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(Review Article)

Phytochemistry and Pharmacological Activities of *Silybum marianum*: A Review

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ABSTRACT

Silybum marianum (Milk thistle), a member of the Asteraceae family, is a tall herb with large prickly white-veined green leaves and a reddish-purple flower that ends in sharp spines. Certain phytoconstituents were revealed such as silybin A, silybin B, isosilybin A, isosilybin B, silychristin, silydianin, apigenin 7-O-β-(2''-O-α-rhamnosyl)galacturonide, kaempferol 3-O-α-rhamnoside-7-O-β-galacturonide, apigenin 7-O-β-glucuronide, apigenin 7-O-β-glucoside, apigenin 7-O-β-galactoside, kaempferol-3-O-α-rhamnoside, kaempferol, taxifolin and quercetin. The plant is exclusively used as anti-diabetic, hepatoprotective, hypocholesterolaemic, anti-hypertensive, anti-inflammatory, anti-cancer, and as an anti-oxidant. Seeds of the plant are also used as an anti-spasmodic, neuroprotective, anti-viral, immunomodulant, cardioprotective, demulcent and anti-haemorrhagic. The plant is also serves as a galactagogue and used in the treatment of uterine disorders. This review paper focuses mainly on phytochemistry and pharmacological activities of the legendary plant milk thistle.

Key Words: *Silybum marianum*, Silybin, Silychristin, Phytochemistry, Anti-diabetic, Hepatoprotective

INTRODUCTION

Medicinal plants are important to the global economy. In 1980, WHO estimated the world trade at US$500 million[1]. Traditional medicine is an important part of African culture. More than 80% of Africans rely on plant-based medicine. Latin Americans also rely on traditional medicines for their health care needs[2]. In India, about 2500 plants have been reported to be used in ethno-medicine[3]. *Silybum marianum*, commonly known as 'milk thistle' belonging to family Asteraceae / Compositae is one of the oldest and thoroughly researched plants in the treatment of liver diseases[4]. It is being used as a general medicinal herb from as early as 4th century B.C. and first reported by Theophrastus[5]. Extract from the seeds of the milk thistle is being used traditionally as a herbal remedy against hepatotoxicity and acute and chronic liver diseases[6]. Silymarin effects have also been indicated in various illness of different organs such as prostate, lungs, CNS, kidneys, pancreas, and skin[7].

PLANT PROFILE

Milk thistle (*Silybum marianum*), is an annual or biennial native to the Mediterranean regions of Europe, North Africa and the Middle East and in some parts of USA[8]. In India, it is commonly found in Jammu and Kashmir [5]. It grows to a height of three to ten feet with an erect stem that bears large, alternating, prickly-edged leaves. The common name, milk thistle, is derived from the "milky white" veins on the leaves, which, when broken open, yield a milky sap. Each stem bears a single, large, purple flower ending in sharp spines. The fruit portion of the plant is glossy brown or grey with spots. The plant grows at an altitude of 1800-2400m in rocky or sandy soil. The plant cherish with flowers in monsoon season from June to August[9].

Taxonomical Classification[10]

Domain	:	Eukaryota
Kingdom	:	Plantae
Subkingdom	:	Viridaeplantae
Phylum	:	Tracheophyta
Subphylum	:	Euphyllophytina
Infraphylum	:	Radiatopses
Class	:	Magnoliopsida
Subclass	:	Asteridae
Superorder	:	Asteranae
Order	:	Asterales
Family	:	Asteraceae
Genus	:	Sylibum
Species	:	Marianum
Botanical name	:	*Silybum marianum*

Vernacular Names[11]

Dutch : Mariendistel, Vrouwendistel
English : Holy thistle, Lady's thistle, Milk thistle
French : Artichautsauvage, Chardon marie
German : Feedistel, Mariendistel, Silberdistel
Greek : Silybon
Italian : Cardodel latte, Cardomariano
Malta : Blessed thistle
Romanian: Armurariu
Russian : Ostropestro
Spanish : Cardolechal, Cardolechero
Swedish : Sempertin

