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RESEARCH ARTICLE

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SEED YIELD AND IMPORTANT SEED CONSTITUENTS FOR NATURALLY AND CULTIVATED MILK THISTLE (*SILYBUM MARIANUM*) PLANTS

ABSTRACT:

Milk thistle (*Silybum marianum*) is a wild plant grows as weed in the poor land on high way roads and as weeds in some crops in Egypt. The plant represents a problem for some farmers due to its flush grow and rich in thorns. Moreover, the plant occupies a large area of land. This work aimed to study physical and chemical properties as well the nitrogenous compounds content of the milk thistle seeds grow as a wild or cultivated in north Delta of Egypt. The obtained results revealed that milk thistle seed is a good source of lipids (29.68%), true protein (25.25%), total carbohydrates (38.16%) and crude fibre (29.95%). The seeds of wild plants acquired greater amounts of the lipids, crude fibers and less content of carbohydrates and proteins. The amino acids were similar in both wild and cultivated plants but their contents varied. The seed protein contained markedly amounts of essential amino acids such as lysine, isolucine, leucine, valine and threonine compared with sunflower seeds meal. But the seeds are poor in sulphur containing amino acids. Amino acid score identified threonine with the highest amino acid score 490 while phenylalanine + tyrosine as the first limiting amino acids followed by tryptophan as the second one.

KEY WORDS:

Milk Thistle, Sliymarin, Chemical Composition, Protein, Amino Acids

Note:

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INTRODUCTION:

There were one out of 10 people in developing countries are undernourished and suffered from malnutrition's. Even though the world’s poor, those who are already spend 60 to 80% of their income on food (FAO, 2012).

Food not only supplies human beings with nutritive components that are indispensable for maintaining a healthy daily life, but also contains many types of biofunctional components that help to maintain good physical and mental health (Pietta, 2000; Wu *et al.,* 2004).

Milk thistle (*Silybum marianum* L. Gaertn) is an annual or biennial plant belongs to family Asteraceae (Composite) and usually grows in dry, sunny areas. The plant native to Mediterranean area and now has spread to other warm and dry regions (Li *et al.,* 2012). The plant is usually found and widespread in Egypt on roadsides, waste ground and in cereal crops as a weed. Milk thistle has a

stem 40-200 cm high, glabrous or slightly downy, erect and branched in the upper part of plant (Montemurro *et al.*, 2007). The basal leaves are alternate, large and glabrous with spiny margins. The leaves can be 50-60 cm long and 20-30 cm wide (Gresta *et al*., 2007). Each steam ends in a flower head about 5 cm of diameter of red-purple or white colour (Montemurro *et al.*, 2007).

Milk thistle seed contains a relatively high amount of oil. Where, oil is the first by-product of silymarin production and has to be removed from seed (Li *et al.,* 2012). However, the seed flour is the secondary by-product and is discarded as waste. This flour may contain health beneficial components such as protein, carbohydrates especially crude fibres, minerals and some phytochemicals that have antioxidants or antimicrobial properties (Parry *et al.,* 2008).

One of the important issues that the plant may be accepted as a safe herbal product, since no health hazard or side effects till now is known (Montvale, 2000).

The seeds are rich in protein, lipids and total carbohydrates, whereas their concentrations in the seeds were flocculated from 19.1 to 30.0%, 20.0 to 30.0%, and 24.2 to 26.3%, respectively (Wichtl and Bisset, 1994; Abu Jadayil *et al.*, 1999; Khalil, 2008).

Most of the existing researches on milk thistle plant were deal only with pharmacological and medicinal studies due to production of Silymarin and its uses to heal some hepatic diseases. They do not pay any attention to gain any knowledgeable about its physical and chemical characteristics as well nutritional properties of the plant. Consequently, this work was carried out to throw some lights on the physical, chemical and nutritional characteristics of wild milk thistle seeds that collected from the high way road side near of Alexandria city, Egypt.

MATERIAL AND METHODS:

Milk thistle seeds were collected from Abees zone, Alexandria Governorate, Egypt. The seed were washed with tap water and finally with distilled water then dried. The seeds were milled by Brown Multi-quick blender (Germany) at speed 2 for 3 min to pass through 60 mesh sieve. The powder was kept into polyethylene bags and stored at -20°C in a deep freezer for further analysis (Atta and Imaizumi, 2002).

