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COMPARATIVE STUDY ON LUPINE AND FENUGREEK SEEDS THAT GROW IN EGYPT

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ABSTRACT: ***Main chemical composition, bioactive constituents and antioxidant activity of Egyptian lupine and fenugreek seeds were investigated. The obtained results showed that white lupine seeds have higher amount of carbohydrate and ash than that in the other seeds. Meanwhile yellow lupine seeds showed the highest percentage of fibers among all tested seeds, while fenugreek seeds were characterized by large amount of crude protein, oil and moisture comparing with both kinds of lupine seeds.***

On the other side, analysis of bioactive constituents revealed the presence of total phenolics, total flavonoids as well as saponins in the highest amount in fenugreek seeds, whereas the highest values of alkaloids and tannins were belonged to yellow lupine. It is noteworthy that white lupine exhibited the lowest amounts of all tested bioactive components among all studied seeds.

And finally, in vitro antioxidant activity using two deferent methods, showed a correlation between total phenolics and total flavonoids on one hand and antioxidant activity on the other hand, where it showed the highest values for fenugreek seeds followed by yellow and white lupine seeds respectively.

Key words: ***lupines (white and yellow) and fenugreek seeds-Alloxan-Diabetic rats-Hypoglycemia - Antioxidant enzymes.***

INTRODUCTION

Nutritional value of white and yellow lupine as well as fenugreek seeds was studied by many researchers, and it was found that white lupine seeds contain, carbohydrate 3.27%, protein 35.8%, oil 9.4%, crude fiber 10.6% (Cowling *et al,* 1998), and that yellow lupine seeds contain 1.38%, 37.9%, 33.68% and 4%, respectively for the same components, while fenugreek seeds presented for such components percentages of 45.2%, 29.3%, 7.9% and 7%, respectively Birhane, (2012)

Meanwhile, studies on bioactive constituents in the tested seeds demonstrated the presence of phenolics, flavonoids, alkaloids, saponins and tannins in varying proportions (Patel and Dhanabal, 2013; Duke, 1992; Kalogeropoulos *et al.,* 2010).

In this context a great number of in vitro methods have been developed to measure the efficiency of natural antioxidants for methanolic extracts of white lupine seeds owing to their content of phytoestrogens such as flavonoids (Adlercreutz, and Mazur, 1997), yellow lupine seeds which have high levels of phenolic compounds mainly tannins and flavonoids (Zia *et al.,* 2001) and fenugreek seeds which their antioxidant property is attributed to their high content of phenolic constituents (Chatterjee *et.al.,* 2009).

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MATERIALS AND METHODS

1-Plant collection and identification:

The seeds of white lupine (sweet lupine), yellow lupine (bitter lupine) and fenugreek were obtained from research center department of medical and aromatic plants Giza, Egypt; the seeds were identified in horticulture department, faculty of agriculture, Menoufia University.

Seeds samples were washed and air-dried for 24 hours, then dried at 50^0C. The dried sample was grinded into fine powder and kept in refrigerator for analysis.

2-Main chemical composition of seeds

Total nitrogen was determined (dry basis) according to the modified micro-kjelahl method as described by the association of official Analytical Chemists, A.O.A.C., (2000). The crude protein contents were calculated using the conversion factor 6.25. Total lipids and moisture were determined according to A.O.A.C., (2000). Total carbohydrate were estimated according to the method of Dubois *et al.,* (1956), while crude fiber was determined according to the method illustrated in A.O.A.C., (2008) , and finaly ash content was determined by ignition of dried sample at 550^0C until a constant weight according to (A.O.A.C., 1990).

3-bioactive constituents in tested seeds

Determination of hydrolysable tannins (HTs):

HTs were determined by the method of Cam and Hisil (2010). 1 ml of 10-fold diluted methyl extracts and 5 ml of 2.5 % KIO_3 were added into a vial and vortex for 10 sec. Optimum absorbance of the red colored mixture was determined at 550 nm versus the prepared water blank. Optimum absorbance, defined as the time to gain maximum absorbance value , was determined and tannic acid solutions (100 to 1600 mg/l) were used for calibrations .The final results were expressed as mg tannic acid equivalent per g of dry weight (mg TAE/g DW).

