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Chemical Composition, Antioxidant and Antimicrobial Activities of Oat, Barley, Sweet Lupin and Lima Bean

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ABSTRACT

Oat flakes, lima beans, sweet lupins and grounded Barley "Talbina" are used as functional foods. The present study aimed to determination of the chemical composition, total phenolic content, total flavonoids, antioxidant scavenging DPPH, the reducing power and the antimicrobial effects on some pathogenic bacterial strains for the extracts of talbina, lima bean, sweet lupin and oat. Sweet lupin recorded the lowest value of moisture content. Sweet lupin obtained the highest total protein content whilst talbina recorded the lowest total protein content. Talbina showed the lowest fat content. However, sweet lupin indicated the highest fat content. Fiber content in sweet lupin was 10.78% while lima bean recorded 1.15%. Ash content of lima bean was 4.12% while oat recorded 1.15%. Sweet Lupin presented the lowest value of K and the highest of Zn. Talbina had the highest value of Fe content and the lowest of Zn. The highest level of total phenolic content was found in barley extract, while the lowest value in sweet lupin extract. Sweet lupin extract recorded the high reducing power. while lima bean extract recorded the lower reducing power compared with ascorbic acid. Barley extract inhibited only *Bacillus cereus and Staphylococcus aureus* bacteria. oat extract had no inhibitory effect at low concentrations (25 and 50 mg/ml) *with Bacillus cereus* and *Escherichia coli*. Sweet lupin extracts potently inhibited the growth of *Bacillus cereus* bacterium at all concentrations. While, it only inhibited *Staphylococcus aureus* at higher concentrations (75 and 100mg/mL). Whereas, it had no antibacterial activity on *Escherichia coli*.

Keywords: Antioxidant; Antimicrobial; oat; barley; sweet lupin; lima bean.

INTRODUCTION

Oat, *Avena sativa* L. is considered a good source of beta glucan, dietary fiber and it is rich in phenolic compounds which reduces the risk of developing heart diseases through decrease of cholesterol, reducing diabetes symptoms and prevent chronic diseases such as stroke, cancer and obesity (Tiwari & Cummins, 2011). Oat phenolics had great potential as nutraceuticals while some others are powerful antioxidants. The oat powder was used as antioxidant in order to extend the shelf life of some cereal products for many years Webster (2002). Many studies clarify the antibacterial effect of oat as a result of phenolic compounds (Uchihashi *et al*., 2011).

Barley, *Hordeum vulgare* L., is considered an annual cereal grain. It is one of the ancient crops in the Arabian Peninsula. Barley was considered as a functional food. Barley contains antioxidants and vitamins to fight free radicals which cause the destruction of the cell membrane and subsequently the incidence of certain types of cancer. Barley extract had an antimicrobial effect on pathogenic bacteria such as *staphylococcus aureus* and *Bacillus subtilis* (Sheela and suganya, 2012; Madineni, 2012 and Suriano *et al*., 2018).

The legume sweet lupin, *Lupinus albus* L had minimal amounts of bitter alkaloids such as saponins, lectins, phytates, at levels of <200 mg/kg, which found in many traditional crops. Legumes have an economical dietary source of protein and are also higher in protein (especially lupins) than most other plant foods. Thus, they are high in nutrients and phytochemicals, which made them an important and inexpensive food staple in many developing countries. The antioxidant and anti-microbial activities of sweet lupin as reported by (Ranilla *et al*., 2009 and Lampart-Szczapa *et al*., 2003; Dikmen *et al*., 2011 and Kouris-Blazos & Belski, 2016).

