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Phytochemical Profiling of the Methanol Extracts of *Silybum marianum* (Milk Thistle) by FTIR and GCMS Analysis

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Abstract

Milk thistle is an annual or biennial plant belongs to family *Asteraceae.* All parts are used to cure many diseases such as treating toxin poisoning, hepatitis, cirrhosis, fibrosis of liver and stimulate liver Regeneration. From the period of time immemorial, this plant is used to cure all types of liver diseases. Their properties are due to presence of phytocompounds silymarin. It is a mixture of flavonoid this study aimed to analyze complexes, is the active component that protects liver and kidney cells from toxic effects of drugs, including chemotherapy. The seeds contain the highest amount of silymarin, but the whole plant is used medicinally. Hence, this study aimed to analyze the FTIR and GCMS profiles of bioactive phytocompounds present in the seed extract of *Silybum marianum*. The FTIR results of the present study showed the functional groups such as amine salts, ester, sulfoxide, alkene and halocompounds. The GCMS analysis showed the phytocmpounds such as 3-Octadecanone, Undecanoic Acid, 9,15, Octadecadienoic Acid, Methyl Ester, (Z,Z)-, 6-Octadecenoic Acid, Methyl Ester, (Z)-, Hexadecanoic Acid, 15-Methyl-, Methyl Ester, 8-Heptadecyne, 1-Bromo, and 9,12-Octadecadien-1-Ol, (Z,Z)-.

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Introduction

As one of the most important organs in the body, the liver plays a key role in carbohydrate, protein, and fat metabolism [1]. Liver diseases cause over two million deaths annually, accounting for 4% of global mortality (from cirrhosis, viral hepatitis, and liver cancer) (1 out of every 25 deaths) [2]. Every year, liver diseases cause death to over 20 lakh persons (10 lacs from liver cirrhosis, 10 lacs from viral hepatitis, and 10 lacs from liver cancer) [3]. Liver disease and its most serious complications of acute liver failure, cirrhosis, and liver cancer are leading and rising causes of mortality worldwide

[4]. Liver toxicity can be caused by allopathic medications, such as oral contraceptives, ciprofloxacin, paracetamol, diclofenac, fluconazole, amoxicillin, and chlorpromazine. These medications have the potential to cause benign neoplasms, hepatic vein blockage, liver cell death, and fulminant inflammation of the liver. Aflatoxin, alcohol, and carbon tetrachloride are among the harmful substances that can also cause liver damage [5]. Throughout human history, plants have been used for food, fuel, and pharmaceutical purposes [6]. Plant products, as parts of foods or botanical portions and powders, have been used with varying success to cure and prevent diseases throughout history [7]. However, the rapid effectiveness of allopathic medicines and the influence of Bristish colonial culture and western culture, most people worldwide rely on synthetic chemical-based allopathic treatments to cure their diseases. Allopathy treatment such as immunization and certain medications, such as steroids and antivirals, are available to prevent and manage liver illnesses; nevertheless, they are not only expensive but also have undesirable side effects [8]. Due to the side effects of drugs derived from synthetic substances, the tendency towards drugs with natural bioactive components is increasing steadily [9] and the peoples worldwide turned to alternative medicine called traditional medicinal plants treatments with the development of modern medicine [10]. Research on plants to identify natural bioactive components and to find new natural drug raw materials is increasing currently [11]. For example, the plants Terminalia arjuna (Arjuna), Withania somnifera (Ashwagandha), Bacopa monnieri (Brahmi) and Rauvolfa serpentina (Indian snakeroot) are used for treating hypertension; Phyllanthus emblica (Amla), Momordica charantia (Karela),Cinnamomum camphora (Kapur), Tinospora cordifolia (Giloy) and Syzygium cumini (Jamun)used for curing diabetes.In india, the plants Glycyrrhiza glabra, Picrorhiza kurroa, Phyllanthus amarus and *Sylibum marianum* are using to treat the all kind of liver diseases from the very old period.