Determination of saponins:

The defatted seeds flours (residue after oil extraction) were kept at room temperature overnight. The next day, 30 ml methanol was added to the tubes and left on the shaker all night, followed by centrifugation .The second and third extractions by methanol was also carried out. At the end, all supernatants of methanol extracts were pooled and the methanol was evaporated using rotary evaporator. Finally, a yellowish crystal powder of crude saponins was obtained which was determined according to the method of Uematsu *et al.,* (2000).

Determination of alkaloids:

The plants material (100g) were ground and then extracted with methanol for 24 hrs. In a continuous extraction (soxhlet) apparatus, the extraction was filtered and methanol was evaporation on a rotary evaporated under vacuum at a temperature of 45^{o}C to dryness. A part of this residue was dissolved in (2 N) HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 gm chloroform (3 times). The pH of this solution was adjusted to neutral with (0.1 N) NaOH. Then 5 ml of bromocresol green solution and 5 ml of phosphate buffer (pH 4.7) were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to the

adjusted volume with chloroform, the absorbance test of the complex in chloroform was measured at 470 nm against blank and standard solutions according to the method of Fazel *et al.,* (2008).

Determination of total phenolics:

The amount of total phenolics in the studied extracts was determined with the Folin-Ciocateu reagent. Gallic acid was used as standard and the total phenolics were expressed as mg gallic acid equivalents (GAE/g dry weight). 10 ml of samples were extracted in methanol, 0.5 ml of each sample and standard were introduced into test tubes and mixed with 2.5 ml of Folin-Cicalteu reagent diluted to 10 fold and 2 ml of 7.5 % sodium carbonate. The tubes were covered tightly and allowed to stand for 30 min. at room temperature before the absorbance which was read at 760 nm spectrometrically Kim *et al.,* (2003).

Determination of total flavonoids:

The total flavonoids content was determined using the method reported by Djeridane *et al.,* (2006). Briefly, an aliquot of 250 µl of each methanolic extract or a standard solution was mixed with 1.25 ml deionized water, followed by 75µl of a 5% $NaNO_2$ solution after 6 min., 150 µl of 10 % $AlCl_3$. $6H_2O$ solution was added to each mixture, after 5 min. 0.5 ml of 1 M NaOH was added, and the total volume was adjusted to 3.0 ml with deionized water. Catechin was used as a standard using absorbance at 510 nm for the measuring which was corrected using a blank, the results were expressed as mg of catechin equivalents (CE) /g dry weight.

Qualitative and quantitative analysis of phenolics using GC/MS method:

The analysis of plant extracts was carried out using a GC (Agilent Technologies 7890 A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with an apolar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m×0.25 mm i.d. and 0.25 um film thickness) the carrier gas was helium with the linear velocity of 1.0 ml/min.

The identification of components was based on comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature Partricia, *et al.,* (2013).

4-In-vitro antioxidant activity:

Which was determined by two different methods because of the complex nature of phytochemicals (Chanda and Dave, 2009), in order to evaluate the antioxidant activity capacity of plant materials and these methods include:

4-1 Reducing power assay using potassium ferricyanide:

It is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then react with ferric chloride to form ferric-ferrous complex that have an absorption maximum at 700 nm. The reducing power of different extracts was determined according to the method of Ebrahimzadeh *et al.,* (2008), where 2.5 ml of extract (200 µg/ml) in water were mixed with a phosphate buffer (2.5 ml, 0.2M, pH6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated

at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm against blank. Increased absorbance of the reaction mixture indicated increased reducing power.

4-2 ββ`-diphenyl-α-picrylhydrazyl (DPPH) radical scavenging activity:

The antioxidant activity of both plant extracts and standard was assessed on the basis of the radical scavenging effect of the stable ββ`-diphenyl-α-picrylhydrazyl (DPPH) by modified method of Braca *et al.,* (2002). The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard. 0.004% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution (100 µg/ml) and standard solution (100 µg/ml) separately. These solution mixtures were kept in dark for 20 min. and optical density was measured at 517 nm using spectrophotometer. Methanol (1 ml) with DPPH solution (0.004%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = A – B /A x 100

Where A = optical density of the blank and B = optical density of the sample.