Lima bean, *Phaseolus lunatus* belongs to the family leguminosae and is predominately cultivated in South America. Lima bean like all other legumes are food resources which offer various optimum nutritional and/or health benefits. It is known that dehusking, soaking, germination, cooking and roasting inactivate the antinutrients including (trypsin inhibitors, phytic acid, saponins, haematoglutinns and tannins) that interfere with absorption and utilization of important minerals such as calcium, iron, zinc and magnesium). Other studies indicated that there were an antioxidant and anti-microbial activities of lima bean which reducing heart and kidney diseases, lowering the sugar indicators of diabetic patients, increasing in satiety, and reducing the occurrence of cancer (Boateng *et al*., 2008; Ezeagu and Ibegbu, 2010; Qayyum *et al*., 2012 and Ortega *et al*., 2013).

This work was planned to determine the chemical composition, antioxidant scavenging DPPH, the reducing power and the antimicrobial effects on some pathogenic bacterial strains of grounded barley, lima bean, sweet lupin and oat.

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MATERIALS AND METHODS

Materials

Oat flakes, lima beans and sweet lupins were bought from "Harraz" company, Cairo, Egypt. Grounded Barley "Talbina" was purchased from "El-sherbiny" market in Damietta Governorate, Egypt. All chemicals and reagents used were of analytical grade.

Methods

Those materials were prepared as follows: oat flakes (*Avena sativa* L.) were ground into powdered form in (*High Speed Multi- funcition Grinder /25000 rpm/min/ China*) Machine. Talbina powder (*Hordeum vulgare* L.) was used as-is without any preparations. Sweet Lupin (*Lupinus albus* L.) was prepared as described by (Levent and BilgiÇli, 2011). The first step is "The Debittering Process" at 60-70ºC for 90 min in tap water and subsequent soaking for four days with refreshed the water four or five times during the soaking period in order to removing alkaloids (Yorgancilar *et al*., 2009). Then, seeds were dried in an oven at 50ºC for 10 hours, after that the dried lupin seeds were ground in the mill such as wholegrain powder. Lima Bean seeds (*Phaseolus lunatus* L.) were prepared according to the method described by (Hassan and Bello, 1988), the "perfect and healthy" seeds were soaked in water for six hours in order to make easy the disposing of tannins components (Miano and Augusto, 2018). Many soaked grains were self-hulled during soaking in water after that, drying in the oven at 55 to 60ºC for 24 hours. After drying, they were ground into powder.

Chemical Analysis:

The chemical composition of lima bean, sweet lupin, talbina and oat were determined as described by AOAC (2012). carbohydrate content was calculated by difference as follows:

% carbohydrate = 100 – (% protein + % moisture + % ash + % fats + % fibers).

Minerals such as iron, zinc and magnesium were estimated by using Atomic Absorption Spectrophotometer (Pectin-Elmer, PinAAcle™ 500 AA) according to APHA (2005) while potassium was determined using flame photometer according to (Hesse and Hesse, 1971). Total calories were calculated by using the equation mentioned by (Livesey, 1995), while the caloric value was calculated using values of 4 Kcal/g for carbohydrate, protein and 9 Kcal/g for fat.

Biochemical analytical methods:

Determination of the phenolics content (PCs):

