

Some Physio-Chemical Properties of *Silybummarianum* Seed Oil Extract



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Abstract

Silybummarianum seed extract have promising effects in nutrition as well as in therapeutics. Therefore, present study was designed to evaluate composition and physio-chemical effects of *Silybummarianum* seed oil in PMAS-AAUR, Rawalpindi Pakistan. Proximate seed analysis of *Silybummarianum* indicated the increase in dry matter (15.75%), crude fiber (14.4%) whereas moisture (8.2%), crude protein (15.75%), crude fat (24%) total ash (4%) did not proved to significant increase in present study. In case of physico-chemical parameters saponification value (126.2mg KOH/g) and ester value (121.15mg/g) were increased while peroxide (7.056meq. Peroxide/g), free fatty acid (2.53mg/g), iodine (2.79mg/g), acid (5.049mg KOH/g), wax (6.89mg/g) pH is 8.09 and specific gravity (0.8129) have lower values. High in saponification and low acid values make this oil suitable for soap industry also increases quality and volatility of oil. Low Peroxide value shows its low oxidation, rancidity and high antioxidant activity.

Introduction

Silybummarianum (Milk thistle) belongs to the family Asteraceae. It is well known since the time of "Theophrastus" and "Dioscorides" [1]. It is native to the North Africa and Mediterranean area and found as annuals or biennials [2,3]. *Silybum* species are highly medicinal and contributes a lot to ethnomedicine [4-6]. It is highly tolerant to drought conditions [4,7].

Milk thistle can also be cultivated for several purposes [8]. Because of its attractive, beautiful spiny and creamy foliage it is cultivated ornamentally [9]. Its stems, roasted seeds and inflorescence are consumed either raw as well as cooked [10]. In *Silybummarianum* flowering continue throughout the whole life cycle [11]. So it is a major problem for selecting harvesting period of *Silybummarianum* [12].

Seeds are common and traditional sources of oil. With increasing demand of better quality oils, Milk thistle seeds oil is cheap, healthy and beneficial for human consumption [13], having no side effects [14, 15]. These seeds oils are rich in flavonoids, amines, trace elements, vitamins and some other factors [16]. Its seeds oil contain relatively high amount of unsaturated fatty acids principally (21-36 percent) oleic acid and (42-54 percent) linoleic acid [2,17-20]. It was reported that under drought condition oil content decreases by 4 percent while a significant increase was observed in polyunsaturated fatty acids [17].

Silymarin is a major component of seeds extract of milk thistle and it is a polyphenolic healthy component with highly

medicinal properties [21]. "Silybin" is the main component of silymarin containing 30 percent calcium and is extracted from fruit of milk thistle [22,23]. It is hepatoprotective and used for the treatment of liver disorders [14,15,24-27]. Silymarin is recognized as antioxidant, anticancer. Anti-metastasis, anti-inflammatory [9,28-30] and also enhances the immunity against hepatitis C [21]. Silymarin prevents infiltration of neutrophils and control spread of inflammatory agents [31].

Compounds derived from *Silybummarianum* seed extract are found to have a promising effect in nutrition as well as in therapeutics. Previous studies on *S. marianum* focus on proximate analysis, physiochemical parameters and its medicinal uses. Therefore, present study was designed to check and compare the proximate values like moisture, protein, dry matter, total ash, protein, fat and crude fiber content, Physiochemical parameters such as saponification, peroxide, free fatty acid, specific gravity, ester, pH, wax content, acid and iodine value and anti-oxidant activity of oil to promote its importance in pharmaceutical and good industry.

Materials and Methods

Sample collection and preparation

Silybummarianum seeds were collected from the fields of PirMeher Ali Shah Arid Agriculture University, Rawalpindi. These seeds were sterilized under sun light. After that the sample was grounded into powered form with the help of laboratory grinder. For further analysis, the sample was kept in fine plastic bags and stored in refrigerator at 4 °C.

Extraction of oil

The seed powder was weighed and 12g sample was placed in extraction thimble filter paper. The thimble was kept in loading chamber and extracted with 125ml of n-hexane that was kept in the 250ml reflux flask, for about 6h in soxhlet apparatus. The change in color was observed when oil is transferred into the solvent [32], the solvent was separated after extraction by soxhlet apparatus using a vacuum evaporator at 45 °C. The remaining n-hexane was allowed to evaporate by allowing the extract to stand at room temperature for 24h. The remaining extract was weighed to determine the amount of oil from 12g of seeds. This process was repeated for three times and oil was stored at 4 °C for further analysis.

