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Phytochemical study of milk thistle, *Silybum marianum* (L.) Gaertn.

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ABSTRACT

The milk thistle Silybum Marianum (L.) Gaertn. is one of the medicinal plants with many therapeutic properties. Silymarin is the active ingredient of the milk thistle plant, which is rich in flavonoid and flavonolignan compounds, and its therapeutic effects have been widely mentioned in medicine. The seed extract of this plant, known as silymarin, protects the liver against various types of poisoning, including Amanita mushrooms and alcohol. However, the results of several studies have shown that silymarin also inhibits the development of prostate and liver cancer. This plant is of great importance due to its medicinal flavonolignans, which are effective in treating various liver diseases, hepatitis, blood lipids, diabetes, cardiovascular diseases, cancer, etc. Therefore, the present study investigates the pharmacological, phytochemical, and physiological properties of the milk thistle plant and its biologically active compounds. The phytochemical extract was examined in three parts of the plant: seeds, leaves, and stems, and its anticancer properties were examined. The finding illustrated that the highest amount of total flavonoids was found in the seeds and stems, followed by the leaves of the plant. Also, these results showed that the effect of time on the amount of flavonoids in this plant and the interaction effect of its harvest month factor on the flavonoids level were significant. It can be concluded that the highest rate of activity in neutralizing free radicals of cancer cells was observed in the seed extract of the plant.

Keywords: Phytochemical, Milk thistle, *Silybum marianum*, Flavonoid, Herbal medicine.
Article type: Report.

INTRODUCTION

Using plants for treatment has a history as old as human one. In recent years, the use of medicinal plants has increased due to their lower costs and side effects than synthetic drugs. Almost eight thousand known plant species grow in Kazakhstan, most of which may have medicinal properties (Abd-El-hady & Arafa 2019). Many medicinal plants are found naturally in their specific habitats. Since these plants are of particular importance in the world for nutrition and disease treatment, identifying their organic compounds, especially the indigenous species of the country, has attracted the attention of researchers and scholars. With its large size and diverse climate, Kazakhstan has a very diverse vegetation cover (Agidew 2022). Milk thistle, *Silybum marianum* is a one- or two-year-old herbaceous plant from the Compositae family. The seeds of this plant mainly contain the active pharmaceutical ingredient called silymarin. These active ingredients are used in the treatment of liver problems and some other diseases. The leaves of this plant are prickly and have side spots around the veins. Milk thistle is a self-pollinating plant with pink or purple flowers; its flowering time is in May and June. This plant's propagation method is through

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seeds, and all parts of this plant, including roots, aerial parts, and seeds, are used for medicinal purposes (Aziz *et al.* 2021). Milk thistle grows in Mediterranean regions and flat plains with warm climates and light sandy soils. The stem height of this plant is 430 to 920 cm, and its leaves are broad and lanceolate and include purple tubular petals (Bijak 2017). The fruit of this plant is a brown or gray hazelnut (Drouet *et al.* 2020). Milk thistle contains substances called silymarin, which itself contains four main isomers called silybin, isosilybin, silydianin, and silykrijatin, attributing the most effects of the plant to this group of substances (Elhassaneen & Mahran 2024). In addition, the seeds contain other flavonolignans, and oils are also present (flavonolignans, betaine, apigenin, silandrin, silymonin, silybonol, proteins; Bhattacharya 2020). Silymarin is a mixture of flavonoids shown to have hepatoprotective and antioxidant effects (Fanai *et al.* 2024). Due to the presence of important secondary metabolites, including flavonoids such as silymarin, this plant exhibits therapeutic properties.

