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Properties of the seed oil of a dwarf cultivar of the pharmaceutical silymarin producing plant *Silybum marianum* (L.) Gaertn. developed in India

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Seeds of *Silybum marianum* cv SMB-5, an Indian cultivar of dwarf habit and high seed yield, were characterized for their oil content and was examined for its physico-biochemical properties. The seed oil content was low at 20±2 %. The oil had acid value of 1.5, specific gravity at 27 ^{0}C was 0.885, saponification value as 199 and iodine value equal to 97. The main fatty acid composition was: palmitic acid, 9.6 %; stearic acid, 4.7 %; oleic acid, 3.9 %; linoleic acid, 45.8 % and linolenic acid, 5.3 %. The properties of the oil of Indian genotype largely correspond with those of *S. marianum* oil of Canadian, Greek, Egytian, Iraqi, Iranian and Pakistani genotypes. In comparison to the commercial oils of soybean, Indian mustard, canola, groundnut, sunflower, safflower and flex, the fatty acid compositional properties of *S. marianum* oil were found to be unique being rich in linoleic acid.

Keywords: Linoleic acid - rich oil, Milk thistle, Oil quality, Seed oil content, Silybum oil, Vegetable oil.

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Introduction

Silybum marianum (L.) Gaertn., common name – milk thistle, of the family Asteraceae is a herbaceous annual species of considerable medicinal importance[1,2]. Having origin in Mediterranian regions of North Africa, Middle East and Europe, the species is now found distributed as a weed world wide[3-5]. All parts of the plants are edible. Germinated seeds raised as sprouts, young fleshy stems and leaves have been in use as antioxidant rich salad[6]. Seeds of the plant are known to be used in traditional medicine for more than 2000 years for the treatment of liver and gall bladder ailments and to protect liver from poisoning by toxins, such as from alcohol, toxic mushrooms, insect stings and snake bites[7-9]. The medicinal properties in *S. marianum* are mainly related to the silymarin flavonolignans, principal one among them is silybin and others are isosilybin, dehydrosilybin, silychristin and silydianin[8,9]. Although present in all parts of the plant, silymarin is richly contained in seeds[8-10]. The preparations of silymarin/silybin are prescribed for use in the treatment and prevention of cirrhosis, hepatitis and other liver diseases associated with consumption of poisonous substances (including alcohol), nephropathy, cardio-pulmonary and skin related problems. Silymarin is also finding use against a variety of cancers and neurodegenerative diseases such as multiple sclerosis, Parkinson's and Alzheimer's diseases[8,9,11-16]. Silymarin is widely used in veterinary medicine as a liver disease protectant[7,8,17]. *S. marianum* seeds and silybin comprise a very active area of physiological, pharmaceutical and clinical research.

S. marianum being a weed plant, its seeds for the extraction of silymarin are collected from the wild. In recent years, with increase in demand for silymarin, certain *S. marianum* accessions have begun to be cultivated in several countries in America, Europe, Middle East and South Asia and in Australia[6,18-25]. Silymarin is extracted from its seeds after the fatty oil has been extracted from them[10]. Various cultivated accession have been reported to contain oil to the extent of 17 to 31 %[Ref. 9,21-25]. Therefore, seed oil is the second important product from this plant. The oils present in different accessions have been found to

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have similar fatty acid composition: linoleic acid > oleic acid > palmitic acid > stearic acid[19,21-25]. It can be used variously and is reported to be edible and suitable for cooking. Research on *S. marianum* to uncover its various morphological, physiological, agronomic and economic properties is progressively growing[26].