Proximate Analysis of *Silybummarianum*

Moisture, dry matter, total ash, protein and fat contents were determined by standard AOAC, 1990 method. Whereas crude fiber content was determined by the standard method of Duke and Atchley (1984) and results showed in percentage. Moisture and dry matter content both were calculated separately by the following formula;

$$W_1 - W \times 10 / \text{Sample weight}_3$$

(W_1 =weight of empty Petri dish, W_2 =weight of empty Petri dish + wet weight, W_3 =weight of empty Petri dish + dry weight)

Total ash value was calculated by following formula;

$A - B \times 100 / C$ (A=weight of crucible with sample, B=weight of crucible with ash, C=weight of sample)

Protein and fat content was determined by using following formulas.

$$\text{Protein content} = (V_1 - V_2) N \times 14 \times 25 \times 100 / W$$

(V_1 =volume of plant sample in ml, V_2 =volume of blank in ml, N=Normality of standardized H_2SO_4 , W=weight of sample)

$$\text{Fat content} = \text{Initial weight} - \text{Final weight} \times 100 / \text{initial weight}$$

Determination of physiochemical parameters

Determination of the saponification value (Protocol reference): Oil sample of 2g was poured in conical flask. 30ml of 0.5M alcoholic potassium hydroxide (KOH) was added, weighed and heated for 30 mints to ensure that sample will be fully dissolved. One milliliter 1 percent phenolphthalein indicator was added. Out of this solution 1ml was taken and introduced into hot mixture of reaction. This solution was titrated with 0.5ml hydrochloric acid (HCL) until it become color less, a blank titration was also done side by side which was the measurement of volume of HCL which was used to neutralize the blank. For sake of accuracy, the process was repeated three times to get saponification value of oil by following formula:

$$SV = V_b - V_s \times 28 / W$$

Determination of peroxide value (Protocol reference): Take 6g of sample, 20ml of chloroform, 30ml of glacial acetic

acid and 2ml of freshly prepared saturated aqueous solution of potassium iodide were added into flask. The flask will be shaken consistently in the dark for 5 mints then 150ml of distilled

Water was added. This mixture was mixed thoroughly. 10ml of this mixture was poured in separate flask and 2ml of one percent soluble starch solution was introduced as indicator. After the addition mixture was titrated by using sodium thiosulphate solution of 0.02 molarities. A reagent blank determined was used. The following formula was use to determined peroxide value.

$$\text{Peroxide value} = 126 (V_b - V_s) \times 10 / W$$

Determination of free fatty acids: The contents of free fatty acids present in oil was found by using AOAC [33] method. 3.5g of the oil is poured in a clean dry flask containing 225ml neutralized alcohol. The resultant mixture was titrated against 0.25N solution of NaOH until a faint pink color appeared. A blank titration was carried out to estimate the volume used to titrate the blank volume of the 0.25N NaOH used was note. The percentage of free fatty acids was determined by using the formula given below:

$$\text{Percentage of free fatty acids} = (V_b - V_{s_2}) \times N \times 28.2 / W$$

Determination of specific gravity (Protocol reference): Oil specific gravity was determined through specific gravity bottle. Gravity bottle of 50ml was filled up to the mark and weighed before and after insertion of stopper. Now the bottle was dried and again filled up to the mark with oil, stopper was inserted and bottle was weighed. The oil specific gravity was determined using the formula:

$$\text{Specific gravity} = W_{\text{oil}} - W_{\text{bottle}} / W_{\text{water}} - W_{\text{bottle}}$$

Density of oil was determined by multiplying the specific gravity of oil with density of water.

Determination of ester value (Protocol reference): Ester value was determined from saponification value and acid value by using the following formula

$$\text{Ester value} = \text{saponification number} - \text{acidity index}$$

Determination of pH of oil (Protocol reference): Oil sample was mix with 10ml of warm distilled water and place this solution into the cool water bath to decrease its temperature to 25 °C. pH of this solution was measured by using pH meter.

