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Characteristics of *Silybum marianum* as a Potential Source of Dietary Oil and Protein

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Abstract. Seeds of *Silybum marianum* (milk thistle) plant were collected from Peshawar valley and evaluated. Study revealed 26.65% ± 0.25 fixed oil and 23.0% ± 0.38 protein. The oil extracted has linoleic acid (64.59%), oleic acid (23.59%), palmitic acid (12.62%), and stearic acid (5.9%) as major fatty acids. Proximate principals, minerals and oil quality indices were determined. The carbohydrate content is quite high (37.72 ± 1.64%), while it has low crude fibre contents (4.55 ± 0.23%). The seed oil shows the following physicochemical characteristics: 13.67 ± 0.23 meq/kg of peroxide value; 194.78 ± 0.23 of saponification value; 103.89 ± 0.56 g/100 g of iodine value and acid value of 1.93 ± 0.34%. The unsaponifiable matter (UNS) of the oil was 2.36% ± 0.45. The results of nutritionally valuable minerals indicates that seeds contains Mg 2,225 ppm, Ca 778.5 ppm, Cu 108.3 ppm, Fe 74.3ppm, Zn 69.4 ppm, Pb 44.3 ppm, Ni 35.5 ppm, Mn 23.5 ppm, Cr 6.8 ppm and Cd 3.2 ppm was found in lowest concentration. The qualitative analysis by thin layer chromatographic (TLC) technique for the protein hydrolyzate revealed ten amino acids. Among these aspartic acid, glycine, glutamic acid and cysteine were the major amino acids. Results of this preliminary investigation indicated that the oil might be edible as inferred from its chemical composition and fatty acid profile.

Keywords: *Silybum marianum*, protein, fixed oil, fatty acid, mineral elements, physicochemical properties

Introduction

Pakistan is a heavily populated country, facing a serious shortage of edible oils for the past several years. Population growth, economic progress and urbanization lead to an increase in the consumption of oils and fats. Domestic production meets 30% of its requirements, while, 70% is imported. The average annual consumption of edible oil in Pakistan during last quarter of the 20^{th} century has increased more than six folds i.e. from 0.3 million tons to 1.8 million tons. Population growth, economic progress and urbanization lead to an increase in this consumption. Oil seed crops grown in Pakistan are generally classified as conventional and non-conventional. The Government of Pakistan has established the "Pakistan Oilseed Development Board (PODB)", which is trying to replace imported oil with the local product for about a decade.

Fats and oil are indispensable food factors (Tooley, 1971). As dietary components they play an important nutritional role by serving as a concentrated source of energy, providing essential fatty acids (EFA), acting as carrier of fat-soluble vitamins, imparting flavour and taste to foods and fulfilling the requirements for normal functions of the body (Mason, 1981; Triebold and Aurand, 1963). Currently, despite the relatively high oil and seed meal production in the United States, USDA (US Department of Agriculture) continues to investigate the non-conventional seeds. The example set forth by USDA, is worth emulating by the developing countries that are more in need of alternative oil sources (Ajayi *et al.*, 2004). According to Balogun *et al.* (1990), there is lack of information on the composition and utilization of the many varied oil seed indigenous to the tropic.There exist already abundant data in literature, on the proximate composition, mineral contents and other characteristics of the more conventional oil seeds but this is lacking on the non-conventional oil seeds.

The seeds of different species of *Silybum marianum* yield good oil, which contains 26-39% fixed oil, composed of unsaturated fatty acid such as oleic 23-47%, linolenic 9-58%, and saturated fatty acid, as palmitic 12-34%, stearic 6-15%. Other constituents of oil are mono, di and triglycerides and free fatty acids .The defatted seeds contain an excellent quality and quantity of protein, ranging from 20-24%. The qualitative analysis of protein hydrolyzate revealed ten amino acids. Among these aspartic acid, glycine glutamic acid, lysine, and cystein were the major amino acids. *Silybum marianum* (milk thistle) is an annual or biennial herb that belongs to the family Acteraceae. The seeds, fruit, and leaves of milk thistle (*Silybum marianum*), a plant native of Europe, have been in use since the Roman period, as a liver tonic, excellent food for cattle and horses. Apart from its use as nutritional edible oil, the standardized extract of silymarin could be used in supportive therapy of liver diseases. It has been proven that