Physical properties of seed:

The seed dimensions (length, width and thickness mm), mass of 1000 seeds in grams (g), as well volume (cm^3) and bulk density (g/cm^3) of the seed were determined according to El-Raie *et al*. (2004).

Gross chemical composition:

Ether extract (official method no 935.38) using petroleum ether (60-80°C) in a Soxhlet apparatus, total nitrogen following kjeldahle method (official method no 950.36), ash content (official method no 930.22) in muffle furnace at 450 - 500°C and crude fibres of samples (official method no 950.37) were determined as described in AOAC (2000). Crude protein content was calculated by multiplying total nitrogen content by the factor 6.25. Non-protein nitrogen (NPN) was determined according to the method of Luman and Reyes (1982) while total amino acids content of milk thistle seed flour was determined using Eppendorff LC3000 amino acid analyzer AAA (Germany) following the method of Bailey (1967). Whereas, AA analysis conditions were flow rate 0.2 ml/min, pressure of buffer 0 to 50 bars, pressure of reagent 0 to 150 bars and reaction temperature 123°C. Chemical score of milk thistle seed flour was calculated according to FAO/WHO (1990) as follows:

$$\text{Chemical Score} = \frac{mg \text{ of essential amino acid in test protein}}{mg \text{ of essential amino acid reference protein}} \times 100$$

Statistical analysis:

Values in the results are represented as the mean and standard deviation (M ± SD) for three replicates calculated by Microsoft excel. Analysis of variance (ANOVA) was applied for evaluating the significant of variances due to the used treatments and the differences between means were further tested using the Dunc's squire multiple range. All the statistical methods were as outlined by SAS (2004).

RESULTS AND DISCUSSION:

Morphological characteristics of milk thistle leaves:

The photos in figure 1 reveals that whole plant of milk thistle acquires spiny leaves and the spines are sharp and long in the wilds plants while in the cultivated plant leaves have short spines and are more wider and not dissected greatly as those leaves of the willed plant.

Fig. 1. Photograph of the milk thistle leaf (cultivated plant left and wild one right)

Physical and Chemical characteristics of milk thistle seeds:

The results in table 1 and figure 2 reveal that whole plant of milk thistle could be carried about 20 to 50 flowers. Number of seeds in each flower was fluctuated from 125 (as minimum) to 170 (as maximum) seeds with an average of 150. The mean values of Milk thistle dimensions' seed were 6.9 ± 0.02mm in length, 3.0 ± 0.01mm width, 2.00 ± 0.01 mm thickness. However, the average of weight and volume of 1000 seeds were 25.23 ± 0.2 g and 35.66 ± 0.04 cm^3, respectively. Also, the mean of seed bulk density was 0.72 ± 0.04 g/cm^3.

Table 1. Some physical properties of milk thistle seeds

parameter	wild	cultivated
Flower/plant	35.00 ± 0.06	22.0 ± 0.06
Seed/flower	150.00 ± 0.033	80.0 ± 0.08
Seed length (mm)	6.90 ± 0.02	7.00 ± 0.03
Seed thickness (mm)	2.00 ± 0.01	1.90 ± 0.04
Seed width (mm)	3.00 ± 0.01	3.00 ± 0.08
Weight of 1000 seeds (g)	25.23 ± 0.20	27.4 ± 0.05
Volume of 1000 seeds (cm^3)	35.66 ± 0.04	35.8 ± 0.02
Bulk density (g/cm^3)	00.72 ± 0.04	0.77 ± 0.12

Milk thistle flower

milk thistle seeds

Fig 2. Milk thistle flower and seeds morphology

These results are at the base line of those reported by Sindel (1991), who reported that milk thistle plant have 10–50 flowers whereas each flower produces approximately 100 seeds, with an average of 3000 seeds per plant. Also, Perez *et al.* (2007) found that wild sunflower seed had a length of 4-6 mm, 1-3 mm width, 1-2 mm thickness and weight of 1000 seeds was ranged from 6.4 to 7.3 g with bulk density of 0.34-0.35 g/cm^3.

Chemical composition of milk thistle seeds:

Data of table 2 display that milk thistle seed is a rich source for protein, lipids and crude fibers. Since, crude protein, ether extract and crude fibers content are 27.54%, 29.68%, and 29.95%, respectively. Ash is 4.5% and total carbohydrate is 38.16%.