Determination of wax content of oil: Wax contents of the oil were determined by [34]. 5g of oil and 25ml of acetone were added in flask then placed in freezer for 24h at 4 °C to crystal the wax. The insoluble part of the solution (acetone) was filtered by using pre-weighed What man No.1 filter paper. Filter paper was dried in vacuum oven at 45 °C and weighed to obtain the wax contents.

Acid Value: Sample of 1g was dissolved with 50ml of ethanol in conical flask. Two drops of Phenolphthalein were used as indicator and titrated it with 0.1 N potassium hydroxide solution

(KOH) to pink end point. Acid value was calculated by using following formula [35].

$$\text{Acid value} = 56.1 \times V \times C / M$$

(56.1=Equivalent weight of KOH, V=Volume in ml of standard volumetric KOH solution used, C=Concentration in KOH solution used (0.1N), M=Mass of sample)

Determination of iodine value of oil (Protocol reference):

Oil sample of 0.25g along with 10 ml of chloroform were added in flask then 30ml of Hanus solution was added and closed the flask by Aluminum foil then left it for 30 minutes with continuous shaking. Added 10ml of 15 % of Potassium Iodide (KI) and 100ml of distilled water. Titrated the iodine solution against 0.1N of Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) till yellow color appeared, then added two to three drops of starch solution and blue color was appeared, then continued titration till blue color disappeared. Iodine value was calculated by using following formula.

$$\text{Iodine Value} = (V_b - V_s) \times N \text{ of Na}_2\text{S}_2\text{O}_3 \times 127\text{g/meq} \times 10 / W$$

(V_b =Volume of blank, V_s =Volume of sample, N=Normality of

Sodium thiosulphate, 127=Mass equivalent of Iodine, W=Weight of sample)

Antioxidant activity

Antioxidant properties of *Silybummarianumu* seed oil was determined by reducing power assay. This assay was described by Chung [36]. In this assay different dilutions were prepared from *Silybummarianumu* seed oil. 2ml of each dilution was mixwith2ml of potassium ferricyanide (1mg/ml) and 2ml of 0.2M of phosphate buffer (pH 6.6) in test tube. Incubated it at 50 °C for 20 minutes and then cooled it. Added 2ml of tris acetic acid (1%), the mixture was then centrifuged at 3000g for 10 minutes. The supernatant (0.25ml) was mixed with 0.25ml distilled water and 0.1% FeCl_3 (0.5ml) and left it for 10min. Measured its absorbance at 700nm. Increased absorbance by reaction mixture showed the higher antioxidant activity.

Statistical analysis

Analysis of each parameter was carried out by using the mean and standard deviation.