Morphology

Roots: Usually taproots, sometimes fibrous.
Stems: 20-150 cm high, rarely shorter, glabrous or slightly downy, erect and branched in the upper part.
Leaves: Alternate, large, white veined, glabrous with strongly spiny margins.
Inflorescences: These are large and round capitula, solitary at the apex of the stem or its branches, surrounded by thorny bracts.
Florets: Florets are hermaphrodite, tubular in shape with a red-purple corolla.
Fruits: Hard skinned achenes 6 to 8 mm long, generally brownish in color with a white silk like pappus at the apex [10, 12].

Microscopy

Pericarp epidermis a colourless palisade layer of cells (about 75 mm long and 8 mm wide) with a strongly thickened outside wall, which reduces the lumen in that part of the cell to a slit; sub-epidermal layer composed of colourless, thin-walled, parenchyma cells or groups of parenchyma cells alternating with a variable number of pigmented cells; innermost layer mostly collapsed and containing cigar-shaped or monoclinic prismatic crystals of calcium oxalate. Testa epidermis consists of large, lemon-yellow, palisade-like, elongated cells (about 150 mm long) with striated walls and narrow lumen widening slightly at the ends; sub-epidermal layers have lignified and pitted cells[13, 14].

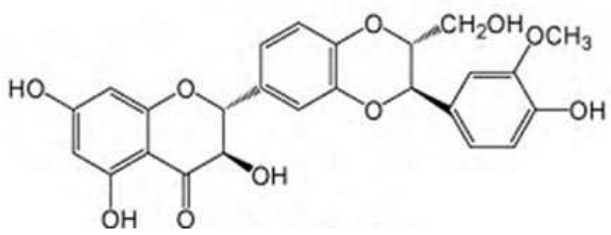
TRADITIONAL USES

In Europe, milk thistle is used in jaundice and other biliary affections. As a diet or in infusion it is said to be a reliable galactagogue. Silymarin is often used as supportive therapy in food poisoning due to fungi.
Root: Root is eaten boiled as a pot herb.
Herb: Herb is used for intermittent fevers, dropsy & uterine troubles. A decoction of it is said to be beneficial as an external application in cancer.
Leaves: Leaves are sudorific and aperient. Young leaves serve as salad and flowering heads are consumed by diabetics.

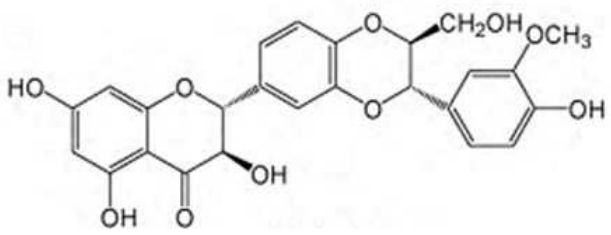
Seeds: Seeds are pungent, demulcent and antispasmodic. They are used for the treatment of jaundice and calculi of liver and gall-bladder and are useful in controlling haemorrhages. Alcoholic extracts of the seed and to a lesser extent of the plant also, increase peristalsis of the small intestine and galenical preparations, both of the seed and oil are mild purgative. Seeds are used as a substitute for coffee[15].

PHYTOCHEMISTRY

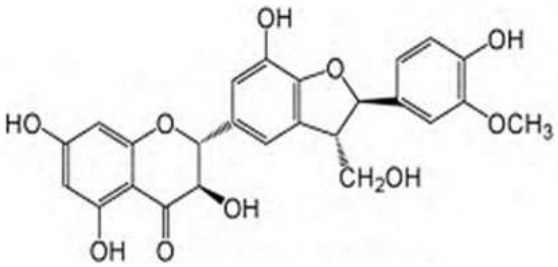
The pharmacological active ingredient present in the plant is the flavonoid complex silymarin, which is the main constituent with about 80% of the extract. Silymarin consists of a large number of flavolignans including silybin (or silybinin), isosilybin, silydianin and silychristin[16, 17]. Besides these taxifolin, quercetin, betaine and silybonol have also been isolated by Kren V, *et al*[18, 19].