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silymarin prevents the development of dietary induced hypercholesterolemia in rats (Krecman *et al.*, 1998), and both silymarin and silibinin possess LDL antioxidant activity *in vitro* (Skottova *et al.*, 1999). However, *in vivo* effects of silibinin are limited by its low bioavailability, which can be improved by complex formation of silibinin with phosphatidylcholine (Morazzoni *et al.*, 1993). Milk thistle fruits contain a mixture of flavonolignans, a large group naturally occurring antioxidant polyphenols. They have received considerable attention during the last decade (Lairon and Amiot 1999) In humans the flavonoids contribute greatly to the dietary sources of antioxidants, and their intake decreases the risk of coronary heart disease (Keli *et al.*, 1996; Knekt *et al.*, 1996; Hertog *et al.*, 1993). Silibin, one of silymarin constituents, could decrease synthesis of cholesterol in liver, by inhibition of 3-hydroxy-3-methyle glutaryl coenzyme A (HMG-Co A) reductase, a key enzyme of cholesterol synthesis (Nassuato *et al.*, 1991). Dietary polyunsaturated fatty acids (PUFA), particularly PUFA of the *n*-3 sub-category, have been shown to positively effect atherogenic indices of vascular functions, and lipid metabolism such as cholesterol and triglycerols (TAG) lipoprotein profile, in the plasma (Harper and Jacobson, 2001; Nestel, 1998).

An acute shortage of traditional edible oil in Pakistan, has created considerable interest in developing and exploring new sources of oils and fats in evaluating their nutritional properties, to establish their suitability for edible purposes. In response to these needs, the present study was focused to some non-conventional sources of dietary oils. *Silybum marianum* seems to be a potential source of edible oil and protein. Therefore, present study was conducted on chemical and nutritional properties of *Silybum marianum* (milk thistle), grown in Peshawar, north west frontier of Pakistan (NWFP).

Materials and Methods

Extraction of oil. The mature fruits of *Si!ybum marianum* (L.). Gaertn (wild grown), were collected from different localities of Peshawar. Seeds were separated by mechanical thresher, while the sunflower seeds were obtained from Agriculture Research Institute Tarnab, Peshawar, for comparative study. The air-dried seed were mechanically crushed and its oil contents were extracted in the soxhlet apparatus using *n*-hexane as solvent. The oil obtained, after distilling of the hexane, was stored in amber glass bottles at room temperature.

Proximate composition of seed. Seed moisture was determined in an oven at 105 °C for 6 h according to Femenia *et al.* (1995). Crude protein (N × 6.25) contents of defatted seeds was estimated by micro Kjeldhal method, while analysis for ash and crude fiber were according to the methods of AOAC (1980). Carbohydrate contents was determined by difference [100% - (protein + crude fiber + ash) (AOAC, 1990)]. The unsaponifiable matters (UNS), were determined as deseribed by Fedeli *et al.* (1966), using gas liquid chromatography sigma 3B equipped with dual flame ionization detector.Trace elements of the seeds were studied by atomic absorption spectrophotometer according the method of Idouraine *et al.* (1996).

Physicochemical characteristics of seed oil. Peroxide value (POV), free fatty acid (FFA), iodine value (Wijs method), and saponification value, were estimated according to AOAC (1985), whereas β-carotene was determined according to methods of AOVC (1966). The oils were converted into corresponding methyl esters (by esterification), prior to the gas chromatographic analysis. The esterification of all the oils was performed according to the method of Lutz *et al.* (1998). Total amino acids were extracted from the defatted samples using 6N HCl according to Gehrke *et al.* (1985). The protein hydrolysate was monitered to their amino acid constituents by TLC (thin layer chromatography) techniques. The stationary phase was silica gel plates, and the mobile phase system was *n*-bentanol: acetic acid: H_2O (4: 1: 5 v/v/v). The visualization was achieved using ninhydrin (0.2 g/100 ml acetone). Rf values were recorded for every amino acid.