Results

Proximate analysis

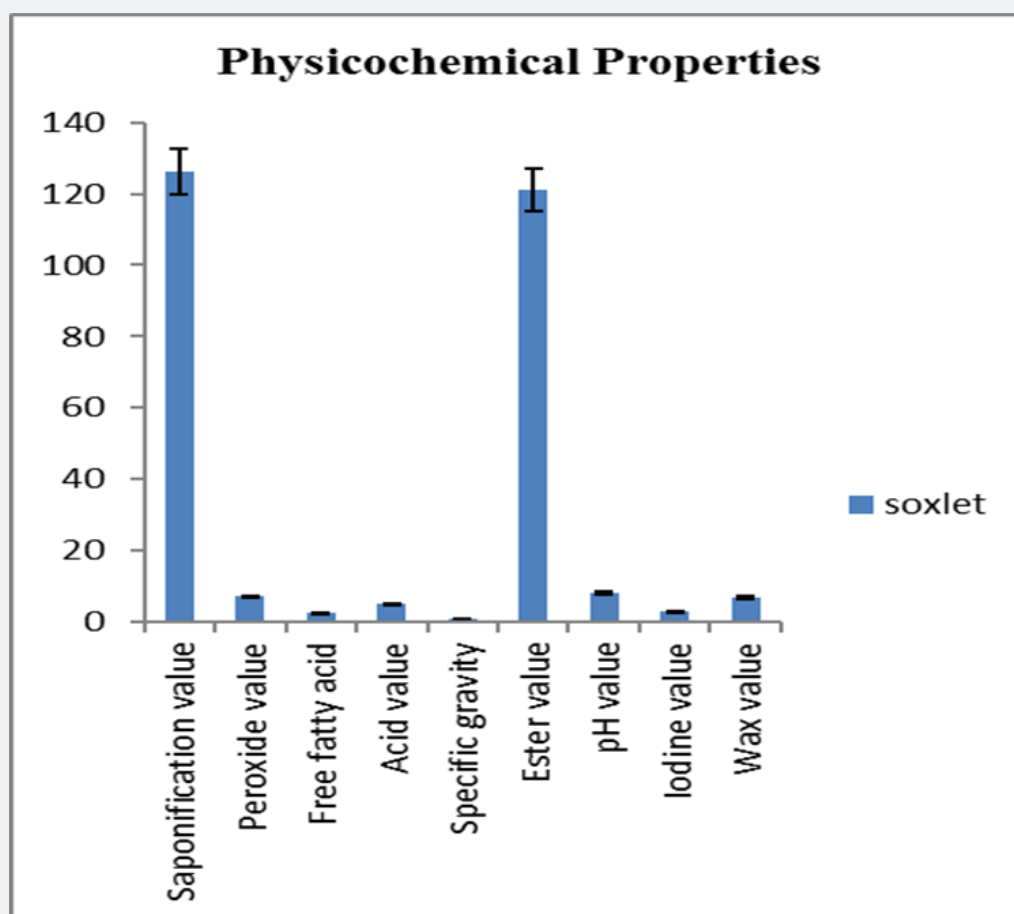


Figure 1

Proximate analysis of *Silybummarianum* is shown in the Figures 1. Results of analysis showed that *Silybummarianum* has maximum content of dry matter (91.8 %) and crude fiber (24 %). A significant amount of protein (15.75 %) was also found. Total ash (4%) was found in the lowest amount. Crude fat were 14.4 % and moisture content was found to be 8.2 %. Proximate analysis of *Silybummarianum* seed is shown in Table 1.

Table 1: Proximate analysis of *Silybummarianum* seeds.

Proximate Analysis (%)	Values
Moisture content	8.2±0.52
Dry matter content	91.8±0.52
Ash value	4±0.40
Crude Protein	15.75±0.52
Crude Fat	14.4±0.50
Crude Fiber	24±0.36

Each value represents the average of three replicate analysis±S.D

Physicochemical parameters

Result of the analysis shows that *Silybummarianum* has peroxide value (7.056meq. Peroxide/g), saponification value

(126.2mg KOH/g), ester value (121.15mg/g), free fatty acid (2.53mg/g), iodine value (2.79mg/g), Acid value is 5.049mg KOH/g, wax value is 6.89mg/g, pH is 8.09 and specific gravity is 0.8129. Results of physicochemical properties are shown in Table 2 and in Figure 1.

Table 2: Physicochemical properties of *Silybummarianum* seed oil.

Physicochemical Property	Values
Saponification value	126.2±0.46
Peroxidase value	7.05±0.05
Free fatty Acid	2.53±0.03
Acid value	5.04±0.27
Specific gravity	0.81±0.01
Ester value	121.15±0.53
pH value	8.09±0.10
Iodine value	2.79±0.11
Wax value	6.89±0.10