In India, *S. marianum* is found growing along road and rail sides and in wheat fields during autumn to early summer. Seeds collected from the wild habitat are used for silymarin production in India[27,28]. Indian agroclimate appears to be suitable for its productive cultivation in marginal and rainfed areas. Towards the domestication of indigenous *S. marianum*, the genetic resources collected from different geographical locations of Indo-Gangetic plains area were phenotyped for seed yield traits and several of them field tested. This work has led to development of a suitable preliminary agrotechnology. In continuation of this work, seed oil of a dwarf cultivar called SMB-5, which demonstrated potential for yielding 3.3 tonnes of seeds/ha was evaluated for its quality. The results of this study wherein physico-biochemical properties of the oil were characterized are described in this report. The oil present in about 20±2 % concentration in seeds demonstrated unique properties similar to different vegetable oils.

Materials and Methods

To assess the quality of seed oil in milk thistle of Indian origin, the genotype SMB-5 was used. The genotype had origin in the accession collected from Hoshiarpur, a foot hill region in the state of Punjab. SMB-5 was isolated as a true breeding dwarf genotype and following its characterization the strain has been called as *S. marianum* cv SMB-5. The genotype bears purple coloured capitula of relatively small size with low seed yield. However, because of smaller size of plant, thick sowing of the genotype gave highest yield of 3.3 tonnes/ha among the various Indian accessions that were field tested at Lucknow and New Delhi. The silymarin content in the seeds was found to be 2.7 %. The genotype is depicted in Plate 1. The above mentioned seed yield of SMB-5 crop is perhaps the best among the genotypes studied in different countries or the present work. A crop of *S. marianum* cv SMB-5 was taken from the CIMAP farm in Lucknow. The seeds were sown on 15 October in lines 20 cm apart, keeping plant to plant distance at 20 cm, accommodating about 2,50,000 plants/hectare. The crop was harvested on 15 April. Seeds were separated from chaff, crushed and subjected to oil extraction in petroleum ether, using soxhlet apparatus, as described by Bajpai *et al*, 1999[Ref. 9]. The fatty acid methyl esters were prepared using the procedure of BIS[30]. Their presence was quantified using gas chromatograph (GC) Hewlett Packard, 6890 equipped with a flame ionization detector fitted with DB 225, 30m × 0.25 mm ID, 0.25um film capillary column. The GC was programmed at temperature of 90 °C (5 min) to 130 °C at 3 °C/min and 130 °C (12 min) to 230 °C at 2 °C/min, split 50;1, injector vol 2µL and nitrogen as carrier gas was used with flow rate 30 mL/min. The injector and detector temperatures were 230 and 260 °C, respectively. The physical and chemical tests of the fatty oil were carried out using standard procedures of BIS[31].

Plate 1—a) Seeds of milk thistle (black coloured achenes), (b) An inflorescence (Capitulam), (c) A view of the field grown crop

Results

The oil was solvent extracted from bulk seeds which bore seed coat but were free of other kinds of chaff. The moisture content of the seeds was 4.2 %. Yellow coloured oil was obtained in 20±2 % concentration on the whole seed basis. The oil remained liquid at room temperature. The specific gravity, refractive index, acid value, saponification value and iodine value of the oil were 0.89, 1.46, 25.83, 199 and 97, respectively (Table 1, present study). The GC analysis (Fig 1 and Table 2) of the oil showed the presence of palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid in 9.6, 4.7, 30.9, 45.8 and 5.2 % concentration. Behemic acid was identified as one of the minor fatty acids; it was present at 2.4 % concentration.

Discussion

The seed oil content and quality characteristics of the *S. marianum* Indian cultivar SBM-5 as compared to these properties described for the genotypes of the species cultivated in Canada[25], Greece[23], Egypt[19], Iraq[32], Iran[22,33] and Pakistan[21] are briefly described below.

The seed oil content of the genotype SBM-5 was observed to be 20±2 %, which is lower than 31 % reported for an Iranian genotype. The Indian accessions have been observed to possess oil content in their seeds in the range of 18-22 % (Unpublished observations). There is need for genetics based selection breeding in *S. marianum* to improve seed yield, silymarin and oil contents. Seeds of several of the oil crops contain 40-45 % oil (Table 1).