Table 2. Chemical composition of Milk thistle seeds (g/100 g based on dry weight bases)

Constitutes	wild	cultivated
Ether extract (lipids)	29.68 ± 0.15	28.53 ± 14
Ash	04.50 ± 0.10	03.25 ± 25
Crude fibers (CF)	29.95 ± 0.49	26.59 ± 32
Nitrogen free extract (NFE)	08.21 ± 0.69	09.23 ± 15
Total carbohydrates (TC)	38.16 ± 0.03	42.35 ± 21
Nitrogenous compounds		
Total nitrogen (TN)	04.41 ± 0.14	04.85 ± 0.24
Crude protein (CP) (N x 6.25)	27.54 ± 0.14	22.50 ± 0.15
Non protein nitrogen (NPN)	00.37 ± 0.03	00.22 ± 0.04
Protein nitrogen (PN)	04.04 ± 0.02	04.34 ± 0.02
True protein (TP) (N x 6.25)	25.25 ± 0.02	22.23 ± 0.02

M ± SD = means and standard division of triplicate trails.

These results are in agreement with those reported by Wichtl and Bisset (1994) who stated that milk thistle seeds contained 20-30% oil and 25-30% protein. Also, Wallace *et al.* (2005) and Abenavoli *et al.* (2010) reported that the plant seeds contained 15-30% lipids and about 30%

protein. Furthermore, these results are closed to outcomes of glop artichoke fruit which belongs to the Asteraceae family where it contained 25% oil and 18-20% protein (Perez *et al*., 2007). Similarly, Economides (1998) stated that sunflower seed meal contained 34.00% crude protein, 6.71% ash and 26.00% crude fibres. At the meantime, Beis *et al*. (2002) stated safflower seed contained 40.0% oil, 19.0% protein, 20.0% crude fibres and 2.2% ash. Also, table 2 indicates that nitrogenous components were 4.41% total nitrogen, 4.04% nitrogen protein (about 91.6% from total nitrogen) and 0.37% non-protein nitrogen. In contrast, these results are higher than those reported by Abu Jadayil *et al.* (1999) who pointed out that milk thistle seeds contained 19.1%, 26.3%, 25.4%, and 24.3% for Protein, crude fat, crude fibres and nitrogen-free extract, respectively. Wallace *et al.* (2005) reported that seeds of milk thistle contained 25% w/w of oil. Also, very low values for 1.5 crude fibers (1.5%), ash (0.7%) crude protein 22% were reported by Khalil (2008).

In conclusion, milk thistle seeds contain high amounts of protein, lipids and total carbohydrates including crud fibers which may could be used as a novel source of plant protein, oil and crude fibers. Also, it could be utilized as suitable food ingredient in low fiber containing food such as meat products**.**

Amino acids content of milk thistle seed protein:

The amino acids composition of milk thistle seeds is given in table 3. The results show that milk thistle protein considered a poor source of proline (0.37%) and histidine (1.44%). However, threonine is the higher (16.66%). Also data indicate that milk thistle contained high amounts of lysine, isolucine, leucine, valine, and threonine than that of sunflower seed meal. Taking up adequate essential amino acids are very important for health since they are building blocks of proteins, which carried functions of human body Zho (2007). Tryptophan is used for synthesis of neurotransmitter serotonin and relief depression. Tyrosine is for dopamine, norepinephrine and adrenalin synthesis. Isoleucine is necessary for the synthesis of hemoglobin in red blood cells. Leucine has beneficial effect for skin, bone and tissue wound healing and promotes growth hormone synthesis. Lysine and valine are essential for muscle proteins. All these essential amino acids can be found in plant foods (McDougall, 2002). Phenylalanine may be useful against depression and suppressing appetite.

Table 3. Amino acids composition of milk thistle seeds flour (as g amino acid/100 g protein) compared with sunflower meal

Type	Amino acids	Wild Milk thistle	Cultivated Milk thistle	Sunflower meal*
Indispensable amino acids	Lysine	5.18	5.18	1.06
	Isoleucine	4.41	4.40	1.66
	Leucine	6.64	4.64	2.38
	Phenylalanine	1.73	1.93	2.28
	Arginine	1.51	2.51	3.02
	Histidine	1.44	1.04	1.07
	Valine	4.03	2.03	1.89
	Threonine	16.66	1.66	1.43
Dispensable amino acids	Aspartic	7.53	6.53	3.14
	Serine	2.77	2.37	1.70
	Proline	0.37	0.30	3.25
	Glycine	20.53	20.50	2.51
	Tyrosin	1.48	1.18	0.96

* Sunflower meal according to Mérida *et al*. (2010)

The chemical scores:

The first effort to evaluate directly on the basis of their ability to meet amino acids requirements was chemical score procedure. The main object of the procedure is to calculate the percent of each amino acid in tested protein compared with the amount of standard protein, where egg protein were initially used as standard to evaluate food proteins. The amino acid that is in greatest deficit could then be identified and the amount, expressed as a percentage of egg protein, give the value for the chemical scores (Whitaker and Tannenbaum, 1977).