Each value represents the average of three replicate analysis±S.D

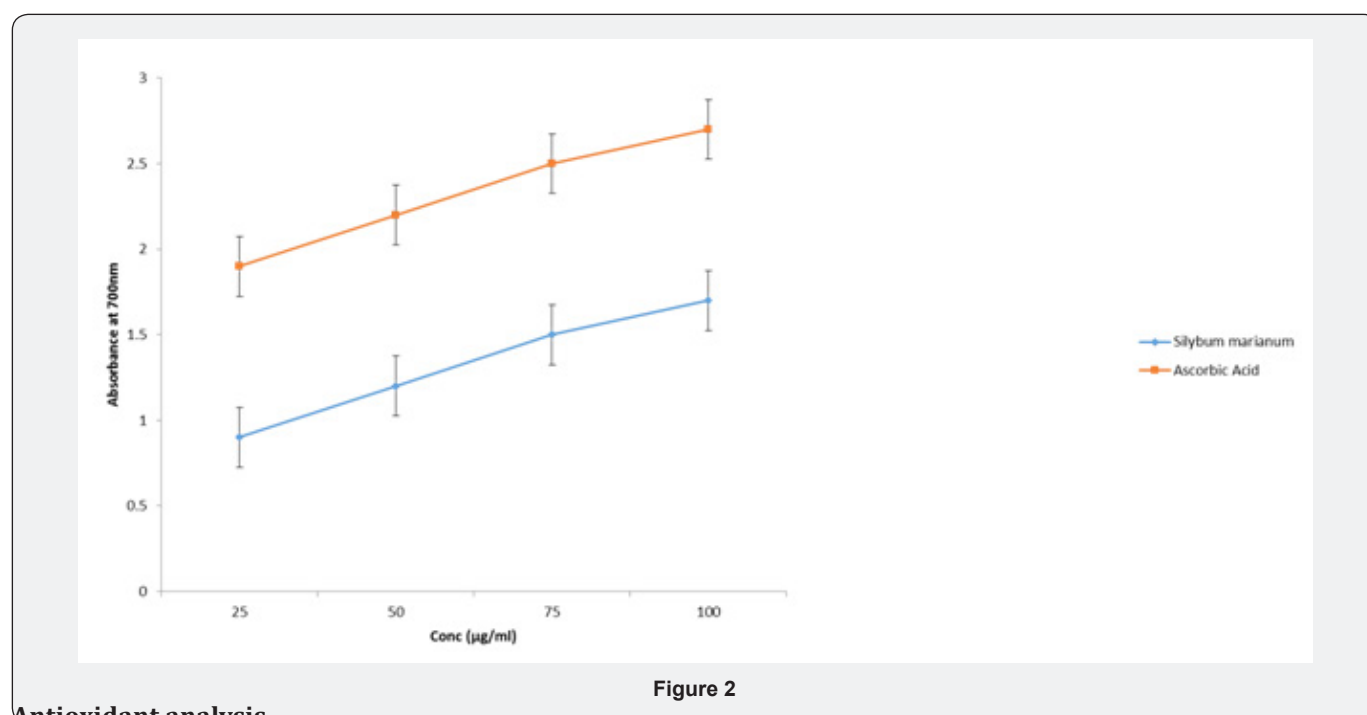


Figure 2

Antioxidant analysis

In the present study reducing power assay was performed to determine the antioxidant potential of *Silybummarianum* seed oil. Concentrations of 25, 50, 75 and 100µg/ml were used for the analysis of antioxidant activity of both oil and standard. Reducing power ability was determined according to the absorbance of spectrophotometer. Finally the results of the assay were compared with ascorbic acid. It was used as a standard. A significant difference was found between absorbance values at different concentrations. Absorbance of *Silybummarianum* seed oil (0.9,1.2,1.5,1.7) and ascorbic acid (1.9,2.2,2.5,2.7). Results of reducing power assay are shown in Figure 2.

Discussion

Proximate analysis

Proximate analysis of *Silybummarianum* shows resemblance to other studies conducted on milk this tle having moisture 4.6%, protein 17.64%, fiber 25.32%, ash 5.10% [37]. In other studies, the reported moisture, ash and protein content of *Silybummarianum* are 4.48%, 1.93%, 23.80%. Value of crude fiber (5.48 %) shows variation due to geographical and environmental variations [20].

Moisture content calculated for *Silybummarianum* is 8.2% which shows a close resemblance with moisture content of *Erucasativa* (5.23% and 4.1%), black olive (5.77-27.77%) and *Annonamuricata* (11.01%) [38-41]. *Silybummarianum* shows relatively low moisture content. It is beneficial to enhance the shelf life and to reduce the pathogenic attack [42].

Protein content calculated for *Silybummarianum* (15.75) shows close resemblance with Sun flower (16.9-25.1% and 20%), *Eruca sativa* (27.4%), *Sonchusasper* (13.2%) and *Annonamuricata* (25%) content [39,41,43-45]. Protein is very important as a source of energy, precursor of enzymes, hormones, important structures and involve in defense functioning of the body [46]. *Silybummarianum* leaves can be considered as a good source of protein. It can be used as protein supplement to meet the need of protein.

Ash value (4 %) calculated for *Silybummarianum* also shows close resemblance with some other plants like 3.5% for sun flower, 6.6% for *Erucasativa*, 3.50% for *Moringaoleifera* and 4.76 for *Catharanthusroseus* [39,44,47,48].