Fatty acid composition-wise the SMB-5 oil was nearest to the oil of Iranian accessions. These oils contained Behemic acid, but in Indian oil arachidic acid was not present (Table 2). In the oils of accessions examined in seven countries, more than 90 % of the fatty acid composition conformed with the already reported feature of the *S. marianum* oil: linoleic acid (range 45.4-66.4; mean 53.8) > oleic acid (range 21.3-31.6; mean 26.6) > palmitic acid (range 7.3 - 12.0; mean 9.2) > stearic acid (range 2.0-6.8; mean 5.0) (Table 2).

The oil had specific gravity marginally lower than that of soybean, mustard, canola, groundnut, sunflower and safflower oils and much lower than that of flax oil. Its saponification value was marginally higher than that in soybean, canola, groundnut, sunflower, safflower and flax oils, but much higher than that of the mustard oil. The *S. marianum* oil shared its iodine value with that of mustard and groundnut oils. Its iodine value, however, was considerably lower than that of soybean, sunflower and safflower oils and much lower than that of flax oil. Although, sunflower, safflower and *S. marianum* plants belong to the same family Asteraceae and sunflower and safflower oils

Table 1—Physical properties of the seed oil of milk thistle *Silybum marianum* cv SMB-5, as compared to those of some commonly used edible oils

Oil seed crop	% content of oil in seeds	Oil quality characters[e]		
		Specific gravity (temperature ˚C)	Saponification value	Iodine value
Soybean[a] (*Glycine max*)	15 - 20	0.916 - 0.922 (25)	189 - 195	128 - 134
Indian mustard[a] (*Brassica juncea*)	33 - 45	0.906 - 0.910 (25)	169 - 176	96 - 112
Rape seed[a] (Canola, *B. napus*)	40 - 44	0.916 - 0.921 (25)	182 - 198	110 - 126
Groundnut[a] (*Arachis hypogaea*)	45 - 55	0.910 - 0.915 (25)	188 - 195	84 - 100
Sunflower[a] (*Helianthus annus*)	25 - 35	0.915 - 0.919 (25)	188 - 194	125 - 140
Safflower[a] (*Carthamus tinctorius*)	25 - 37	0.919 - 0.924 (25)	188 - 194	128 - 134
Flax[a] (*Linum usitatissmum*)	40 - 44	0.931 - 0.938 (15)	189 - 196	170 - 180
Milk thistle (*Silybum marianum*[c], SMB-5)	18 - 22	0.885 (27)	199[d]	97 [d]

a = 35; b= 36 and 37; 38; c = present study; d= The iodine value and saponification values of *S. marianum* oils reported from Pakistan were, respectively 109 and 191 (21); e = IS: 548 (Part I)-1964