The methanolic extraction of oat powder, lima bean powder, sweet lupin powder and talbina powder were done according to (Skotti *et al*., 2014). After preparation of the materials as mentioned earlier, the dried powdered materials (200g) of each one was mixed with 99% methyl alcohol (1.0 L) in a closed flask and kept for 3 days. Shaking frequently during the first 12 hours and allowed to stand until the end of period soaking. Thereafter, they were filtered rapidly through filter paper (Whatman NO. 4) taking precautions against the loss of the solvent. The methanolic extracts were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (50-60ºC). The extracts were stored in a refrigerator at 4ºC till further use. Then, the total polyphenols were determined against (Gallic Acid) as standard curve by using Folin-Ciocalteu reagent spectrophotometric method according to (Lin and Tang, 2007). Briefly, about 0.1ml of extract was mixed with about 2.8ml of distilled water, 2.0ml sodium carbonate 2% (w/v) and 0.1 ml of folin-ciocalteu reagent 50% (v/v) were added. After shaking, the mixture was incubated at room temperature for 30 minutes and the absorbance of the resulting color was measured at 750, using PG Instruments T60 UV-Vis spectrophotometer. Total phenol contents were expressed as milligram gallic acid equivalent (mg GAE) per gram of extract. The total flavonoids contents were estimated by a photometric method, aluminum chloride colorimetric method was used for quantitative flavonoids determination (Chang *et al.*, 2002). Briefly, the different samples (0.1 ml) were mixed with 1.5 ml of methanol, then (0.5 ml) of the solution was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride ($AlCl_3$), 0.1 ml of 1.0 M potassium acetate (CH_3COOK) and 2.8 ml of distilled water. The mixture was kept at room temperature for 30 minutes, the reaction mixture absorbance was measured at 415 nm with using the double beam Perkin Elmer UV/Visible spectrophotometer (Lamda 2S). The concentration of flavonoids was determined from the standard curve; Quercetin was chosen as standard of flavonoids to make the standard curve (0-50mg/L). The results of TFCs were expressed as mg of quercetin equivalents (mg QE) per gram of the raw materials extracts. Reducing power assay was determined as described in the method of (Sharma and Gujral, 2011). Briefly, ascorbic acid was used as reference standard. Each extract (1.0 ml) was added to 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. Then the mixture was incubated at 50 C for 20 min. after 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml deionized water and 0.5 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm against the corresponding blank solution: higher absorbance indicates higher reducing power. DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging capacity was determined according to the technique reported by (Martins *et al.*, 2008). The IC50 value of extracts (concentration of extract required for 50% reduction in absorbance) was calculated by a sigmoid linear regression model. Briefly, Sample extracts were diluted with DMSO and in each reaction mixture, then the solution was mixed with 2.0 ml of 100 µM DPPH. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. in dark. While ascorbic acid was used as standard. De-colorization of purple DPPH was determined by measuring the absorbance at 517 nm with the double beam Perkin Elmer UV/Visible spectrophotometer (Lamda 2S).

$$\% \text{ Scavenging Activity} = (A_0 - A_1/A_0) \times 100$$

Where;

A_0: Absorbance without extract.

A_1: Absorbance in the presence of the extract or standard sample.

Antimicrobial assessment:

Bacterial strains: three bacterial strains were used in the study as follows: *Escherichia coli:* gram negative, non-spore forming, rod-shaped and facultative anaerobic bacteria. *Staphylococcus aureus:* gram positive that

potentially pathogenic, cocci and facultative anaerobic bacteria. *Bacillus cereus:* gram positive, rod-shaped, beta-hemolytic, spore forming, motile and obligate aerobic. The agar diffusion method, nutrient agar, was used for cultivation of bacteria as described by (Bagamboula *et al*., 2003) with some modification. In this study, 25, 50, 75 and 100 ppm of each extract were used to be tested on the surface of an agar plate freshly seeded with a standard. After inoculation the plates of bacterial strains were incubated at 37ºC for 24 hours. At the end of incubation period, the inhibition zones were measured.

RESULTS AND DISCUSSION

The obtained data in Table (1) indicated the chemical composition of sweet lupin powder, lima bean powder, oat powder and talbina powder. sweet lupin recorded the lowest value of moisture content, while talbina recorded the highest value and those results agreed with those reported by (Elsamani *et al*., 2014 and Youssef *et al*., 2013). lima bean and oat represented moisture content values at 6.44% and 6.94%, respectively and those values slightly similar to values reported by (Moses *et al*., 2012 and Chappalwar Vijayakumar *et al*., 2013). Sweet lupin obtained the highest total protein content, whilst talbina recorded the lowest total protein content and those are in the same trend with those reported by (Youssef *et al*., 2013 and Elsamani *et al*., 2014). Talbina was the lowest fat content. However, sweet lupin indicated the highest fat content and these results slightly agreed with those recorded by (Youssef *et al*., 2013). In addition, lima bean and oat were recorded values of 1.90%, 6.93%, respectively and those agreed with those mentioned by (Torruco-Uco *et al*., 2009 and Chappalwar Vijayakumar *et al*., 2013). Ash content in oat was consistent with that recorded by Chappell *et al*. (2017), when sweet lupin, lima bean and talbina obtained ash contents which agreed with those values reported by (Youssef *et al*., 2013 and Elsamani *et al*., 2014). Fiber content in sweet lupin was 10.78% and this value is in the same trend with the value recorded by Elsamani *et al*. (2014). Besides, fiber content of talbina was 5.10% and this result is strongly in agreement with those results mentioned by Youssef *et al*. (2013). Furthermore, lima bean recorded 1.15% which disagreed with results reported by Moses *et al*. (2012) and this might be due to the hulling of lima bean seeds during soaking process. Talbina had the highest total carbohydrate content while sweet lupin powder recorded the lowest content. On the other hand, sweet lupin gained the highest value of energy, while talbina powder presented the lowest energy value.