Results and Discussion

The results of the proximate composition of *Silybum marianum* seed are presented in Table 1. The seeds rendered 26.65% ± 0.25 fixed oil, while the protein content of oil-free meal was 23.0% ± 0.38. The results were in agreement with the findings of El- Tahawi *et al.* (1987) and Rizk *et al.* (1970). The protein content of the seed was higher than those of known oil seeds like sunflower (19.50 %), and castor seed (18.90%) reported by Fetuga *et al.* (1974). The protein content in seed was higher than in many commonly consumed oil seeds. Low food production coupled with poor quality of protein is creating a protein gap of alarming proportion (Shah, 1984; UN, 1974). Shortage of animal protein can be replaced

Table 1. Proximate composition of *Silybum marianum* seeds

Parameters*	Mean ± SD
Crude Moisture (%)	4.48 ± 0.24
Protein (%)	23.0 ± 0.38
Fat (%)	26.65 ± 0.25
Ash (%)	3.4 ± 0.15
Fiber (%)	4.55 ± 0.23
Carbohydrate contents (NFE%)	37.72 ± 1.50

* = values are average of three determinations

by utilization of vegetable protein as an alternative source (Pirie, 1976). The oil yield also compares favourably with the oil contents reported for sunflower seeds (22-36%).The seed has low quantity of moisture (4.48% ± 0.24, which was similar to the value reported by Al-Khalifa, 1996). Carbohydrate content of the seed was 37.72% ± 1.50.

The pale yellow oil of test seeds was consistently liquid at room temperature. The physicochemical constants of the oil are presented in Table-2.The acid value of the oil was 1.93 ± 0.75 which was similar to the value reported by Shaban *et al.* (1995) for *Silybum* seed oil ranging (1.26-2.00%). Iodine value gave an idea about the degree of unsaturation. Sunflower oil had higher iodine values (116.7 g/100 g), as compared to *Silybum marianum* oil (103.89 g/100 g). The saponification value was 194.78, which was close to sunflower oil. The unsaponifiable matter was 2.36% ± 0.45.

The role of trace elements in human nutrition and disease cannot be overemphasized. Even though the mineral elements, form a small portion of total composition of most plant materials and of total body weight are not contribute to the energy value of food. They are of great physiological importance, particularly in body metabolism (Schwart, 1975). The results (Table 3) indicate that the seeds of test plant contains Mg 2,225 ppm, Ca 778.5 ppm, Cu 108.3 ppm, Fe 74.3 ppm, Zn 69.4 ppm, Pb 44.3 ppm, Ni 35.5 ppm, Mn 23.5 ppm, Cr 6.8 ppm and the Cd 3.2 ppm showing the lowest concentration, that can be deduced the seeds of *Silybum marianum*, if consumed, could be a good source of minerals.

Rf values of amino acids are presented in Table 4. All samples contain cystein, lysine, aspartic acid, glysine, tyrosine, serine,glutamic acid, thereonine and leucine with Rf values of 0.05 ,0.09, 0.15, 0.18, 0.20, 0.24, 0.28, 0.53 and 0.64, respectively. These findings are similar to the results of Shaban *et al.* (1995) Verma *et al.* (1980).

The fatty acid composition of SMO in comparison to SFO is presented in Table 5 and Fig.1. The fatty acid composition of the seed oil revealed that linoleic acid (64.59%) and oleic acid (23.59%) are the predominant fatty acids. The linoleic and oleic acid contents are similar to that in sunflower oil. Therefore, the importance of SMO can be realized as compared to other conventional edible oils. Results indicated that *Silybum marianum* oil is fairly high in polyunsaturated fatty acids, particularly in linoleic acid. High proportion of mono and polyunsaturated fatty acids is desirable in food, while saturated fatty acids are cholesterol raising nutrients.