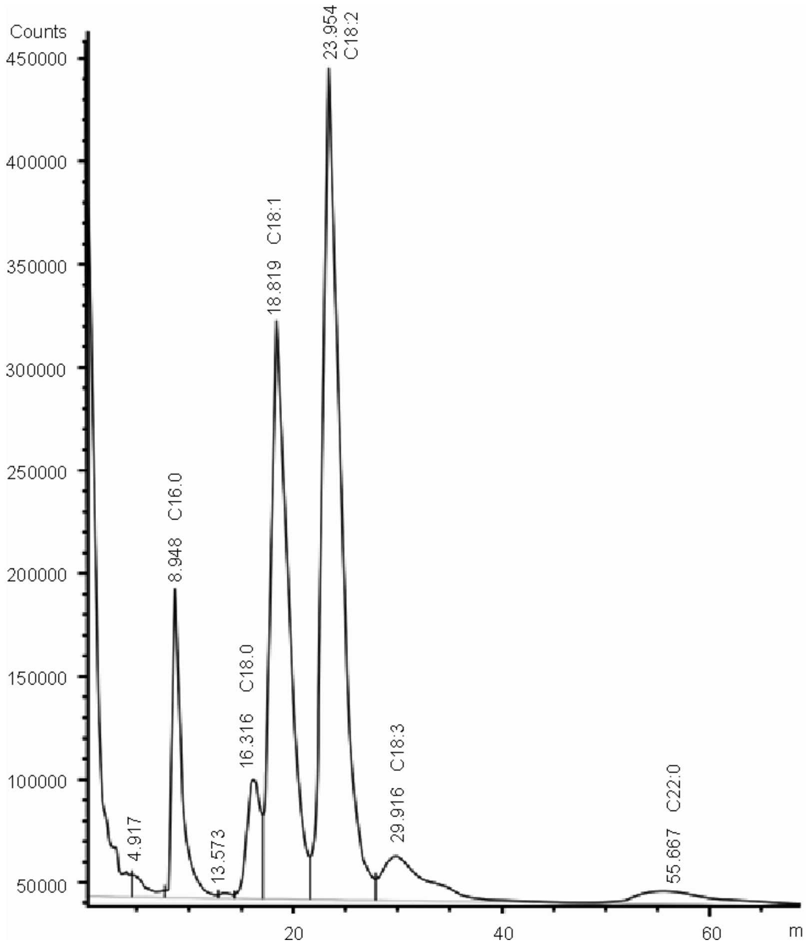

Fig. 1—Gas chromatography/ flame ionization detector (GC/FID) chromatogram of fatty oil obtained from seeds of milk thistle, *Silybum marianum*.

Table 2—Fatty acid composition of the seed oil of *Silybum marianum*, as compared to that reported for oils of the species and accessions grown in different geographical areas.

Fatty acid	Concentration observed/reported in the							Concentration Mean ± SE
	cv SMB-5, present study[a]	Canada material[b]	Greece material[c]	Egypt material[d]	Iraqi material[e]	Iranian material[b]	Pakistan material[g]	
C 16:0 Palmitic acid	9.6	9.0	NR	9.4	12.0	7.3 - 8.4 (7.8)[h]	7.2	9.2±0.7
C 18:0 Stearic acid	4.7	5.0	NR	6.6	6.1	4.6 - 6.8 (5.7)	2.0	5.0±0.5
C 18:1 Oleic acid	30.9	23.0	NR	21.3	30.5	22.8 - 31.6 (27.2)	26.4	26.6±1.1
C 18:2 Linoleic acid	45.8	57.0	50.6-66.4 (58.5)	53.3	48.3	45.4 - 53.4 (49.5)	64.4	53.8±1.9
C 18:3 Linolenic acid	5.2	NR	NR	NR	2.4	0.2 - 0.9 (0.5)	NR	2.7±0.9
C 20:0 Arachidic acid	NP[i]	NR	NR	NR	NR[j]	2.9 - 4.3 (3.6)	NR	3.6±0.0
C 22:0 Behemic acid	2.4	NR	NR	NR	NR[e]	2.3 - 2.9 (2.6)	NR	2.5±0.1
Total	98.6	94.0	NR	90.6	99.3	99.6	100.0	103.4

a = Oil content 20 %; b = Oil content 20-27 % (25); c = Oil content 17.5-21.6 % (23); d = Oil content 20% (19); e = oil content 28-30 % (32); f = Oil content 25 % (22, 33; g = (21); h = value in parentheses is mean; i = NP, not present; j= NR, Not reported.