The fruit and seeds of the milk thistle has been used for medicinal purposes for over 2000 years, most commonly for the treatment of liver disease (cirrhosis and hepatitis) as well as for the protection of the liver from toxic substances [12] and treatment for liver and biliary disorders. The seeds are the medicinal part of the plant, [13]. Hence, the present study have been programmed to find the phytocompounds present in the seeds of *Silybum marianmum* by using the FTIR and GCMS analysis.

MATERIALS AND METHODS

Confirmation of originality of *Silybum marianum* seeds

The seeds of *Silybum marianum* were purchased from the amazon through online order. Seeds of many plants have identical structure, shape and colour with the silybum marianum, therefore, before the seeds make into powder to prepare the seed extract, the originality of seeds were confirmed by observing the seedlings. For this confirmation study, ten seeds were randomly picked out and cultivated in a pot containing soil (25%) and vermicomposte (75%) mixture. Regular watering was done and the leaf and stem appearances were observed to identify and confirm the originality of *Silybum marianum* plant seeds.

Sun drying and grinding of seeds

All the seeds were spread on the newspaper the defective seeds and infected seeds were removed and exposed to direct sunlight for one day to remove if any moisture content in the seeds. After ensuring the complete drying-ness and the seeds were transformed to mixy grinder and made into fine powder. The coarse materials present in the powder were removed by sieve plate.

Soxhlet extraction

Exhaustive extractions in the Soxhlet apparatus were performed using 5 g portions of the material. Precisely weighed powder of *Silybum marianum* seeds were transferred to a paper thimble. The loaded thimble was inserted into a 100-mL Soxhlet extractor. Extractions were performed in the two-step process (n ¼ 3). In the first step of the procedure, the plant material was defatted for 6 h using 75 mL of n-hexane. In the second, *silymarin* was extracted for 5 h with 75 mL of methanol. After cooling to room temperature, the obtained extract was transferred to a 100-mL volumetric flask, which was subsequently filled up to its volume with methanol. Finally, the extract was transferred to open petriplate and air dried under room

Table 1 FTIR Peak Values, Intensities, Functional Groups, and Phytocompounds Identified in Methanol Extract of Silybum marianum

S.No.	Reference Wave number (cm^{-1})	Wave number (cm^{-1}) (Test Sample)	Functional group assignment	Intensity	Phyto compound identified
1.	3000-2800	2926	N-H Stretching	Strong,broad	Amine salt
2.	3000-2800	2854	N-H Stretching	Strong,broad	Amine salt
3.	1750-1735	1739	C=Stretching	Strong	Esters
4.	-	1464	-	-	Unknown
5.	1070-1030	1055	S=O Stretching	Strong	Sulfoxide
6.	995-985	992	C=C Bending	Strong	Alkene
7.	-	928	-	-	Unknown
8.	850-550	836	C-Cl Stretching	Strong	Halo compound
9.	730-665	730	C=C Bending	Strong	Alkene

temperature. The air dried extract was further dried with the help of vacuum pressure.

FTIR and GCMS Analysis

Before the preparation of pellet, all the parts of die were cleaned with chloroform thoroughly to get rid of any dirt and dry them with tissue paper. Similarly, the mortar and pestle were cleaned well with chloroform or acetone thoroughly to get rid of any dirt and dry them with tissue. Scoop out dry KBr into the mortar and dried methanol extract of *Silybum marianum* seed at the ratio 100:1. Grind using the pestle.

Continue for a minute or two to get a homogenous mix. Fix the Pellet Press together. Insert the die into the cavity. Transfer the ground sample mix into the cavity using a metal spatula. Make sure it's evenly spread. Insert the bolt press and rotate into the cavity to distribute the particles. Transfer the whole die set to the hydraulic pellet press and rotate the wheel to secure it tightly.

Close the valve of the hydraulic press. Pull the level to put pressure until handle becomes tight. After 30 seconds, release the die first need to loosen the pressure, then move up the upper wheel of the press and then take out the die.

