetone WsaB (values are the mean of three replicates).

Solvent extracts activity of flour and bran is differ in their antioxidant activity. In a brief conclusion, data showed that acetone extract was great antioxidant active in reducing power and DPPH scavenging radical (Fig. 7) for wheat giza extracts. In wheat seds, acetone in DPPH radical (Fig. 8) and hexane in reducing power were the most potent extract. While for Wheat beni suif samples WbeF and WbaF have reasonable phenolic content as shown in Figure 9. In the same time, WbmB proved to have the highest phenolics among bran extracts. All the extracts of wheat beni suif showed reasonable activity specially for DPPH radical.

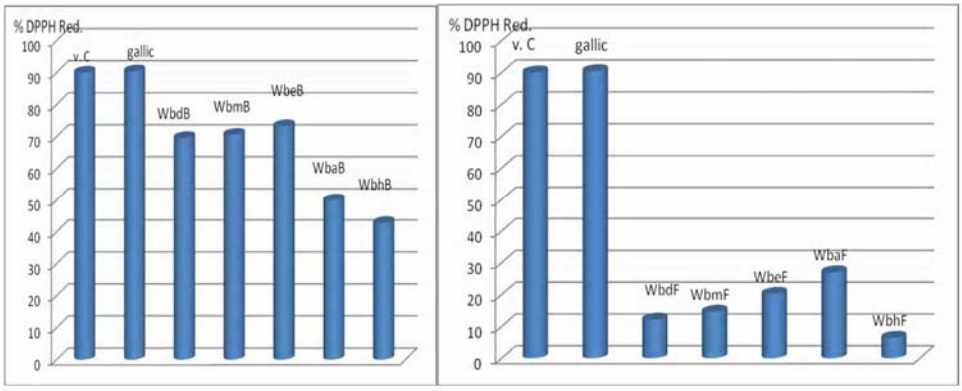

Figure 9: % Reduction of DPPH radical of whole wheat Beni suif (a)& bran (b) defatted WbdB and extracted bran samples by methanol WbmB, hexane WbhB, ethanol WbeB and acetone WbaB (values are the mean of three replicates).

For barley giza, all the extracts showed reasonable activity especially for DPPH radical. Data of phenolic content (Fig. 2b), showed that barley flour samples were higher than that for barley bran except for acetone extract. That does not correlate with scavenging free radical results. On the other hand hexane extract showed good phenolic content in barley flour and bran, and methanol or ethanol extracts have high antioxidant activity in both tested experiments in barley bran (Fig. 10). Polar solvents for in barley grain flour and bran (Fig. 6, 10) showed high reducing power activity.

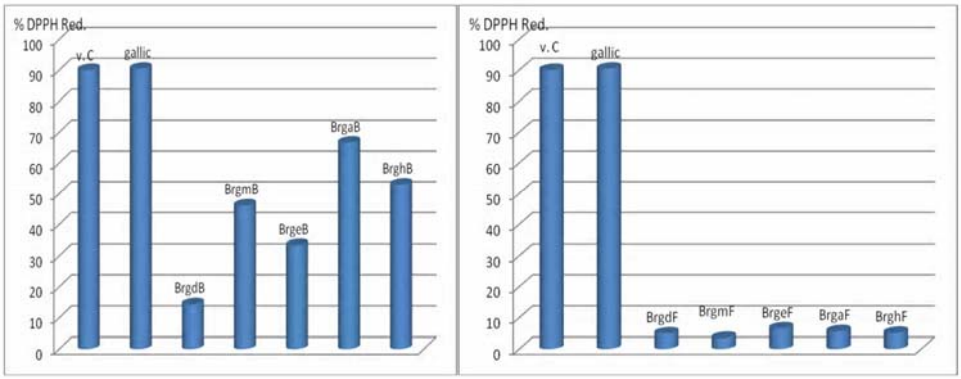

Figure 10: % Reduction of DPPH radical of whole Barley giza (a)& bran (b) defatted BrgdB and extracted bran samples by methanol BrgmB, hexane BrghB, ethanol BrgeB and acetone BrgaB (values are the mean of three replicates).

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مستخلصات الحبوب وأغلفتها كمصادر قيمة للمركبات الفينولية ومضادات الاكسدة

عماد صبرى شاكر ، فوزى سليمان هاتور ، نسرين سيد أحمد
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تعتبر حبوب القمح والشعير مصدر جيد للمركبات الحيوية مثل الفينولات العديدة (الاحماض الفينولية ومشتقاتها). ثلاثة من أصناف القمح (جيزة ١٦٨، سدس ١ ، بنى سويف ١) والشعير (جيزة ٢٠٠٠) تم اختبارهم وتقدير قيمتهم الصحية لدقيق هذه الحبوب ومطحون اغلفتها. تم تقييم مطحون ودقيق حبوب واغلفه أصناف القمح والشعير للمركبات الفينولية ونشاط مضادات الاكسدة وغيرها من الاختبارات غير المذكورة.

تم عمل مستخلصات الأسيتون والهكسان والأيثانول والميثانول لحبوب واغلفة القمح والشعير. وقد وجد أن مستخلص الهكسان لحبوب قمح الجيزة به أكثر كمية من المحتوى الفينولى (اكثر من ١٤٠ مجم مكافئ حمض الجاليك / جرام حبوب جافة) من بين مستخلصات العينات واغلفة قمح الجيزة. قياسات مضادات الأكسدة تم اجرائها عن طريق تقدير القوة الاختزالية وطريقة مسك شق داى فينيل بكريل هيدرازيل. وجد أن مستخلص الاسيتون لاغلفة حبوب قمح الجيزة تحتوى القيمة الاكثر نشاطا للقوة الاختزاليه (اكثر من ٢٠ ميكرومول مكافئ حمض اسكوربيك / جرام مادة جافة) وهذا يتوافق مع المحتوى الفينولى.

من الناحية الاخرى وجد أن اغلفة قمح الجيزة كان الاكثر فى تقليل أصطياد الشق الحر لبكريل هيدرازيل بقيمة اكبر من تلك لدقيق الحبوب المقابلة. وجدت عينات منزوعه الدهن (ذات القيمة المنخفضة) لاغلفة قمح سدس زادت فيها النسبة ٩٠% نسبة نقص للشق الحر وتبعه فى ذلك مستخلصات الايثانول والاسيتون لأغلفة قمح الجيزة. فى هذه التجارب تمت مقارنة النشاط المضاد للاكسدة بنشاط حمضى الجاليك والاسكوربيك كمضادات اكسدة قياسية.