A high correlation was found between chemical scores determined by this way and values obtained by biological assays for protein quality (Hegsted, 1974). Data of table 4 indicates that milk thistle seed protein is a rich source of threonine with a chemical score of 490. It also contain a considerable score for each isoleucine, valine, and leucine. Also, it could be noticed that phenylalanine + tyrosine are the first limiting amino acid followed by tryptophan as a second one. Data cleared that sulphur containing amino acids could not be detected in the milk thistle protein.

Table 4. Chemical scores of milk thistle seeds compared with the amino acid scores pattern for the FAO/WHO/UNU (1985).

Amino acid	FAO/WHO/UNU	milk thistle	% Amino Acid Scores
Lysine	5.80	5.18	89.31
Isoleucine	2.80	4.41	157.5
Leucine	6.60	6.64	100.60
Phenylalanine + Histidine	6.30	3.21	50.95*
	1.90	1.44	75.78
Valine	3.50	4.03	115.14
Tryptophan	1.00	0.58	58.00**
Threonine	3.40	16.66	490
Methionine	2.20	Not detected	

Chemical score was calculated as a percentage of the FAO/WHO/UNU, 1985

*First limiting amino acids. **Second limiting amino acids.

REFERENCES:

Abenavoli L, Capasso R, Milic N and Capasso F. 2010. Milk thistle in liver diseases: past, present, future. Phytother. Res., 24(10): 1423-1432.

Abu Jadayil S, Tukan SK, Takruri HR. 1999. Bioavailability of iron from four different local food plants in Jordan. Plant Foods for Hum. Nutr., 54(4): 285-294.

AOAC. 2000. Association of Official Analytical Chemists, Official method of analysis 17th (Ed), Arlington Virginia, USA.

Atta MB, Imaizumi K. 2002. Some characteristics of crude oil extracted from Roselle (*Hibiscus sabdariffa* L.) seeds cultivated in Egypt. J. Oleo Sci., 51(7): 457-461.

Bailey JL. 1967. Techniques in Protein Chemistry. Elsevier Publishing Co., London, UK.

Beis SH, Onay Ö, Koçkar ÖM. 2002. Fixed-bed pyrolysis of safflower seed: influence of pyrolysis parameters on product yields and compositions. Renew. Energ., 26(1): 21–32.

Economides S. 1998. The nutritive value of sunflower meal and its effect on replacing cereal straw in the diets of lactating ewes and goats. Livest. Prod. Sci., 55(1): 89–97.

El-Raie AE, Hassan HE, Abd El-Rahman AA, Abd El-Hady YB. 2004. A laser optical method for measuring some physical properties and inference surface area equation of faba bean. Misr J. Ag. Eng., 4(1): 25-40.

FAO. 2012. World food situation. Available on the internet at: http://www.fao.org/worldfoodsituation/ wfs-home/ csdb/ en/).

FAO/WHO. 1990. Report of the Joint FAO/WHO Expert Consultation\ on Protein Quality Evaluation.

FAO/WHO/UNU. 1985. Energy and protein requirements, report of the joint FAO/WHO/UNU expert consultation. Technical report series No. 724. FAO, WHO and the United Nations University, Geneva, Switzerland.

Gresta F, Avola G, Guarnaccia P. 2007. Agronomic characterization of some spontaneous genotypes of milk thistle (*Silybuim marianum* L. Gaertn) in Mediterranean environment. J. Herbs Spices Med. Plants, 12(4): 51-60.

Hegsted DM. 1974. Assessment of protein quality. In: "Improvement of Protein Nutriture". Washington, DC: National Academy of Sciences, pp. 64–88.

Khalil JA. 2008. Biochemical studies on Milk thistle (*Silybum marianum*). M Sc., Thesis, Fac. Agric., Cairo Univ., Egypt.