Take out the bottom Plunger and place the die in the ejector. Apply pressure to release the pellet from the die. Finally The translucent pellet was transferred to a pellet holder and positioned in the FTIR machine for analysis. Infrared ryas were applied. The data of infrared transmittance was collected over a wave number ranged from 4000 cm−1 to 500 cm−1. All the samples were analyzed in triplicates with plain KBr pellets as blank. The spectral data were compared with a reference to identify the functional groups existing in the sample. FTIR analysis was done by the instrument FT/IR-6300 type, S. No-A021261024 BMDLABS Company in standard light sources by using TGS detector at resolution 4cm-1 (**Figure 1**,**Figure 2**)

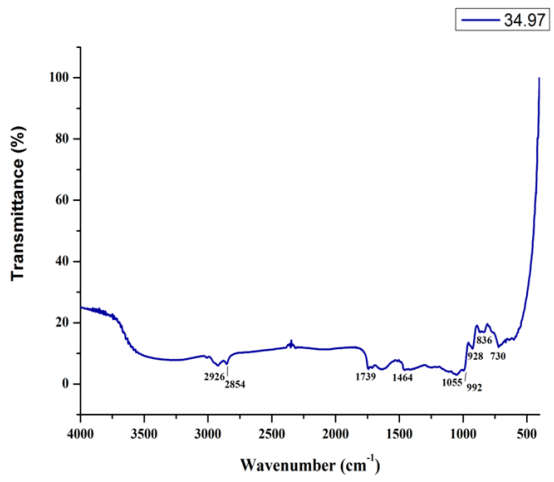

Figure 1 Shows the FTIR spectral peak for methanol extract of the plant Silybum marianum

GCMS analysis

100 mg of dried methanol extract rediluted methanol in an orbital shaker for 72 h. The GCMS analysis was done by GCMS- Perkin Elme.