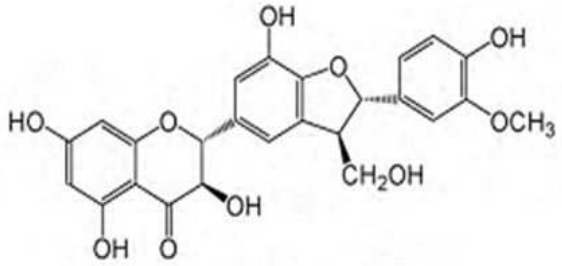
Silychristin



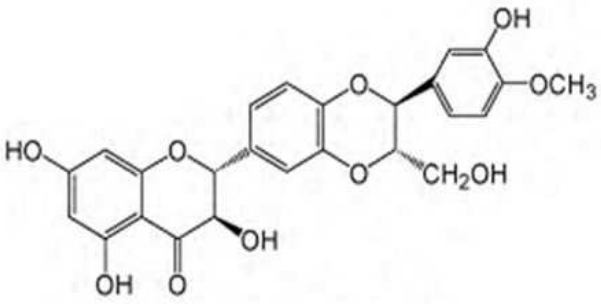
Silydianin



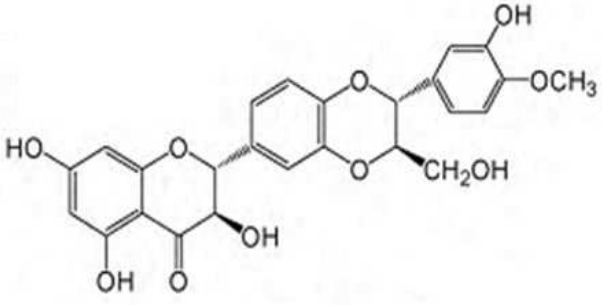
Silychristin A



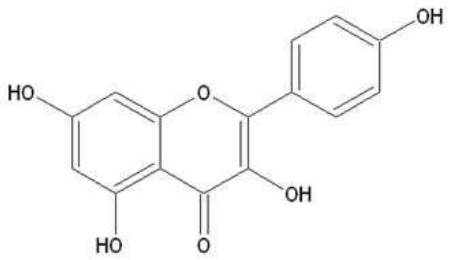
Silychristin B



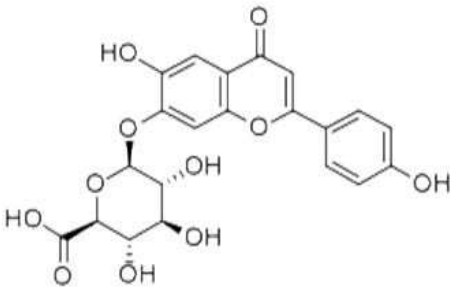
Silybin A



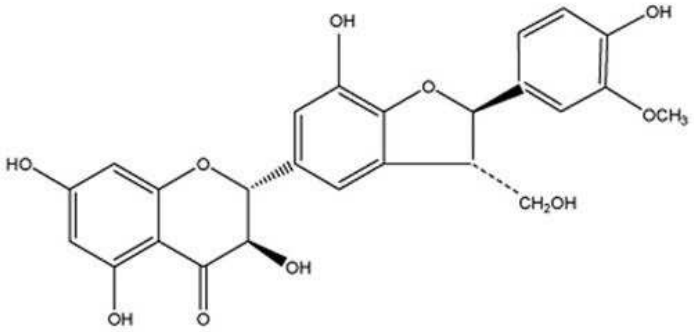
Silybin B



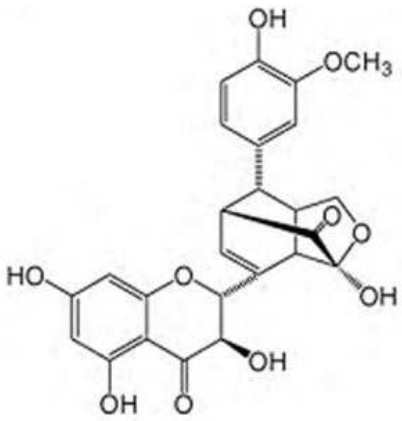
Kaempferol



Apigenin 7-O-β-glucuronide



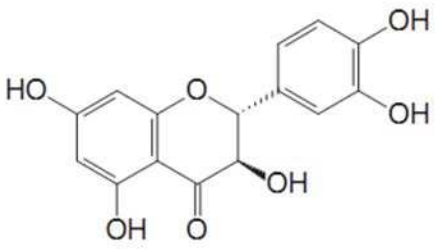
Isosilybin A



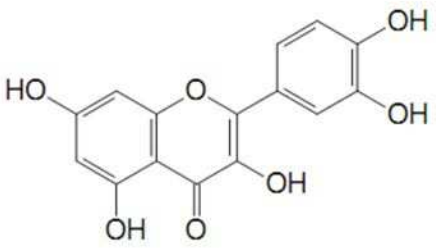
Isosilybin B

Ahmed A, et al. reported seven flavonoids from an aqueous methanol extract of the flowers of *Silybum marianum viz*., apigenin 7-O-β-(2″-O-α-rhamnosyl)galacturonide, kaempferol 3-O-α-rhamnoside-7-O-β-galacturonide, apigenin 7-O-β-glucuronide, apigenin 7-O-β-glucoside, apigenin-7-O-β-galactoside, kaempferol-3-O-α-rhamnoside and kaempferol[20].

Barreto JFA, *et al.* examined the batch extraction of silymarin compounds from milk thistle seed meal in 50, 70, 85, and 100°C water as a function of time. After 210 min of extraction at 100°C, the yield of taxifolin was found to be as 1.2 mg/g of seed, a 6.2-fold increase over the results obtained in a Soxhlet extraction with ethanol on pretreated (defatted) seeds. Similarly, the yield of silychristin was reported as 5.0 mg/g of seed, a 3.8-fold increase. The