Table 1. Chemical Composition of sweet lupin powder, lima bean powder, oat powder and talbina powder (g/DM):

Materials	M %	P %	F %	Ash %	Fib %	Carb %	Energy (Kcal/100g)
Sweet Lupin	5.74	45.76	14.00	3.07	10.78	20.65	391.64
Lima Bean	6.44	27.73	1.9	4.12	1.15	58.66	362.66
Oat	6.94	18.47	6.93	1.95	9.86	55.85	359.65
Talbina	9.07	12.58	1.78	2.78	5.10	68.69	341.10

M%: Moisture%; P%: Protein%; F%: Fat%; Fib%: Fiber%, and Carb%: Carbohydrate% (by difference).

Minerals content

Data in Fig. (1) showed the macro elements content of potassium (K) and magnesium (Mg). Sweet Lupin presented the lowest value of K. While lima bean had the lowest content of Mg and that value was slightly agreeing with that reported with (Ezeagu and Ibegbu, 2010). on the other hand, lima bean had the highest content of K when sweet lupin has the highest content of Mg and those obtain results in agreement with those reported by (Levent and BilgiÇli, 2011 and Ezeagu and Ibegbu, 2010).

Results in Fig. (2) represented the micro elements content of iron (Fe) and zinc (Zn). Talbina had the highest value of Fe content whilst lima bean obtained the lowest Fe content and those results were agreeing with those recorded by (Youssef *et al*., 2013 and Ezeagu and Ibegbu, 2010). Talbina recorded the lowest Zn content while sweet lupin represented the highest Zn content and those values agreed with those detected by (Chappell *et al*., 2017 and De Carvalho, 2005).

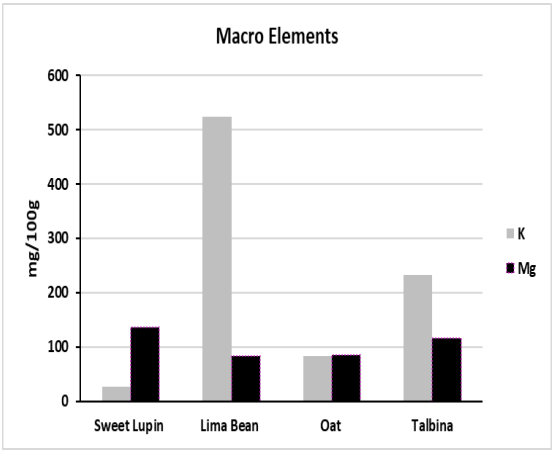

Fig. 1. The macro elements content of K and Mg in sweet lupin, lima bean, oat and talbina.