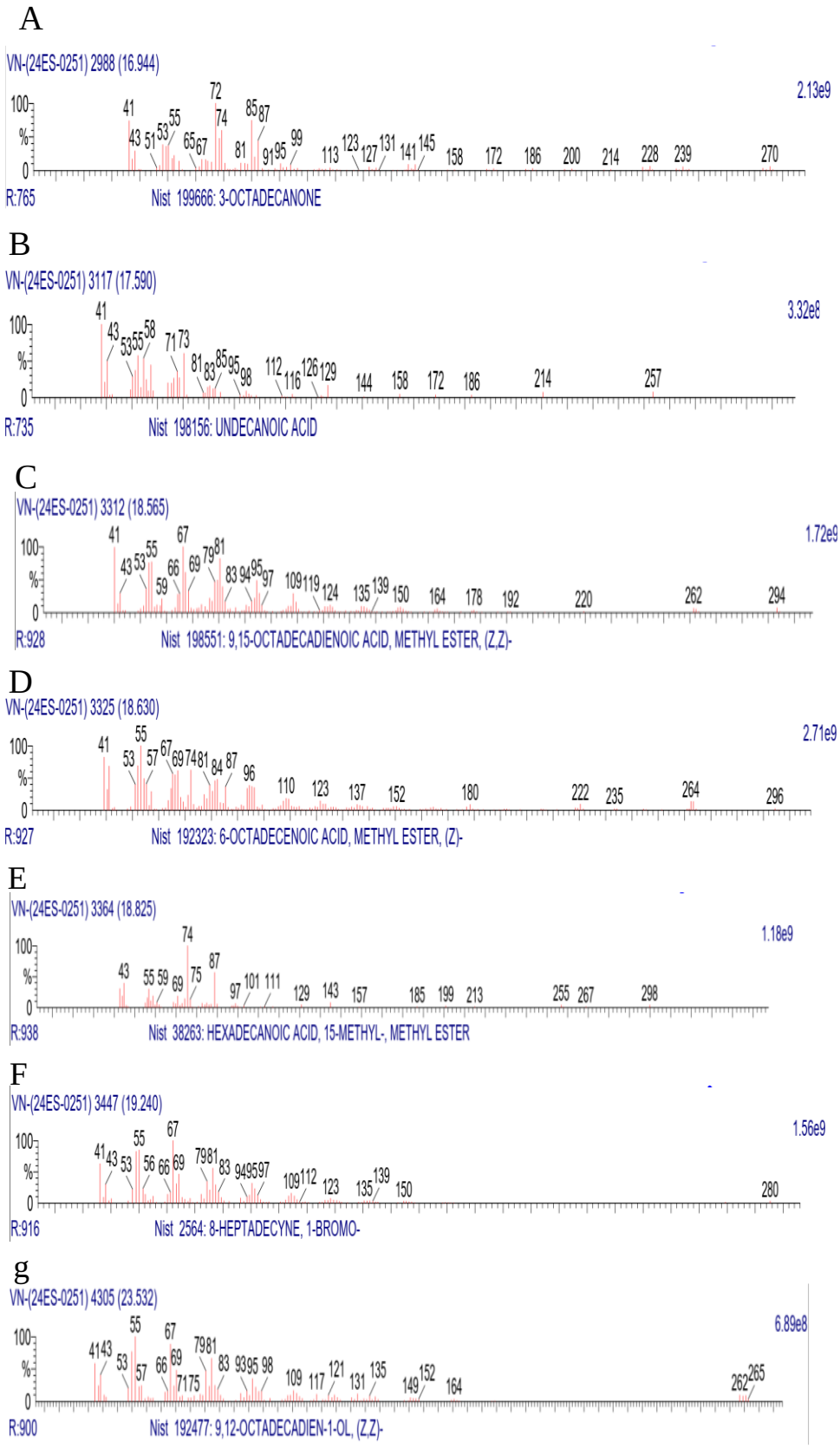

Figure 2 Retention time and related bioactive phytocompounds in the methanolic leaf extract of Silybum marianum

Table 2 Retention time, area%, name , molecular formula, molecular weight and uses of the GCMS analyzed bioactive phytocompounds of Silybum marianum

PK.No	RT	Area%	Chemical Compounds	M.F	M.Wt	Uses
1	16.944	8.657	3-OCTADECANONE	C18H36O	268	Antimicrobial Properties, Anti-inflammatory Effect Analgesic Activity, Skin Heal, Potential Role in Cancer Therapy-inhibit the growth of colorectal carcinoma cells. Antimalarial activity against erythrocytic stages of Plasmodium falciparum the parasite that causes malaria, and also inhibits cancer cell proliferation in vitro.
2	17.590	2.866	UNDECANOIC ACID	C11H22O2	186	Antifungal properties- is used to treat fungal infections of the skin, such as athlete's foot and ringworm, Antimicrobial Activity, Potential in Managing Epilepsy, Skin Health, Weight Management and Metabolic Health, Potential Neuroprotective Effects
3	18.565	16.595	9,15-OCTADECADIENOIC ACID, METHYL ESTER, (Z,Z)-	C19H34O2	294	Anti-inflammatory Properties, Cardiovascular Health Neurological Benefits, Cancer Research
4	18.630	26.709	6-OCTADECENOIC ACID, METHYL ESTER, (Z)-	C19H36O2	296	Antimicrobial Activity, Anti-inflammatory Effects Skin Health, Potential Role in Cancer Therapy, Neuroprotective Properties, Lipid Metabolism
5	18.825	2.062	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	C18H36O2	284	Antibacterial, Antifungal, Anti-inflammatory, Hepatoprotective, Anticancer, Antitumor, Anticoronary Hypocholesterolemic
6	19.240	34.476	8-HEPTADECYNE, 1-BROMO	C17H31Br	314	Antimicrobial Properties, Anti-inflammatory Agents Cancer Research, Neurological Applications, Drug Delivery Systems, Synthetic Intermediates, Potential as Antiviral Agents
7	25.532	8.636	9,12-OCTADECADIEN-1-OL, (Z,Z)-	C17H31Br	314	Antibacterial, Antifungal, Anti-inflammatory, Hepatoprotective, Anticancer, Antitumor, Anticoronary Hypocholesterolemic