yields of silybinin A and silybinin B were 1.8 and 3.3 mg/g of seed, respectively, or roughly 30% of the Soxhlet yield. The more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar compounds (silybinin A and B) were favored at 100°C[21].



Taxifolin



Quercetin

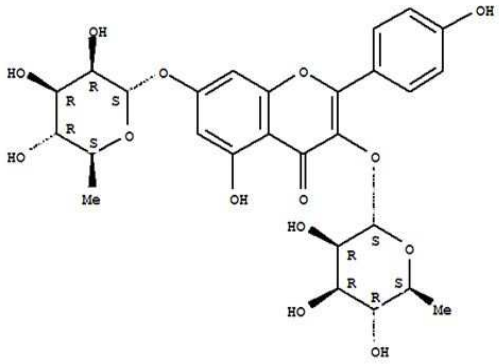
Bilia AR, *et al.* separated all classes of flavonoid (flavones, flavonols, flavanonols, and flavanolignans)

using simple reversed-phase HPLC method. The relative standard deviations obtained from investigation of the repeatability of the method were reported as silybin 2.33%, taxifolin 2.19%, quercetin 2.08% and isoquercitrin 2.21%[22].

Parry J, et al. investigated for FA, tocopherol, and carotenoid compositions, total phenolic contents (TPC), antioxidant activities, oxidative stability index (OSI), color, and physical properties in cold-pressed milk thistle seed oils. The total mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) contents were found to be as 25.2 and 61.1 g/100g of oil, respectively. The ratio of oleic to linoleic acid was 0.4, whereas the carotenoid content was 2.30 μmol/kg. The α-, γ-, and δ-tocopherol contents were reported as 156.3 ± 0.9, 35.1 ± 0.4 and 7.0 ± 0.0 mg/kg, respectively. The oxidative stability index (OSI) and refractive index were found to be as 13.3 ± 0.3h and 1.4335[23].