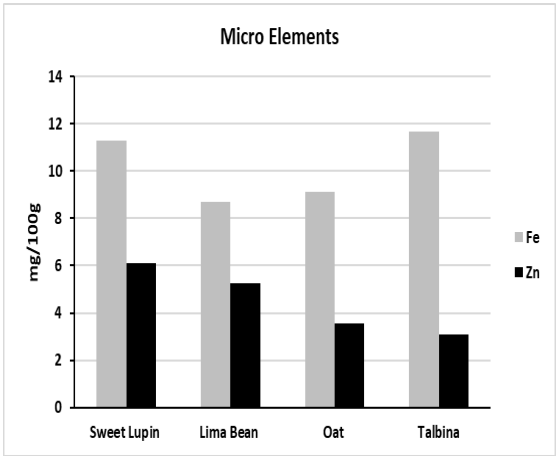

Fig. 2. The micro elements content of Fe and Zn in sweet lupin, lima bean, oat and talbina.

Total phenolic and flavonoid contents of methanolic extracts:

The results in Fig. (3) showed the measured total phenolic content, using gallic acid as standard curve and for total flavonoid content, using quercetin as standard reference. There were no differences in total phenolic content values observed between all extracts. The highest level of total phenolic content was found in barley extract,

which slightly agreed with that reported with (Madhujith and Shahidi, 2009) and disagreed with high results mentioned by (Baba *et al*., 2016), while the lowest value of total phenolic content was obtained in sweet lupin extract, while lima bean extract come in the second place after barley extract which was slightly similar to total phenolic contents (TPCs) in that lima bean reported by (Granito and Paolini, 2008) and was in disagreement with that lower TPCs value by (Boateng *et al*., 2008), followed by the oat extract. In addition, the TPCs in oat methanolic extract was higher than those reported by (Ryan *et al*., 2011) and slightly similar to the study by (Cai *et al.*, 2011). TPCs content in sweet lupin was higher than those values reported by (Ranilla *et al.*, 2009) and (Wang and Clements, 2008). These variances in TPCs results reported might be due to differences in the genetic factors, agricultural conditions, methods of extraction and/or type of solvent (Suriano *et al*., 2018).

As shown in Fig. (3), the obtained total flavonoids content of methanolic extracts for barley, oat, lima bean and sweet lupin. Total flavonoids of barley, lima bean and sweet lupin were strongly in agreement with those reported by Boateng *et al.* (2008) and Siger *et al.* (2012). Which were part of phenolic contents and played a great role as natural antimicrobial and antioxidant.

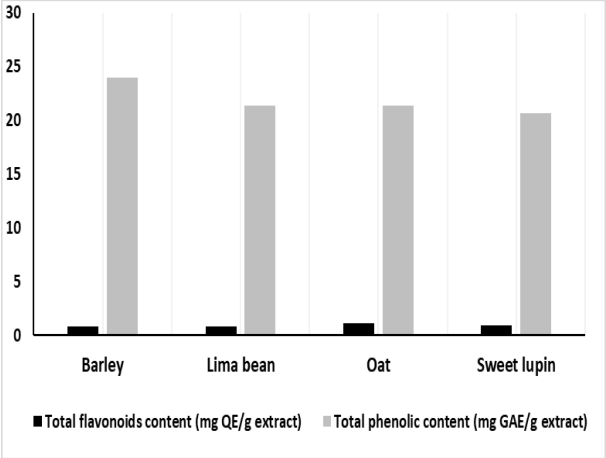

Fig. 3. Total phenolic and total flavonoid contents of methanolic extracts for barley, oat, lima bean and sweet lupin.

Radical scavenging activity of barley, oat, lima bean and sweet lupin methanolic extract using DPPH:

The antioxidant capacity of barley, oat, lima bean and sweet lupin methanolic extracts (scavenge DPPH free radicals) observed in Fig. (4). Also, Fig. (5) showed that gradual increasing of vitamin C is leading to free radical scavenging. Lima bean extract exhibited the highest free radical scavenging activity compared with ascorbic acid. That result disagreed with the results obtained by Fan and Beta (2017), then followed by the barley extract, which had a lower value recorded by Rao *et al*. (2018). In contrast, previous studies which were conducted by Yoshida *et al*. (2010) achieved a higher IC50. While the study on oat and sweet lupin extracts achieved the lowest DPPH value compared with ascorbic acid. Those results disagreed with those reported by Siger *et al*. (2012) who found low DPPH value in oat extract. While higher value recorded by Ranilla *et al*. (2009). Those results might be due to the total phenolic and high flavonoid contents.