Grundy (1997), stated that substitution of dietary saturated fats by oils, containing high amounts of polyunsaturated fatty acids has beneficial effects on hypercholesterolemia. It is well known that dietary fats rich in linoleic acid prevent disorders such as coronary heart disease, atherosclerosis, and high blood pressure. Linoleic acid derivatives also serve as structural components of the plasma membrane and as precursor of some metabolic regulatory components (Viles and Gottenbos, 1989). The other fatty acid found in SMO is palmitic acid 12.62% and stearic acid 5.9%. The fatty acid

Table 2. Physicochemical constants of *Silybum marianum* oil (SMO) and sunflower oil (SFO)

Parameters*	SMO	SFO
Peroxide value (meq/kg)	13.67.1 ± 0.23	18.6 ± 0.24
Acid value (%)	1.93 ± 0.75	8.3 ± 0.15
Iodine value g/100 g	103.89 ± 0.56	116.7 ± 0.25
Saponification value	194.78 ± 0.23	194.57 ± 0.23
Anisidine value	1.41 ± 0.21	1.8979 ± 1.50
Colour (OD)	0.341 ± 0.35	0.279 ± 1.50
β-Carotene (ppm)	18.76 ± 0.32	13.49 ± 0.38

* = values are average of three determinations

Table 3. Mineral element composition of *Silybum marianum* seeds

Element*	Mean ± S.D (ppm)
Mg	2,225 ± 1.50
Ca	778.5 ± 0.38
Cu	108.3 ± 0.23
Fe	74.3 ± 0.25
Zn	69.4 ± 1.50
Lead	44.3 ± 0.23
Ni	35.5 ± 0.15
Mn	23.5 ± 0.24
Cr	6.8 ± 1.50
Cd	3.2 ± 0.38

* = values are average of three determinations

Table - 4 Amino acid composition of *Silybum marianum*

Amino acid	Rf value
Cystein	0.05
Lysine	0.09
Aspartic acid	0.15
Glycine	0.18
Tyrosine	0.20
Serine	0.24
Glutamic acid	0.28
Thereonine	0.53
Leucine	0.64

Table 5. Fatty acid profile of *Silybum marianum* oil (SMO) / sunflower oil (SFO)

Fatty acids	% Composition	
	SMO	SFO
$C_{16\text{-}0}$ Palmitic	12.62	7.23
$C_{18\text{-}0}$ Stearic	5.9	2.55
$C_{18\text{-}1}$ Oleic	23.59	23.25
$C_{18\text{-}2}$ Linoleic	64.59	66.44
Saturated	18.52	9.78
Unsaturated	88.18	89.69
TU/TS*	4.76	9.17

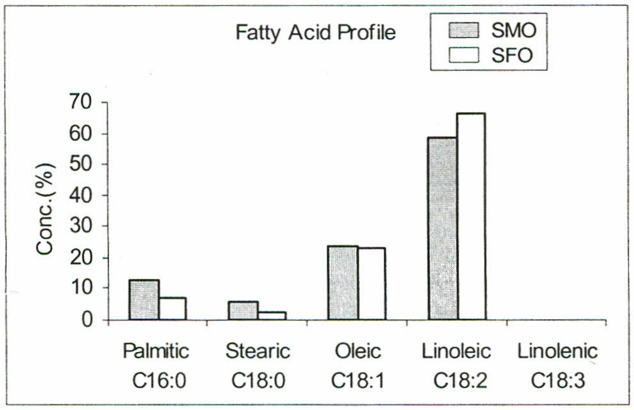

Fig 1. Fatty acid profile of SMO/SFO.

composition is comparable to that of any other edible oil. Some earlier researchers (Bandopadhyay *et al.,* 1972) found similar results regarding fatty acid composition of *Silybum marianum* seed oil. The percentage of saturated fatty acid in the oil is 18.52%, while the percentage of the unsaturated fatty acid is 88.18%, which is of great nutritional significance, and could serve nutritional purposes. It can be used to lower serum cholesterol, and prevent coronary heart disease. The SFO exhibited higher 18:2/18:1 and higher total unsaturated fatty acids to saturated ones (TU/TS) ratio as compared to SMO. These results indicate that the oil compares favourably with sunflower oil.

Conclusion

The results of the present preliminary study on *Silybum marianum* oil, indicated that the seed oil may be edible, as inferred from its chemical composition and fatty acid profile. It appears to be a good source of linoleic acid. The oil has high content of unsaturated fatty acids and therefore, could be served as substitute for highly unsaturated fatty oils. However, refining and long-term nutritional studies are necessary to establish its safety, before it can be recommended as edible oil for human consumption. The defatted seed contains an excellent quality and quantity of protein and dietary fibers, which could be used in animal feeds.

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