RESULTS AND DISCUSSION

The FTIR examination of the methanolic seed extract of revealed the presence of a total of nine peaks indicate the presence of nine bioactive compounds The seven known peaks were observed at 2926 and 2854 (strong and broad bands with N-H stretching) indicates the presence of amine salts have a concordancewith the peak observed at 416.15 with C-N-C bend in the methanolic extract of plant *Sphaeranthus indicus* [14] peak at 2854.69 with strong N-H stretchy for amine salts in the methanolic extract of fresh wet leafless tems of *Sarcostemma viminalea* [15] peaks at 3410, 3371 and 3317 with C-H stretch in the methanolic extract of *Leucas aspera* of primary and secondary amines at peaks 3942, 3873, 3803 (O-H stretch) in the methanolic extract of *Leucas aspera* of fraction- II of primary ,secondary amines and amides [16]. The amines are very important in medicinal chemistry, and any number of legal (and illegal) drugs contains the amine salt functional group. The reason for this is water solubility; a water-soluble molecule is more easily taken in by the human body, and is more bioavailable than a water insoluble molecule. [17] (**Table 1**).

The spectral peak at 1739 (strong C=stretching) in the present study indicating the presence of ester has a concorant results with the methanolic leaf extract of *cassia alata* has peaks at and peaks at 1452.14 with C-C for ester [18]. It is an orally bioavailable isopropylester cytidine analog used to treat COVID-19. A vitamin important for retinal function that is used clinically to correct vitamin A deficiency and Zuretinol acetate has been used in trials studying the treatment of Impaired Dark Adaptation, RP (Retinitis Pigmentosa), Retinitis Pigmentosa (RP), and LCA (Leber Congenital Amaurosis). Some kind of esters that are employed more for its preservation properties than for its distinctive smell. It is widely used in cosmetics, toothpastes, hair care products, moisturizers, and deodorants. It is also used as a food preservative and as a product to protect pharmaceuticals against fungal decay (**Table 1**).

Spectral peaks at 992 and 730 with strong C=C indicating the presence of bioactive compounds such as alkene showed a relationship with peaks at 1452.14 with C-C bond observed for alkenes in the methanolic leaf extract of *cassia alata [18]*and peak at 923.68 with strong C=C bond in the fresh and wet photosynthetic leafless stem of *Sarcostemma viminalea* for alkenes [15]. The alkenes are unsaturated hydrocarbons containing at least one C=C bond. It is used n the treatment of severe psoriasis as an oral retinoid,in myocardial perfusion scintigraphy and to treat supraventricular tachycardia.

Peak at 836 (strong C-Cl stretching) indicate the presence of halocompounds showed similarity with the peak at 605.72 with C-CL stretchy halo compounds observed in the methanolic extract of fresh and wet photosynthetic leafless stem of *Sarcostemma viminalea* [15]. Halo compounds are hydrocarbons with halogen atoms replacing some or all of their hydrogen atoms. It is used as antibiotic to treats typhoid fever (Chloramphenicol), to treat malaria (Chloroquine), reduces the risk of cardiovascular death and heart failure hospitalization in certain high-risk patients (Vericiguat), Chiral alkyl halide anesthetics that are reversible and cause loss of consciousness (Isoflurane and halothane), immunosuppressant that treats atopic dermatitis (eczema) (Pimecrolimus), inhibits cancer cell growth (Spongistatins),antifungal and insecticidal (Jaspamide), antifungal, nematicidal, and antimicrobial (Lachnumone),powerful fungicides and insecticides (Pyrrolnitrin and dioxapyrrolomycin) and also used as flame retardants, propellants, solvents, refrigerants, and fire extinguishants.

The peaks at 1055 (strong S=O stretching)indicates the presence of sulfoxide has some concordance with the the peaks at 1167.69 with S=O bond indicates the presence of sulfones, sulfonyl chlorides, sulphates and sulphonamides in the of methanolic extract of *cassia alata [18]* peaks at 1357.89 NO2 antisym stretch NO2 in salfonamides in the leaves of *Mollugo lotoides* [19], peaks at 1382.96 SO2 antisym stretch of sulfonyl chlorides and 1072.42 with symstretch SO3H of sulfonic acide in the raction I and II of *Indigofera tinctoria* [20], peaks at 1068.56 with SO3 H Sym Stretch of sulfonic acids and 1381.03 SO3H with SO2 antisym stretch of sulfonic acids in the leaf *of Tinospora cardifolia* [21].