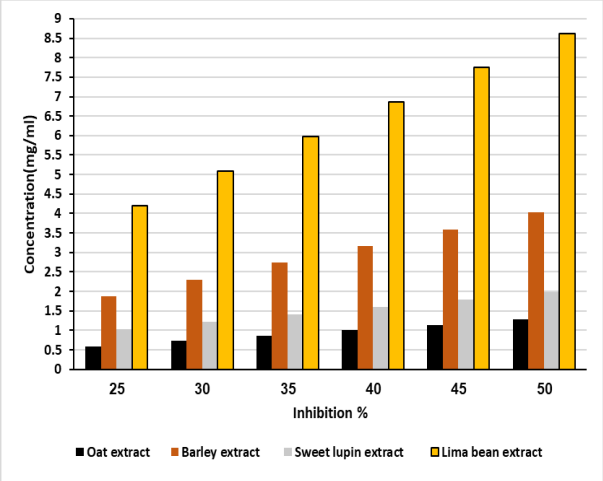

Fig. 4. Changes in DPPH radical scavenging activities of barley, oat, lima bean and sweet lupin extracts comparable with ascorbic acid at absorbance 522 nm.

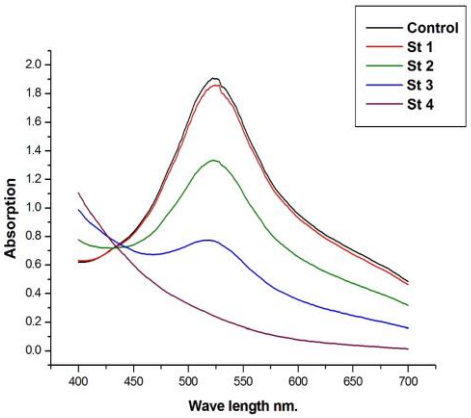

Figure 5. Effect of gradual concentrations for Vitamin C on the UV-visible spectra of DPPH in DMSO solvent at 25oC.

Reducing power of various concentrations of methanolic fortifying materials extracts:

The analysis of reducing power of barley, oat, lima bean and sweet lupin methanolic extracts at 40 µg/mL indicated variable absorbance ratios in Table (2). Sweet lupin extract recorded the high reducing power followed by oat then barley while lima bean extract recorded the lower reducing power compared with ascorbic acid.

Results in Table (2) for barley and oat extracts are respectively agreement with values which indicated by (Liu and Yao, 2007 and Shah *et al*., 2017) who illustrated that reducing power of barley extract seem to be the result of its antioxidant activity. It is presumed that the phenolic compounds may act in a similar way as reductions by donating electrons and reacting with free radicals for converting them to more stable products and terminating the free radical chain reaction. On the other hand, results of lima bean extract reducing power disagreed with values revealed by Amarowicz *et al*. (2008) that might be due to the dehulling process of lima bean seeds during the soaking.

Table 2. Reducing power of various concentrations of of barley, oat, lima bean and sweet lupin methanolic extracts.

Methanolic material extract concentrations (µg/ml)	Fe^{+2} reducing power (absorbance)				
	V.C	OE	BE	SLE	LBE
5	0.5832	0.1357	0.0865	0.2168	0.065
10	1.0537	0.1542	0.1075	0.2423	0.071
20	1.9947	0.1912	0.1495	0.2933	0.083
30	2.9357	o.2282	0.1915	0.3443	0.095
40	3.8767	0.2652	0.2335	0.3953	0.107

V.C: Vitamin C; OE: Oat extract; BE: Barley extract; SLE: Sweet Lupin extract; LBE: Lima Bean extract

Antibacterial activity:

Antibacterial activity of barley extract: Data in Fig. (6) shown that no inhibition zone was noticed at all concentration against *Escherichia coli* strain. On the other hand, it inhibited *Bacillus cereus* bacteria in higher degree more than *Staphylococcus aureus* bacteria at all used concentrations. The greater concentration of barley extract, the greater antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*, this antibacterial activity may be due to its content of total phenolic and flavonoid compounds. Those results were in the same trend with (Sheela and suganya, 2012 and Madineni, 2012).