Wallace SN, *et al.* evaluated the milk thistle seeds containing flavanolignan and dihydroflavanol compounds. The maximum yields of taxifolin, silychristin, silydianin, silybinin A, and silybinin B in ethanol were reported as 0.6, 4.0, 0.4, 4.0, and 7.0 mg/g of defatted seed, respectively[24].

Duan L, *et al.* extracted milk thistle seeds with hot water at 100, 120, and 140° C using the same water flow rate (0.30 mL/min) and seed meal particle size (0.4 mm). The yields of taxifolin, silychristin, silybinin A and silybinin B at 140° C were reported as 0.5, 2.4, 1.2 and 2.0mg/g seed[25].



Kaempferol-3-O-α-rhamnoside

PHARMACOLOGICAL ACTIVITIES

Anti-diabetic Activity

Huseini HF, *et al.* had worked on the valuable effect of antioxidant nutrients on the glycemic control of diabetic patients in experimental and clinical studies. The average fasting blood glucose level in the silymarin group at the beginning of the study was reported as 156 ± 46 mg/dL, which decreased significantly ($p<0.001$) to 133 ± 39 mg/dL after 4 months of silymarin treatment. The average fasting blood glucose level in the placebo group at the beginning of the study was 167 ± 47 mg/dL, which increased significantly ($p<0.0001$) to 188±48 mg/dL after 4 months of placebo treatment[26].

Hepatoprotective Activity

Silymarin protect sliver cells against many hepatotoxins in humans and animals. Desplaces J, *et al.* examined severe *Amanita* poisoning in 60 patients treated with infusions of 20 mg/kg of slibinin with excellent results showing no death of the patients treated. Silymarin also offers liver protection against tetracycline, d-galactosamine and thallium-induced liver damage and erythromycin estolate, amitryptiline, nortryptiline and tert-butyl hydroperoxide exposure of neonatal hepatocytes[27, 28].

Vogel G, *et al.* reported the action of silybinin for anti-hepatotoxic activity against *Amanita phalloides*, ethanol, paracetamol (acetaminophen) and carbon tetrachloride-induced liver injury. Silybinin also produced hepatoprotective effects in acute viral hepatitis, alcohol related liver cirrhosis at doses ranging from 280 to 800 mg/day[29].

Madani H, *et al.* treated with the polyphenolic extracts of *Silybum marianum* which reduced the level of serum aminotransferases activities including SGOT and SGPT and also alkaline phosphatase (ALP) activity and the level of total bilirubin, comparing with thioacetamide group. The extracts were injected to

the rats, at the dose of 25 mg/kg body weight together with thioacetamide at the dose of 50 mg/kg body weight. It was reported as the liver cells around central veins showed relatively a high number of necrosis and apoptosis. Some acute and chronic inflammatory cells were also seen around the necrotic cells. In the groups treated with polyphenolic extracts of the plant, central veins were congested and dilated[30].

Hypocholesterolaemic Activity
Skottova N, *et al.* reported the hypocholesterolaemic activity of silymarin on the basis of experimental evidence showing that silybin inhibits HMG-CoA reductase activity in vitro; and silymarin improved the binding of low density lipoproteins (LDL) to rat hepatyocytes, decreased the liver cholesterol content in rabbits fed with a high-cholesterol diet, decreased the plasma-cholesterol and LDL-cholesterol levels in hyperlipaemic rats[31].