Li F, Yang L, Zhao T, Zhao J, Zou Y, Zou Y, Wu X. 2012. Optimization of enzymatic pre-treatment for n-hexane extraction of oil from *Silybum marianum* seeds using response surface methodology. Food Bioprod. Process., 90(2): 87-94.

Luman BO, Reyes PS. 1982. Chemical composition studies on winged bean tubers. J. Food Sci., 47(3): 821-824.

McDougall J. 2002. Plant foods have a complete amino acid composition. Circulation, 105 (25): e197.

Mérida SN, Tomás-Vidal A, Martínez-Llorens S, Cerdá MJ. 2010. Sun flower meal as a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diets: Amino acid retention, gut and liver histology. Aquaculture 289: 275-281.

Montemurro P, Fracchiolla M, Lonigro A. 2007. Effects of some environmental factors on seed germination and spreading potential of *Silybum marianum* Gaertener. Ital. J. Agron., 2(3): 315-320.

Montvale NJ. 2000. Milk thistle (*Silybum marianum*) in PDR for herbal medicines. Med. Econ. Comp., pp. 516-520.

Parry JW, Cheng Z, Moore J, Yu LL. 2008. Fatty acid composition, antioxidant and antiproliferative capacity of selected cold-pressed seed flours. J. Am. Oil Chem. Soc., 85(5): 457-464.

Perez EE, Crapiste GH, Carelli AA. 2007. Some physical and morphological properties of wild sunflower seeds. Biosyst. Eng., 96(1): 41- 45.

Pietta P. 2000. Flavonoids as antioxidants. J. Nat. Prod., 63(7): 1035-1042.

SAS. 2004. SAS users Guide, Version 9.1 Statistical Analysis Systems Institute, Cary, NC.

Sindel BM. 1991. A review of the ecology and control of thistles in Australia. Weed Res. 31(4): 189-201.

Wallace S, Carrier DJ, Clausen E. 2005. Batch extraction of flavolignans from Milk thistle (*Silybum marianum* (L) Gaertner). Phytochem. Anal., 16(1): 7-16.

Whitaker JR, Tannebaum SR. 1977. Food proteins. The Avi publishing company, Inc. Connecticut, U.S.A.

Wichtl M, Bisset NG. 1994. Herbal drugs and phytopharmaceuticals. Stuttgart: med. pharm. sci. publishes.

Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United states. J. Agric. Food Chem., 52(12): 4026-4037.

Zho J. 2007. Nutraceuticas, nutritional therapy, phytonutrients and phytotherapy for improvement of human health: a perspective on plant biotechnology application. Recent pat. Biotechnol., 1(1): 74-97.

إنتاجية البذور ومركبات البذور المهمه للنباتات البرية والمزروعة من نبات شوك الجمل

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نبات شوك الجمل نبات ينمو بريا كحشيشة على جوانب الطرق والترع والمصارف وفى بعض الحقول فى مصر. ويمثل النبات مشكله للفلاحين لسرعة نموه وتكوين مستعمرات تشمل مساحات واسعة من الأرض. وتهدف الدراسة الى دراسة الخواص الفيزيائية والكيميائية وكذلك المركبات النتروجينية لبذورنبات شوك المنتشر بريا والمنزرع بمنطقة دلتا النيل بمصر. وقد أظهرت نتائج الدراسة ان نبات شوك الجمل مصدر جيد للبيدات (29.68%) والبروتينات الحقيقية (25.25%) والكربوهيدرات الكليه (38.16%) والألياف الخام (29.95%). وقد وجدت تلك المركبات بكميات أكبر فى النباتات البريه عنها فى المزروعة ماعدا الكربوهيدرات الكلية والبروتينات التى كانت أقل بها. وكانت نوعية الأحماض الأمينية متماثله فى النباتات البرية والمزروعة ولكن اختلفت فى كمياتها. احتوت بروتينات بذور النبات على الأحماض الأمينية الأساسية بكميات عالية مثل الليسين والأيزوليسن واللبوسين والفالين والثريونين . ولكن كانت بروتينات بذورالنبات فقيرة فى الأحماض الأمينية الكبريتية . وقد سجل للثريونين اعلى معدل امينى 490 فيما سجلت للفنيل الآنين + التيروسين اول الأحماض الأمينية المحددة تلاها التريبتوفان فى المرتبة الثانية.