Fig. 1. Milk thistle, *Silybum marianum.*

Several *in vitro* and clinical studies have shown that silymarin protects the liver against toxicity caused by carbon tetrachloride, acetaminophen, and carbon tetrachloride (Ghanbari Moheb Seraj *et al.* 2021; Fraschini *et al.* 2002; Fanai *et al.* 2024). It was presented that silymarin exerts hepatoprotective effects through multiple procedures, including stimulation of DNA polymerase, stabilization of cell membranes, inhibition of free radicals, and increased cellular glutathione concentration (Hosseinabadi *et al.* 2019; Javeed *et al.* 2022). Stimulation of DNA polymerase by silymarin increases ribosomal RNA synthesis and, as a result, liver cell regeneration. Increased cellular glutathione concentration also stabilizes cellular superoxide dismutase and glutathione peroxidase. Silymarin also reduces hepatic inflammation by inhibiting the lipoxygenase cycle and inhibiting the leukotrienes and free radicals in the production of the rat. In addition, silybin prevents the production of lipid peroxidation and cell damage in rat hepatocytes (Javeed *et al.* 2022). Numerous studies on living animals indicate that silymarin keeps liver cells against various injuries, containing viruses, chemicals, and natural toxins such as the *Amanita* mushroom poison and alcohol. Pretreatment of laboratory animals with silymarin protects against lethal poisoning caused by *Amanita* mushroom. In dogs, administration of silybin (50 mg kg^{-1}) protected them against poisoning caused by a lethal dose of *Amanita* mushroom even after 40 hours of administration (Kachel *et al.* 2023). In addition, it significantly prevents liver toxicity and lipid peroxidation induced by halothane, thallium tetrachloride, and acetaminophen. Silymarin inhibits hepatic fibrosis induced by bile duct obstruction in rats (Katiyar *et al.* 1997). It is used in traditional medicine worldwide to treat various liver illnesses (Kaur & Agarwal 2007). Clinical studies indicate that the consumption of 120 mg silybin (complexed with phosphatidylcholine twice daily for 2 months significantly reduced serum AST and ALT in liver patients (Fanai *et al.* 2024). Silymarin is widely used in the treatment of poisoning caused by *Amanita* mushrooms. This treatment has reduced mortality by 60-80% in patients (Kristenson *et al.* 1983). Intravenous silybin administration (25-60 mg kg^{-1} day^{-1} for 1 week) within 48 hours of *Amanita* poisoning completely inhibits liver damage. In a study of 205 individuals poisoned by *Amanita* mushrooms, 46 deaths were reported in the untreated group. No deaths were reported in the 16 individuals who were administered silybin (Le *et al.* 2018). However, in a double-blind study of patients with cirrhosis, silymarin administration at doses of 280 and 150 mg three times a day did not decrease mortality in comparison to the control one (Lucini *et al.* 2016). In another study of 116 patients with alcoholic hepatitis, silymarin administration at 420 mg/day was not linked to a significant reduction in mortality in comparison to the control one. It showed no significant improvement over the control group for three months, but in this study, about half of the cases could stop drinking alcohol (Maaloul *et al.* 2024). *In vitro* data indicate that silymarin, particularly silybin, has

chemopreventive impacts on epidermal, prostate, and breast cancer cells in mice and animals (Maaloul *et al.* 2024). Silymarin has a cytoprotective effect on human prostate and breast cancer cells exposed to carcinogens (Roberts *et al.* 2002). Pre-inoculation of cancer cells with silybin before exposure to adriamycin enhances the effect of adriamycin in inhibiting cell growth. Given the potent antioxidant effects of milk thistle, there is concern that this plant may interfere with the stabilization of the cytotoxic action of chemotherapeutic drugs that act through peroxidative biochemical pathways, however, in the human breast and ovarian cancer cells, silybin potentiates the cytotoxic effects of cisplatin and doxorubicin, and there is no evidence of interference with their cytotoxic effect. Animal data confirm that silymarin protects against carcinogenesis in various mouse models of epithelial tumors. For example, giving silymarin to mice protected them against the effects of carcinogens and UVB radiation (Saller *et al.* 2008). The stimulatory impacts of silymarin on liver DNA are on non-cancerous cells. In research on rats bearing hepatomas, silymarin did not stimulate tumor development. The present study investigated milk thistle's pharmacological, phytochemical, and physiological properties and its biologically active compounds in different parts and at different harvest times. It also presented a method for obtaining the most flavonoids.