RESULTS AND DISCUSSION

Main chemical composition of tested seeds :

Data obtained from Table (1) indicate that white lupine seeds contain, carbohydrate 38.046%, protein 24.476%, oil 7.745% crude fiber 29.161%, ash 0.452% and moisture 2.15% and that yellow lupine seeds contain 38.218% 22.304%, 5.851%, 33.143%, 0.404% and 0.08%, respectively for the same aforementioned components, while fenugreek seeds presented for such components percentages of 35.471%, 25.28%, 8.489%, 30.183%, 0.437% and 0.14% in the same order.

The results are in accordance with those of Duke., (1992); vats *et al.,* (2003); sujak *et al.,* (2005); Erbas *et al.,* (2005) and Martinez-Villaluenga *et al.,* (2006).

Table (1): Main chemical composition of white lupine, yellow lupine and fenugreek seeds.

Chemical composition	Type of seeds			
	White lupine %	Yellow lupine %	Fenugreek %	
Total carbohydrate	38.046	38.218	35.471	Dry weight
Crude protein	24.476	22.304	25.28	
Oil	7.745	5.851	8.489	
Crude fiber	29.161	33.143	30.183	
Ash	0.452	0.404	0.437	
Moisture	0.12	0.08	0.14	

Bioactive constituents in tested seeds

The obtained results in Table (2) showed that alkaloids and tannins were in high content in yellow lupine (2.798 and 1.523 mg/100gm dw), followed by fenugreek (1.861 and 1.05 mg/100 gm dw), while the minor contents were for white lupine (1.352 and 0.596 mg/100 gm dw). For saponins, it was found that fenugreek recorded the highest value (5.5 mg/100 gm dw) and thereafter yellow lupine (4.8 mg/100 gm dw), whereas white lupine showed the lowest value (3.5 mg/100 gm dw).

The results are in the same line with those described by Zia *et al.,* (2001); Schryver, (2002); Vats *et al,* (2003) and Siger *et al.,* (2012).

On the other hand, date indicate that fenugreek seeds have higher percentages of both total phenolics and flavonoids (0.848% and 0.05%, respectively) than that in lupine seeds. In respect to lupine seeds, it was found that yellow lupine seeds, showed high amount of total phenolics and flavonoids (0.732% and 0.021%) comparing, with that in white lupine seeds (0.371% and 0.017%. Theses results are in parallel with those obtained by Rao *et al,* (1996); Skaltsa, and petropoulos (2002); Lu, *et al.,* (2008) and Siger, *et al.,* (2012).

In connection with the above, it was found that quantitative analysis of phenolic compounds as shown in Table (3), exhibited the presence of 25 of phenolic compounds in white lupine seeds and 23 ones only in both yellow lupine and fenugreek seeds and that 5.7.3.4-tetrahydroxy flavone represent the main phenolic compound in white lupine and fenugreek seeds (75.19% and 62.51%, respectively), while it recorded 6.45% only in yellow lupine seeds.

On the other hand, 4-metylcatechol was the principle component in yellow seeds where it amounted 13.94% comparing with 3.44% and 6.98 for white lupine and fenugreek seed, respectively.

Theses results agree with those of Ricardo-Dasilva, *et al.,* 1993; Naidu *et al.,* 2011 and Siger *et al.,* (2012).

Relating to phenolic compounds, antioxidant activity was carried out using two different methods as mentioned earlier. Both of them showed that fenugreek seeds were the highest (Table 4), where their reducing power was 56.225 while antioxidant activity was 82 by the second method followed by yellow lupine where their values in the two methods were 51.4 and 65 respectively and lastly white lupine which showed the lowest percentages in both two methods where they recorded 41.015 and 48% in the same order.

These results are compatible with those reported by Dixit *et al.,* (2005) and Chanda and Dave (2009).

Table (2): Bioactive constituents in white lupine, yellow lupine and fenugreek seeds.

Bioactive phytochemicals compounds	Type of seeds			
	White lupine	Yellow lupine	Fenugreek	units
Alkaloids	1.352	2.798	1.861	mg/100g dw
Saponins	3.5	4.8	5.5	
Tannins	0.596	1.523	1.05	
Total Phenolics	0.371	0.732	0.848	g/100g dw
Total Flavonoids	0.017	0.021	0.05	

Table (3): The phenolic compounds (%) in methanolic extract of white lupine seeds using GC/MS.