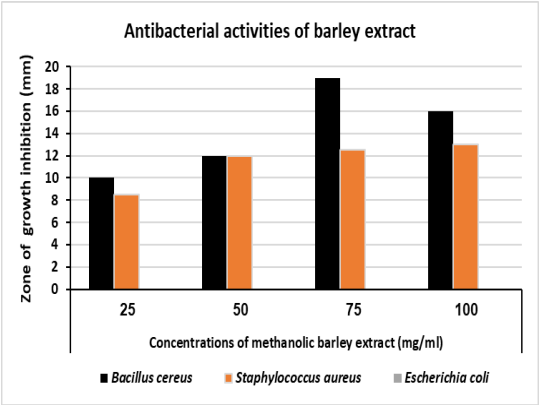

Figure 6. Antibacterial activities of barley extract.

Antibacterial activity of Lima Bean extract: Results in Fig. (7) clearly showed that lima bean extract had no antimicrobial activity at concentration of 25 mg/ml with all tested microbes. Therefore, increasing of lima bean extract concentration increased antibacterial activity and those results were in the same trend as reported by de Jesús Ariza-Ortega *et al.* (2014). That antibacterial activity might be due to total content of phenolic and flavonoid compounds.

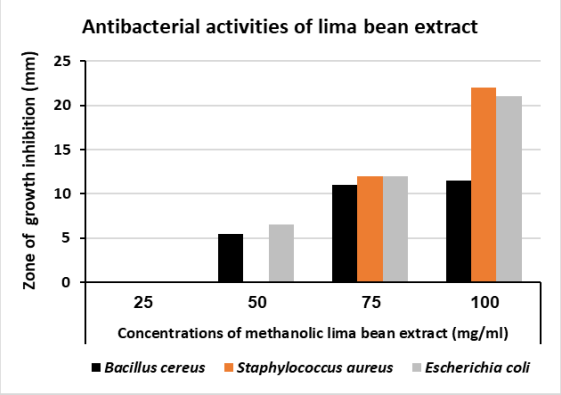

Figure 7. Antibacterial activities of lima bean extract.

Antibacterial activity of Oat extract: From data in Fig. (8), oat extract had no inhibitory effect at low concentrations (25 and 50 mg/ml) *with Bacillus cereus* and *Escherichia coli.* Antibacterial activity acted with increasing of concentrations against all studied bacteria strains. These results were might be due to oat contents of phenolic and flavonoid contents.

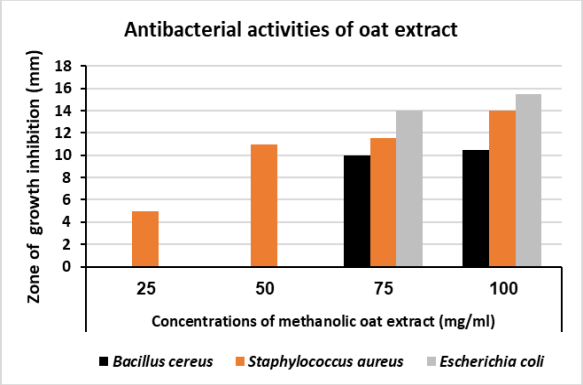

Figure 8. Antibacterial activities of oat extract.