Crude fiber calculated for *Silybummarianum* is 24%. It is similar to the crude fiber of *Annonamuricata* (22.20%) and *Sonchusasper* (18.3%) [41,49]. Fibre is helpful for detoxification in the stomach and maintains cholesterol level [50]. Fat content? of *Silybummarianum* is very close to *Erucasativa* (26±1.2 %) fat content [39]. *Silybummarianum* dry matter content (91.8 %) is closely resembles with *Catharanthusroseus* (91.94) and *Annonamuricata* (88.9%) [41,51].

Physicochemical parameters

Specific gravity of *Silybummarianum* is 0.8129 unit?. However, in plants such as *Butyrospermumparkii* oil, *Dacryodesedulis* and Nigerian pumpkin, the specific gravity was 0.87, 0.895, and 0.830 respectively. The specific gravity of these plants show close resemblance to *Silybummarianum* [52].

Free fatty acid content of *Silybummarianum* is 2.53mg/g. This value shows similarities to *Dacryodesedulis* seed oil (2.78-2.81) [52]. *Silybummarianum* has a lower amount of free fatty acid. Fatty acid content depends on the soil conditions and climatic effects under which a plant is growing [53]. Fatty acids play a vital role in plant cellular activities [54].

Peroxide value of *Silybummarianum* (7.056 meq. Peroxide/g) is very close to peroxide value of *Moringaoleifera* (8.10-15.96 meq. peroxide/g) and *Citrulluslanatu* soil (2.90-5.06 meqO₂ kg⁻¹) [51,55]. Refined oils have low value of peroxide then the unrefined oils [56]. Milk thistle has low peroxide value which shows its low oxidation, rancidity and high antioxidant activity.

Saponification value of *Silybummarianum* is 126.2mg KOH/g which is very close to saponification vale of *Moringaoleifera* (134.3mg KOH/g) [47]. Due to high content of saponins milk thistle it is very good for the soap industry. Increase in saponification value increases quality and volatility of the oil [57].

pH calculated for *Silybummarianum* is 8.09. However, this value is 6.78 for same milk thistle and 6.6 for white variety of *Silybummarianum* [17,58]. Wax content calculated for *Silybummarianum* is in the range of study conducted by Hamid et al. [39] i.e., 17.073±0.050%.

Iodine value of *Silybummarianum* is 2.79mg/g which is very close to iodine value of Nigerian almond i.e., 2.65-153.00g I₂/100g [52]. Oil with low iodine value are resistant to oxidation due to presence of less unsaturated bonds [59]. Oils with low iodine value are nondrying oils due to their susceptibility to oxidation. So these oils are very suitable to be used in soap industry [60].

Ester value calculated for *Silybummarianum* is 121.15mg/g which show resemblance with value of *Chrysophyllumalibidum* (122.74mg/g) and *Vignasubterrenea* (123.88) [52]. Acid value of *Silybummarianum* is calculated to be 5.049mg KOH/g which is very close to 5.56-5.61mg KOH/g for *Dacryodesedulis* and 5.764mg NaOH/g for *Lavandulabipinnata* seed oil [52]. Plants with low acid value are highly antioxidant, edible and can be used in soap industry [59]. Milk thistle has a low acid value so it is highly antioxidant and suitable for the use in soap industry.

Antioxidant analysis

Essential oils of the plants which possess medicinal and antioxidant properties are largely studied and of great interest for their commercial purposes [61]. Reducing power gives a clear cut picture of antioxidant ability of a plant [62,63]. Reducing power action involves the reduction of Fe⁺³ ion into Fe⁺²ion which results in the formation of bluish colour ferri cyanide complex at 700nm [64]. It is concluded that reducing power ability increases with the increase in concentration [65]. Furthermore the results of present study can also be justified by other studies [55,66].

Conclusion

Due to high content of saponin, milk thistle it is very good for the soap industry. Increase in saponification value increases quality and volatility of the oil. Milk thistle has low peroxide and acid values which show its low oxidation [67-69], rancidity and high antioxidant activity and its suitability for the use in soap industry. Reducing power of *S. marianum* increases with the increase in concentration [70-72]. Overall results indicate that *Silybummarianum* is good source of nutraceuticals components and also find its application in pharmaceutical industry. *Silybummarianum* is highly hepatoprotective as well as a good antioxidant.

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