Anti-hypertensive Activity
Jadhav GB, *et al.* evaluated the effect of silymarin (300 mg/kg and 500 mg/kg, p.o, for 4 weeks) in Deoxycorticosteroneacetate (DOCA) salt induced hypertensive rats. It was reported that silymarin (300, 500 mg/kg/day, p.o) significantly ($p<0.05$) reduced systolic blood pressure, heart rate, basal arterial blood pressure and pressor responses to nor-adrenaline, adrenaline, phenylephrine and serotonin (5-HT) in animals treated with DOCA salt as compared with DOCA-salt hypertensive rats[32].

Anti-inflammatory Activity
Dehmlow C, *et al.* reported the effect of silybin which inhibited the synthesis of leukotriene B4 (IC_{50} 15 µmol/l) in isolated rat Kupffer cells, but had no effect on prostaglandin E_2 formation at concentrations up to 100 µmol/l. Minonzio F, assessed the anti-inflammatory activity of silybin in human polymorphonuclear leukocytes in vitro. The mechanism of anti-inflammatory activity involved the inhibition of hydrogen peroxide formation[33, 34].

Anti-oxidant Activity
Haddad Y, *et al.* proved that the production of superoxide anion radicals and nitric oxide after treatment in the isolated rat Kupffer cells with silybin (IC_{50} 80 µmol/l) was inhibited. Treatment with silibinin (200 mg/kg) improved liver steatosis and inflammation and decreased non-alcoholic steatohepatitis-induced lipid peroxidation, plasma insulin and plasma tumor necrosis factor-α (TNF-α). Silibinin also decreased superoxide radical ($O_2^{\bullet -}$) release, and returned the relative liver weight as well as GSH back to normal[35].

Neuroprotective Activity
Kittur S, *et al.* evaluated that milk thistle enhanced nerve growth factor (NGF)-induced neurite outgrowth in PC-12 neural cells and prolonged their survival in culture. Milk thistle extract also protected cultured rat hippocampal neurons against oxidative stress-induced cell death[36].

Anti-cancer Activity
Bhatia N, *et al.* worked on the treatment of different prostate, breast, and cervical human carcinoma cells with silibinin resulted in a highly significant inhibition of both cell growth and DNA synthesis in a time-dependent manner with large loss of cell viability only in case of cervical carcinoma cells. The higher doses (100±200 µM) of silymarin induced programmed cell death specifically in human ectocervical carcinoma A431 cells[37].

Anti-viral Activity
Das SK, *et al.* evaluated the inhibitory action on inflammatory and cytotoxic processes induced by viral infection. It was reported that silibinin strongly inhibited growth of both HepG2 (hepatitis B virus negative; p53 intact) and Hep3B (hepatitis B virus positive; p53 matured) cells with relatively more cytotoxicity in Hep3B cells which is associated with apoptosis induction. Silymarin also showed inhibitory activity against other viruses in different cell lines[38].

Immunomodulatory Activity
Meeran SM, *et al.* reported the ultraviolet radiation-induced immunosuppressive activity of silymarin in experimental rodents. It was found as silibinin inhibited the activation of human T-lymphocyte, human polymorpho-nuclear leucocyte. Silymarin also significantly suppressed the inflammatory mediators,

expression of histocompatibility complex molecules and nerve cell damage. Long-term administration of sliymarin improved immunity by increasing T-lymphocytes, interleukins and reducing all types of immunoglobulins[39, 40].

Cardioprotective Activity

Vereckei AS, et al. worked on the activity of Amiodarone as a very potent antiarrhythmic drug however, its use is limited due to direct cytotoxicity, development of lysosomal phospholipidosis, indirect immunologically mediated toxic effects and membrane destabilization[41]. Administration of silybin together with amiodarone decreased significantly lysosomal phospholipidosis[42] and this effect was further enhanced in combination with vitamin E, as demonstrated by Agoston M, *et al*[43]. During the amiodarone treatment (rats) silymarin itself as well as in combination with vitamin E significantly decreased conjugated diene concentration[44] but not attenuated the antiarrhythmic activity of amiodarone, as reported by Gyonos I, *et al*[45].