It contains a class of organic compounds containing sulfur and oxygen and having the general formula (RR′) SO, in which R and R′ are a grouping of carbon and hydrogen

atoms. The sulfoxides are good solvents for salts and polar compounds. The best-known sulfoxide is dimethyl (or methyl) sulfoxide (DMSO), which is prepared by aerial oxidation of dimethyl sulfide (a by-product of paper manufacture) in the presence of nitrogen dioxide. It is also used as a solvent for drugs and antitoxins applied topically. The sulfoxide functional group occurs in several drugs. Notable is esomeprazole, the optically pure form of the proton-pump inhibitor omeprazole. Another commercially important sulfoxides include armodafinil. Based on its remarkable ability to penetrate animal tissues, DMSO is used as a cryoprotectant because it decreases osmotic stress and cellular dehydration, and thereby enables stem cells to be stored for several years, treatment of interstitial cystitis, and as a penetrating vehicle for various drugs. It is such as treatment of musculoskeletal and dermatological diseases, cryopreservation of stem cells, treatment of interstitial cystitis, treatment of increased intracranial pressure, and many more [22]. Sulfoxides is the most important sulfur antioxidants present in vegetables. Sulfoxides are common in vegetables of the genus Allium, particularly garlic (*Allium sativum*), one of the oldest medicinal plants.

The bioactive phytocompounds in this extract were analyzed using GC-MS. The chromatogram obtained from the GC-MS analysis of the methanol extract of *Silybum marianum* revealed the presence of seven prominent peaks at different retention times such as 17.590, 18.565, 18.630, 18.825, 19.240 and 25.532. The components corresponding to these seven peaks were identified as follows. 3-Octadecanone, Undecanoic Acid, 9,15, Octadecadienoic Acid, Methyl Ester, (Z,Z)-, 6-Octadecenoic Acid, Methyl Ester, (Z)-, Hexadecanoic Acid, 15-Methyl-, Methyl Ester, 8-Heptadecyne, 1-Bromo, and 9,12-Octadecadien-1-Ol, (Z,Z)-. (**Table 2**).

CONCLUSION

This study has demonstrated that the methanol seed extract of *Silybum marianum* (milk thistle) is rich in bioactive compounds with strong potential to support liver health. Using FTIR and GC-MS analyses, we identified several important functional groups, including amines, esters, alkenes, halocompounds, and sulfoxides-each known for their ability to protect the liver, reduce inflammation, and combat oxidative stress. These findings confirm that the plant holds significant promise as a natural remedy for liver diseases.

Interestingly, the traditional use of *Silybum marianum* by indigenous tribes in the Tamil Nadu hills further supports its therapeutic potential. For generations, these communities have consumed the seeds of *Silybum marianum* as a coffee-like drink each morning to help prevent liver diseases. This traditional practice aligns perfectly with the modern scientific understanding of the plant's medicinal properties, reinforcing the value of indigenous knowledge in guiding future research and healthcare practices.

The results from the FTIR and GC-MS analyses not only highlight the plant's ability to promote liver health but also suggest its broader therapeutic applications. Compounds such as amine salts, esters, and alkenes play crucial roles in enhancing bioavailability and reducing inflammation, both of which are vital for liver regeneration and protection. The presence of halocompounds and sulfoxides also points to the potential of *Silybum marianum* in treating a variety of health conditions, including infections and even cancer.

In conclusion, *Silybum marianum* is a plant with great promise, offering a natural and effective solution for liver protection. The combination of traditional use and scientific validation makes it an exciting candidate for further research. With its wealth of bioactive compounds, *Silybum marianum* has the potential to become an important part of both preventive and therapeutic approaches to liver diseases. More clinical studies are needed to fully understand its benefits and to establish it as a reliable natural treatment in modern medicine.

Ethical Approval

No ethical approval was necessary for this study.

Author Contribution

All authors significantly contributed to laboratory activities; including plant extract preparation, FTIR and GCMS analysis, manuscript preparation, and tabulation. They consented to submit to the current journal, provided final approval for the version to be published and accepted accountability for all aspects of the work. All authors meet the eligibility criteria established by

the International Committee of Medical Journal Editors.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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