NO	RT(min)	Area sum %	Compounds
1	3.424	3.44	4-Methylcatechol
2	4.791	1.04	4-Methoxycinnamic acid
3	6.283	0.79	Sinapyl alcohol
4	6.283	0.77	Caffeic acid
5	6.706	1.05	4-Hydroxybenzoic acid
6	7.166	0.76	Scopoletin
7	7.287	1.705	6-Monohydroxyflavone
8	7.910	0.85	2-Methoxy-5-methylphenol
9	8.023	0.76	Neo dihyrocarveol
10	8.295	0.96	Fisetin
11	8.717	2.16	Quercetin 3,4,7 trimethyl ether
12	9.700	1.03	Methyl salicylate
13	10.557	0.77	2-Allyl-p-cresol
14	11.937	0.78	Apigenin-8-c-glucoside
15	12.685	1.11	2,4-Dihydroxybenzoic
16	12.894	1.715	Juniper camphor
17	15.679	0.91	Cyanidincation
18	15.867	75.19	5,7,3`,4`,Tetrahydroxyflavone
19	16.662	0.97	Probucol
20	17.138	0.89	Zearalenone
21	17.355	0.83	Enterodiol
22	19.120	0.97	Cannabinol
23	21.370	1.11	4-Tert-octyl-o-cresol
24	22,783	1.33	3,5,7-Trimethoxyflavone
25	23.933	0.77	Hydroquinone

Table (3): Cont.

NO	RT(min)	Area sun%	Compounds
1	3.424	13.94	4-Methylcatechol
2	4.791	2.36	4-Methoxycinnamic acid
3	6.283	2.66	Caffeic acid
4	7.166	6.89	Scopoletin
5	7.287	2.62	6-Monohydroxyflavone
6	7.910	2.96	2-Methoxy-5-methylphenol
7	8.023	2.33	Neo dihyrocarveol
8	8.295	2.4	Fisetin
9	8.717	2.18	Quercetin 3,4,7-trimethyl ether
10	9.700	7.76	Methyl salicylate
11	10.557	2.76	2-Allyl-p-cresol
12	10.845	2.31	Tetramethyl phenol
13	11.937	2.72	Apigenin-8-c-glucoside
14	12.685	1.89	2,4-Dihydroxybenzoic acid
15	12.894	1.53	Juniper camphor
16	15.679	4.03	Cyanidin cation
17	15.867	6.45	5,7,3`,4`-Tetrahydroxyflavone
18	16.662	5.8	Probucol
19	17.138	3.54	Zearalenone
20	17.355	5.51	Enterodiol
21	19.120	4.72	Cannabinol
22	21.370	4.74	4-Tert-octyl-o-cresol
23	22,783	7.91	3,5,7-Trimethoxy flavone

Table (3): Cont.

NO	RT(min)	Area sum%	Compounds
1	3.424	6.98	4-Methylcatechol
2	4.791	1.23	4-Methoxycinnamic acid
3	6.283	0.76	Caffeic acid
4	7.166	1.04	Scopoletin
5	7.287	0.85	6-Monohydroxyflavone
7	8.023	0.66	Neo dihyrocarveol
8	8.295	0.76	Fisetin
9	8.717	0.68	Quercetin 3,4,7-trimethyl ether
10	9.700	1.7	Methyl salicylate
11	10.557	0.92	2-Allyl-p-cresol
12	11.937	0.78	Apigenin-8-c-glucoside
13	12.685	1.12	2,4-Dihydroxybenzoic acid
14	12.894	1.38	Juniper camphor
15	15.679	2.29	Cyanidin cation
16	15.867	62.51	5,7,3`,4`,Tetrahydroxyflavone
17	16.662	4.57	Probucol
18	17.138	1.83	Zearalenone
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20	19.120	0.69	Cannabinol
21	21.370	1.9	4-Tert-octyl-o-cresol
22	22,783	2.96	3,5,7-Trimethoxy flavone
23	23.933	2.77	Hydroquinone

Table (4): In-vitro antioxidant activity of tested seeds extracts.