MATERIALS AND METHODS

Botany

Milk thistle is a plant from the chicory family with the scientific name *Silybum marianum*. It is a biennial plant with hairless, dull green, spiny, erected stems. This plant has slightly branched or thick branches that end in a green cap and have longitudinal grooves. It has several ovate-triangular segments. Its flower is pink-purple, ciliated, and spiny, each ending in a wide and spear-shaped appendage, and in the lower part, it is spiny, spiky, and widely turned. The milk thistle was collected from the Almaty in June and the plant species was identified by the medicinal plants research institute of Kazakhestan. The plants collected for the experiment were dried under normal conditions and away from sunlight and ground into powder by a grinder. One of the initial steps in phytochemical studies is to determine the type of secondary metabolites present in the compound. Preliminary phytochemical tests (Wellington & Jarvis 2001) were performed using powdered samples and standard methods to identify active ingredients.

Chemical compositions

The chemical compositions of milk thistle were determined according to the methods described in AOAC (2005). Oil content was determined by (official method no 935.38) using n-hexan in a Soxhlet apparatus. The crude protein content was determined using factor 6.25 (official method no 950.36), crude fibers determined by (official method no 950.37) and ash content determined by (official method no 930.22) in muffle furnace at 450-500 °C. Total carbohydrate was calculated by differences (Abd-El-hady & Arafa 2019). The total phenolics of methanolic MS extract were determined by the method described by Jindal & Singh (1975). Total flavonoids of methanolic extract were determined following the methodology of Zhishen *et al.* (1999), modified by Kim *et al.* (2003). The seed extract of this plant contains many compounds, including: silybin A and B, silydianin, silychristin, apigenin, dihydrosilybin, dioxysilychristin, dioxysilydianin, etc. The dry seed extract of the plant contains 1 to 4% silymarin, which includes flavonoids including silybin A and B, silydianin, silychristin, and dihydrosilybin (Elhassaneen & Mahran 2024). In addition, the dry seed of the plant contains 15 to 20% oil, which has no medicinal properties (Elhassaneen & Mahran 2024). About 20 to 50% of the seed extract of this plant is absorbed by the body after oral consumption. However, the phosphatidylcholine complex has a higher absorption. The absorption of this drug from the digestive tract is rapid and reaches its maximum blood concentration after 12 to 240 minutes. Its elimination half-life is 6 hours (Taleb *et al.* 2018). A rate of 10% of this drug is excreted in the bile, and as a result, this drug has a strong biliary absorption and excretion. The bioavailability of this drug depends on the type of formulation. It has been reported that the bioavailability of the German-made drug Legalon is twice that of other formulations. Silybin is the most effective substance in silymarin, known as an antioxidant and liver protector, and its concentration in bile is 60 times that in blood. Reports of laboratory studies indicate that administering silymarin to laboratory animals with hyperlipidemia prevented the formation of atherosclerotic plaque in their aorta (Valková *et al.* 2020).

RESULTS

The flavonoid complex in the extract of this plant is called "silymarin", which contains several different types of flavonoids. Milk thistle seeds contain approximately 4–6% silymarin; this plant extract contains 65–80%

silymarin and 20–35% fatty acids (Agidew 2022). Silymarin contains seven types of flavonolignans (silybin type A, silybin type B, isosilybin type A, isosilybin type B, silychristin, isosilychristin, and silydianin) and one taxifolin (yellow or yellow dye). Silymarin is produced from the phenylalanine biosynthesis pathway, which has two pathways: one through the production of coniferyl aldehyde by cinnamyl alcohol dehydrogenase and the other through the production of taxifolin by chalcone synthase (Drouet *et al.* 2020). Silymarin also protects the liver from damage by inhibiting the NF-κB gene and subsequently reducing the production of pro-inflammatory cytokines (Elhassaneen & Mahran 2024). Silymarin stabilizes the membrane by affecting intracellular glutathione and preventing membrane lipoperoxidation and related cellular production.