ADVERSE EFFECTS

The main adverse effects reported are headaches, gastroenteritis and dermatological symptoms; amongst them gastrointestinal symptoms at higher dose (> 1500 mg/day) are the most common which involves mild laxative effect due to increased bile secretion and flow, reported by Kren V, *et al*[18, 46]. Allergic reactions to milk thistle have been reported[47]. Burgess CA, reported that silymarin with other drugs that were conjugated by uridine diphosphoglucuronosyl transferase (UGT1A6/9) led to a reduction in the clearance of certain drugs and a potential for increased toxicity[48]. Anaphylactic shock has been reported in a patient ingesting a tea prepared from crude drug[49].

NUTRITIONAL VALUE

Fixed oil (16% to 18%), betaine, trimethylglycine and amines. The seeds contain 1.5-3% flavolignans, collectively referred to as silymarin; 20-30% fixed oil, of which approximately 60% is linoleic acid, approximately 30% is oleic acid, and approximately 9% is palmitic acid; 25-30% protein; 0.038% tocopherol; 0.63% sterols, including cholesterol, campesterol, stigmasterol, and sitosterol; and some mucilage[50].

MARKETED FORMULATIONS[51-53]

Dosage Form	Dose	Supplier	Price	Uses
Tablets (Uncoated)	400mg	Almeta Health Labs	Rs.132.75/-	Stomach and liver tonic
Tablets (Coated)	450mg	Apex Neutraceuticals, LLC	Rs.153.5/-	Antioxidant, anti-inflammatory
Tablets (Coated) (Livergol®)	70mg	Goldaru Pharmaceutical Lab, Iran	N/A	Hepatoprotective, choleretic
Capsules (Thisilyn®)	175mg	Nature's Way, USA	US$ 27	Stomach and liver tonic
Capsules (Legalon®)	70mg	Madaus AG, Cologne, Germany	N/A	In liver toxicity, hepatic cirrhosis
Aqueous suspensions (Siliphos®)	200mg	Indena, Italy	N/A	Hepatocyte protection
Extract	175mg	Dietceutical Supplements, LLC, USA	Rs.177.5/-	Stomach and liver tonic
Powder	275mg	Dietceutical Supplements, LLC, USA	Rs.177.5/-	Stomach and liver tonic
Milk thistle complex	450mg	Dietceutical Supplements, LLC, USA	US$ 125	Stomach and liver tonic

Milk thistle extract is now marketed as silymarin and silybinin capsules and tablets with an improved bioavailability under the trade names like Livergol®, Silipide® (Siliphos®) and Legalon®[54]. Indena, Italy

experimented in rats that after oral administration of 200 mg/kg of silybin, the plasma levels of silybin and its conjugated metabolites were below the analytical detection limit, while, after oral administration of Silipide® (200 mg/kg as silybin) the plasma levels of silybin (free and total) were easily measurable, being well absorbed within minutes when in phytosomal form[55, 56]. Livergol® capsules were tested for the study of acute, viral hepatitis, in which 29 patients were treated with silymarin showing a definite therapeutic influence on the characteristic increased serum levels of bilirubin and liver enzymes compared with a placebo group[57, 58]. In a study of Livergol® capsules in chronic viral hepatitis, silymarin was shown to result in dramatic improvement. Use at a high dose (420 mg of silymarin) for periods of 3-12 months resulted in a reversal of liver cell damage (biopsy), an increase in protein level in the blood, and a lowering of liver enzymes. Common symptoms of hepatitis (abdominal discomfort, decreased appetite, and fatigue) were all improved[57, 59].

CONCLUSION

This article briefly reviews the phytochemistry, pharmacological, therapeutic applications, traditional knowledge and different formulations of the plant *Silybum marianum*. The plant had been extensively used as a medicinal and legendary plant since a long period of time. Some of its branded products in the market along with its dose and price have also been mentioned. This is an attempt to compile and document the information on different aspects of *S. marianum* and highlight the needs for research and development in future.

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