Methods of antioxidant activity	Type of seeds		
	White lupine	Yellow lupine	Fenugreek
Reducing power	41.015	51.40	56.525
DPPH radical scavenging activity	48.0	65.0	82.0

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دراسه مقارنه علي بذور الترمس والحلبه المزروعه في مصر

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الملخص العربي

تمت دراسه التركيب الكيميائي لبذور الترمس والحلبه من ناحية المكونات الأساسية وكذا المركبات النشطه بلأضافه الي النشاط المضاد للأكسده لمستخلصات هذه البذور

وقد أظهرت النتائج التحصل عليها ما يلي :

- وجد أن بذور الترمس الأبيض (الحلو) تحتوي على كربوهيدرات وبروتين وزيت وألياف ورماد ورطوبه بنسب هي على الترتيب ٣٨.٠٤٦٪, ٢٤.٤٧٪، ٧.٧٤٪، ٢٩.١٦٪، ٠.٤٥٪، ٠.١٢٪ وأن نفس هذه المكونات في بذور الترمس الأصفر (المر) كانت نسبها وعلى التوالي (٣٨.٢١٨٪، ٢٢.٣٠٪، ٥.٨٥٪، ٣٣.١٤٪، ٠.٤٢٪ , ٠.٠٨%) بينما في بذور الحلبة كانت هذه النسب للمكونات السابق ذكرها وعلى نفس الترتيب (٣٥.٤٧١٪، ٢٥.٢٨٪، ٨.٤٨٪، ٣٠.١٨٪، ٠.٤٣٪, ٠.١٤٪).
- أما بالنسبة للمركبات الكيميائية النشطة في بذور هذه النباتات فقد ثبت وجود قلويدات وصابونينات وتنينات بالكميات الآتية: ١.٣٥، ٣.٥ ، ٠.٥٩ في بذور الترمس الأبيض مقابل كميات قدرها ٢.٧٩٨ ، ٤.٨ , ١.٥٢ في بذور الترمس الأصفر أما في بذور الحلبة فكانت هذه الكميات وعلى الترتيب ١.٨٦١,٥.٥,١.٠٥ (مج لكل ١٠٠جم بذور) مع العلم بأن كل النتائج مقدرة في ١٠٠ جرام من الوزن الجاف للعينه.

وفي نفس الوقت كانت نسب كل من الفينولات والفلا فونيدات الكلية في بذور الترمس الأبيض هي ٠.٣٧٪، ٠.٠١٪ على الترتيب بينما كانت نسبها في الترمس الأصفر ٠.٧٣٪، ٠.٠٢٪ أما بذور الحلبة فقد أظهرت أعلى نسبة لهذه المكونات حيث وصل إلي ٠.٨٤٨٪,٠.٠٥٪ على التوالي.

وقد أظهرت نتائج التحليل الكروماتوجرافي احتواء بذور الترمس الأبيض على ٢٥ من المركبات الفينولية مقابل ٢٣ مركب فقط في كل من بذور الترمس الأصفر والحلبة وأن المركب الفينولي الأساسي في كل من بذور الترمس الأبيض والحلبة كان ٥، ٧، ٣/، ٤/ رباعي هيدروكسي الفلافون حيث كانت نسبته في بذور النباتين ٧٥.١٩٪، ٦٢.٥١٪ على الترتيب بينما نسبته في بذور الترمس الأصفر كانت ٦.٤٥ % فقط.

وعلى الجانب الآخر كان المركب ٤- ميثيل كاتيكول هو المكون الغالب في بذور الترمس الأصفر حيث بلغت نسبته ١٣,٩٤٪ وذلك مقارنة بنسبته في بذور كل من الترمس الأبيض (٣,٤٤٪) والحلبة (٦.٩٨٪).

- النشاط المضاد للأكسدة المعملي:

وقد تم بطريقتين (طريقه قياس القدره ألأختزاليه وطريقه إزاله الشق الحره DPPH) وقد أظهرت كلتا الطريقتين أن بذور الحلبة هي أعلاهم نشاطا حيث بلغت ٥٦,٢٢٥٪ بالنسبة للقدرة الاختزالية, ٨٢٪ بالنسبة لإزالة الشقوق الحرة يليها بذور الترمس الأصفر حيث كانت نسبته في الطريقتين ٥١,٤٪'٦٥٪ علي التوالي أما الترمس الأبيض فكان أقلهما حيث كانت نسبته في الطريقه الأولي ٤١,٠١٥٪ و ٤٨٪ في الطريقه الثانيه.

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