Table 3—Fatty acid composition of the seed oil of Silybum marianum, as compared to that of some commonly used edible oils of low saturated fatty acids content

Oil seed crop	Fatty acid composition (%)						
	Palmitic (C 16:0)	Stearic (C 18:0)	Oleic (C 18:1)	Linoleic (C 18:2)	Linolenic (C 18:3)	Arachidic (C 20:0)	Other(s)
Soybean[a]	7 - 11	2 - 6	22 - 34	5 - 11	43 - 56	---	---
Indian mustard (*Brassica juncea*)[a]	4 - 8	2 - 5	13 - 23	13 - 40	6 - 10	---	30 – 51[d]
Rape seed[b] (Canola)	4 - 6	1 - 2	50 - 65	20 - 34	7 - 10	---	1 - 2[e]
Groundnut[a]	6 - 9	3 - 6	52 - 60	---	13 - 27	2 - 4	1 - 3[f] 1 - 3[g]
Sunflower[a]	3 - 6	1 - 3	14 - 35	---	44 - 75	0.6 - 4.0	0.8[h]
Safflower[a]	3 - 6	1 - 4	13 - 21	t[i]	73 - 79	0. 2	---
Flax[a]	4 - 7	2 - 5	12 - 34	35 - 60	17 - 24	0.3 - 1.0	---
Milk thistle (*Silybum marianum*)[c]	8 - 12	4 - 7	30 - 32	45 - 49	<1 - 6	0 - 4	0 - 3

a = 35; b = 39-41; c = Table 2; d = erucic acid (42, 43); e = C 20:1, eicosenoic acid; ef= lignoceric acid; g= C 22:0, behemic acid; h = C 22:0, behemic acid; i= t, trace amount (<0.1%).

have similar physical properties, yet the oil of *S. marianum* differed in its physical properties from both sunflower and safflower oils. Clearly, the silybum oil possessed unique combination of properties as compared to the edible oils listed in the Table 1.

One, two or three fatty acids make the bulk of the fatty acid content of the edible oils listed in Table 3. Oleic acid (31 %) and linoleic acid (47 %) together accounted for about 78 % of the fatty acid content in *S. marianum* oil. None of the other major edible oils including soybean, mustard, canola, groundnut, sunflower, safflower and flax shared this property with *S. marianum* oil. In this regard, the nearest oils to *S. marianum* oil are rape seed (canola) and flax oils where oleic acid and linoleic acid make 70-85 %, except that the flax oil has linolenic acid in high concentration (~20 %) which is a minor constituent in Silybum (< 6 %) and canola oils (< 10 %). Another difference between the *S. marianum*, flax and rape seed (Canola) oils is that whereas flax and *S. marianum* oils have more linoleic acid than oleic acid, the rape seed oils have more of oleic acid than linoleic acid. In comparison soybean oil is rich in linolenic acid, lesser so in oleic acid and has linoleic acid in minor concentration. Contrastingly the mustard oil is rich in erucic acid and to lesser extent in linoleic and oleic acids. The fatty acid composition of *S. marianum* oil is highly different from those of sunflower and safflower oils. Both the latter oils are rich in linolenic acid like the soybean oil, contain oleic acid in lower concentrations and demonstrate near absence of linoleic acid. In contrast, the oleic acid is the major constituent in the groundnut oil. Thus *S. marianum* has unique fatty acid composition which does not match with that of most other edible oils. However, the *S. marianum* oil, being rich in unsaturated fatty acids like sunflower, groundnut and soybean oils, could be treated as cardio-protective oil. The richness of essential fatty acids in the *S. marianum* makes the oil suitable for cosmetics and dermatological compositions on account of its antioxidant potential[34]. In this regard, presence of tocopherals has been reported in the seed oil of *S. marianum* of different geographical origins[8,9,21,25].

Conclusion

S. marianum seeds are resource for the much in demand pharmaceutical silymarin and of edible oil, because silymarin is extracted after oil has been separated from seeds. The species is a common weed of worldwide distribution and is undergoing domestication in several countries for making the supply of silymarin sustainable. Properties of the seed oil of a recently developed Indian cultivar SMB-5 were compared with the oils of improved genotypes developed in six other countries. It demonstrated unique properties in comparison to soybean, mustard, canola, ground nut, sunflower, safflower and flax oils; it is richest in linoleic acid (45 %) and linoleic and oleic acids (~ 85 %). The oil is suitable for its conversion into biodiesel.

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