Antibacterial activity of sweet lupin extract: Data in Fig. (9) indicated that sweet lupin extracts potently inhibited the growth of *Bacillus cereus* bacterium at all concentrations. While, it only inhibited *Staphylococcus aureus* at higher concentrations (75 and 100mg/mL). Whereas, it had no antibacterial activity on *Escherichia coli.* These results are slightly agreement with those obtained by (Romeo *et al.*, 2018) who found that there were no antibacterial effects on *Escherichia coli* and *Staphylococcus aureus.* Those results might be due to sweet lupin contents of flavonoid and phenolic compounds.

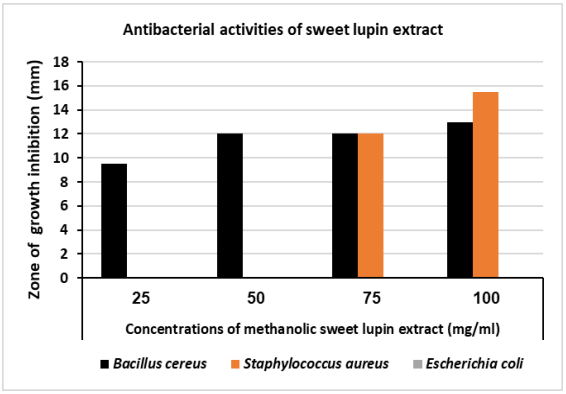

Figure 9. Antibacterial activities of sweet lupin extract.

CONCLOSION

This study was concluded to increse the knowledge about the nutritional importance, antioxidant activities and anti-microbial activeties of barley, oat, lima bean and sweet lupin. And from brevious data, we can recommended to use them in fortifying foods or using them in healthy diets. Data indicated that lima bean, sweet lupin, oat and barley had total pholy phenols and falvonoids contents, had an antioxidant activities as showed by radical scavenging activities (DPPH) and reducing power analysises and also they had an anti-bacterial activities aginest three pathoginic stains such as *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*.

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التركيب الكيماوي والنشاط المضاد للأكسدة والمضاد للميكروبات للشوفان والشعير والترمس الحلو والفاصوليا البيضاء

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في هذا البحث تم دراسة التركيب الكيميائي وتقدير المحتوي الكلي للبولي فينولات والفلافونيدات للمستخلص الميثانولي لكلا من الشوفان والشعير والترمس الحلو والفاصوليا البيضاء. وكذلك دراسة تأثير هذه المستخلصات كمواد مضادة للأكسدة من خلال تقدير القوة الاختزالية وتثبيط الشقوق الحرة, وتقدير قدرة هذه المستخلصات علي تثبيط نشاط بعض البكتريا المرضية مثل *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*. وأوضحت النتائج أن أعلي محتوي من الفينولات كان في مستخلص الشعير وأقل محتوي سجل في مستخلص الترمس الحلو بينما أعلي محتوي من الفلافونيدات كانت في مستخلص الشوفان والأقل كانت في مستخلص الفاصوليا البيضاء في حين سجل مستخلص الفاصوليا أعلي معدل تثبيط للشقوق الحرة بينما الشوفان كان الأقل. وسجل مستخلص الترمس الحلو أعلي معدل امتصاص (القوة الاختزالية) في حين الفاصوليا البيضاء سجلت أقل معدل. لم يظهر أي تثبيط ميكروبي لمستخلص الشعير في جميع التركيزات مع ميكروب *Escherichia coli* بينما لم يظهر أي تأثير تثبيطي لمستخلص الفاصوليا البيضاء عند تركيز ٢٥ مليجرام/مليلتر مع الثلاث سلالات الممرضة المستخدمة تحت الدراسة. مستخلص الشوفان لم يظهر تأثيره علي *Escherichia coli* و *Bacillus cereus* علي التركيزات المنخفضة (٢٥ و ٥٠ مليجرام/مليلتر). في حين لم يظهر تأثير مستخلص الترمس الحلو التثبيطي علي *Staphylococcus aureus* إلا عند التركيزات المرتفعة منه (٧٥ و ١٠٠ مليجرام/مليلتر) ولم يظهر له أي تأثير تثبيطي علي *Escherichia coli* عند جميع التركيزات.