It prevents inflammation by inhibiting the cyclooxygenase cycle, inhibiting leukotrienes, and inhibiting the production of free radicals in cells (Javeed *et al.* 2022). The presence of a methoxy group in one of the phenolic rings of silymarin increases its antioxidant properties. Silymarin can help the antioxidant defense system in several ways: (i) Direct inhibition of free radicals; (ii) Prevent free radicals' formation by inhibiting the enzymes responsible for producing free radicals or by maintaining the integrity of the electron transport chain from mitochondria under oxidative stress conditions; and (iii) by activating a wide range of enzymes belonging to the antioxidant defense system and non-enzymatic antioxidants (Maaloul *et al.* 2024). Silymarin increases ribosomal RNA synthesis by stimulating p1a and rRNA transcription (Javeed *et al.* 2022). It stimulates DNA polymerase, and by stimulating it, it increases ribosomal RNA synthesis and, consequently, liver cell regeneration. Silymarin also inhibits the lipoxygenase cycle and the production of free radicals and leukotrienes in Kupffer cells of the mouse liver and reduces liver inflammation (8). It protects the cell from oxidative stress by reducing the level of reduced glutathione. Silymarin has been reported to act as an antioxidant through various pathways, including direct free radical scavenging activity, preventing the formation of free radicals, helping to inhibit specific enzymes involved in the production of free radicals, and maintaining an optimal redox state of the cell by activating a wide range of enzymatic and non-enzymatic antioxidants, mainly through transcription factors, including NF-(Nrf2) E2-related factor. Fig. 2 shows the chromatogram of a standard methanolic solution of silymarin. Four peaks were observed in this chromatogram, each of which represents one of the flavonolignan compounds present in pure silymarin. Two factors affecting the quality of milk thistle are the time of harvest and the components of the plant itself. Therefore, Fig. 2 shows the amount of flavonoids at different harvest times of the year and also different plant components.

As shown in this Fig., the free radicals of the ethanolic extract of the plant leaves showed no significant difference compared to the treatment group with the ethanolic extract of the plant stem. In different samples harvested in December, the highest amount of total silymarin was observed in the plant. The total amount of silymarin in the leaves and stems did not significantly differ. The study of the constituent components of silymarin also showed that the seeds contained the highest amount of taxifolin and the leaves contained the lowest amount. Fig. 2 also shows that the amount of flavonoid in the components of milk thistle flowers can vary by up to 20% depending on the time of harvest, and the winter season is the best time to harvest. The highest level of free radical neutralization activity of cancer cells was observed in the seed extract of the plant, which acts as an antioxidant. Fig. 3 shows the chromatogram of the standard methanolic solution of silymarin. This chromatogram revealed four peaks, each representing one of the flavonolignan compounds in pure silymarin.

CONCLUSION

Considering the lack of side effects of chemical drugs and the cheap and accessible advantages of using medicinal plants, this study attempted to study the botany and pharmacology of milk thistle and its phytochemical aspects. Considering the antioxidant properties of milk thistle extract, this plant can be used in the treatment of various diseases, including liver diseases and those related to liver enzymes and diabetes. These compounds are made in plants by sunlight and are stored in various organs. The synthesis of these compounds in plants depends on the tissue and organ and is affected by environmental factors, plant age, leaf maturity, etc. The present results have shown that the interaction of two factors, harvesting time and organ in the milk thistle plant, was significant in the amount of flavonoids in this plant, and various factors such as seasonal changes, temperature, light, and plant organs were effective on the number of total flavonoids. Therefore, the amount of flavonoids in milk thistle plant parts at different times of the year was investigated, and the results showed that the cold season and the seed part can provide the highest antioxidant properties.

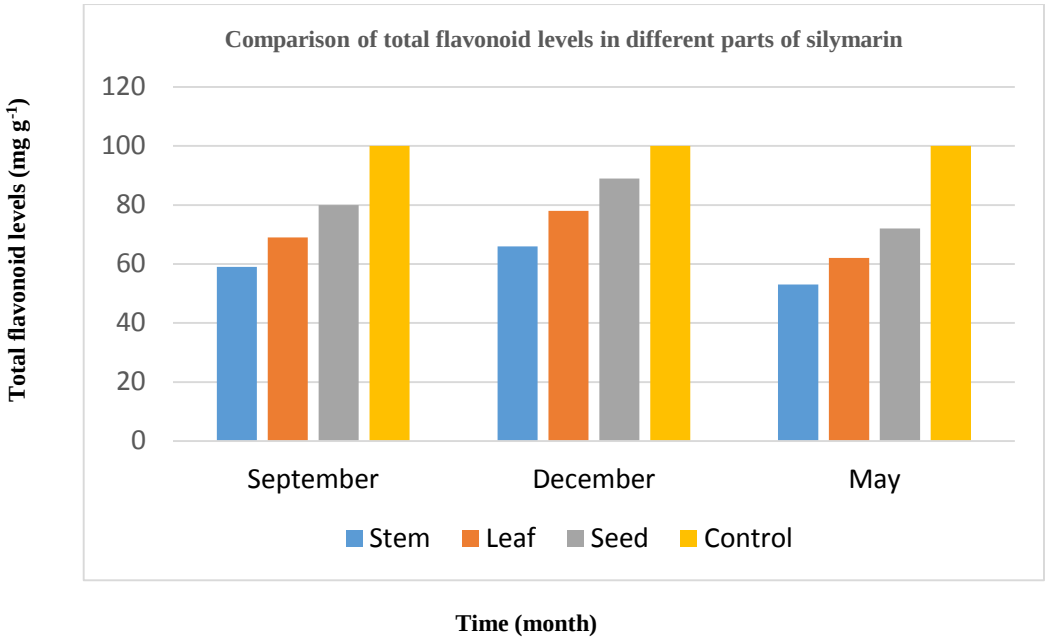

Fig. 2. Comparison of total flavonoid levels in different parts of silymarin during different months.

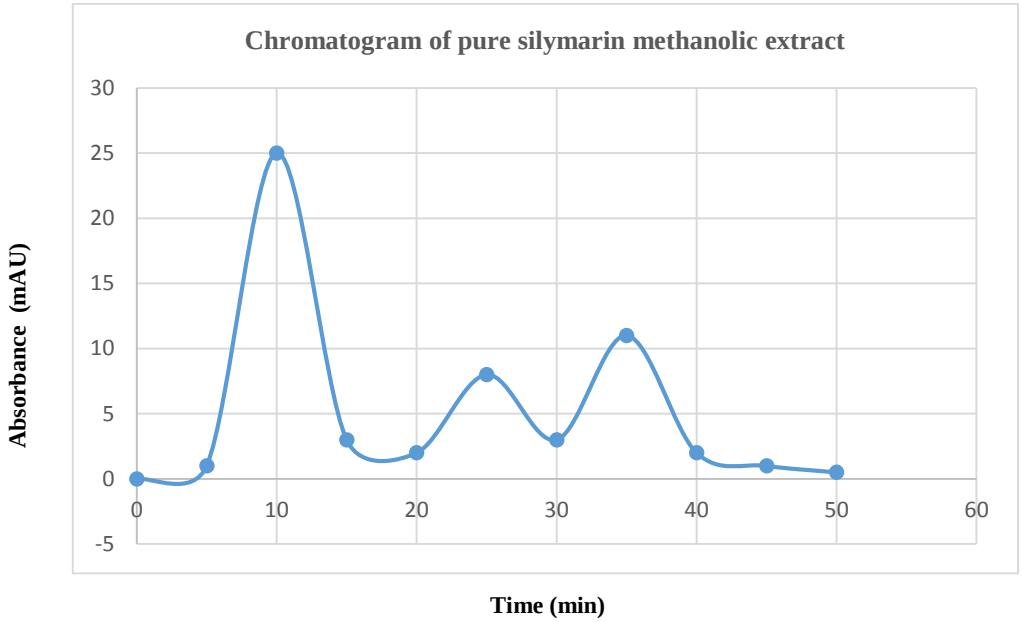

Fig. 3. Chromatogram of pure silymarin methanolic extract.

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