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Phytochemical screening and GC–MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L).

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ABSTRACT

Objective: To investigate the phytochemicals and GC-MS analysis of ethanol extracts of *Silybum marianum*.
Methods: The air-dried leaves were powdered and subjected to selective sequential extraction using solvents of increasing polarity through percolation, ethanol to obtain an ethanolic extract. Then, each of the extracts was further subjected to gas chromatography-mass spectrometry.
Results: Qualitative determination of the different biologically active compounds from crude extracts of *Silybum marianum* using gas chromatography-mass spectrometry revealed different types of high and low molecular weight chemical entities with varying amounts present in each of the extracts. These chemical compounds are considered biologically and pharmacologically important.
Conclusions: The study established the chemical composition and anticancer activity of the plant.

Keywords: *Silybum marianum*, Phytochemicals screening, GC–MS analysis, Bioactive compounds.

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INTRODUCTION

Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments [1]. They can be a source of chemical compounds of biological and pharmacological importance. History reveals that plants are sources of successful drugs and will continuously be important for screening of new lead compounds [2]. The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs [3]. The various parts of the plant are used in the Indian traditional medicine for the treatment of various diseases like asthma, joint pain, lumbar pain and sprains, cough, eczema, malaria, rheumatism, swellings, venereal diseases [4, 5, 6].

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [7]. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides [8]. These compounds were synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are taxonomically and chemically extremely diverse compounds with obscure function. They are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas [9]. In this biochemicals are often referred to as Secondary metabolites which are useful to the traditional medicine system and these biochemicals are identified by using GC-MS technique [10].

Gas Chromatography-Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the quantification and identification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [11]. In the last few years, gas chromatography-mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolites profiling in both plant and non-plant species [12, 13, 14].

Silybum marianum (L) is an essential medicinal plant that has volatile oils and other secondary metabolites. This

common name is Milk thistle. The most common compound in milk thistle is silymarin, which is an isomeric mixture of flavonolignans (silybin, silychristin, and silydanin) present in *S. marianum* (L). Silymarin acts as a strong anti-hepatotoxic, which has been used for chronic inflammatory liver disease and liver cirrhosis [15]. Milk thistle is hepatoprotectants for Cancer Patients. Toleration of cancer therapy may progress from using this herb, the reason is that it is blood and liver toxin clearing agents [16]. It has strong anticancer effects against breast, tumours, ectocervical and prostate [17]. Nowadays, the use of medicinal plants and their bioactive phytocompounds and our scientific information about them comprises the modern field of the photoscience. This is a science created from the incorporation of a range of disciplines that have never been connected before, combining some various areas of economic, social, and political fields, chemistry, biochemistry, physiology, microbiology, medicine, and agriculture. Hence the present study focused on Phytochemical Screening and analysis of Bioactive Compounds of ethanolic leaves Extract of *S. marianum* (L.) using Gas chromatography and mass spectrometry.

MATERIALS AND METHODS

Plant collection and Preparation of plant extracts

Fresh, healthy, and young leaves of *Silybum marianum* were collected from the Himalayan Region, India. The leaves were cleaned and dried in shade for 7 days and then ground well to a fine powder. About 500 g of dry powder was extracted with ethanol (80%) at 70°C by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 hrs, and the ethanolic extract was then filtered and kept in a hot air oven at 40°C for 24 hrs to evaporate the ethanol from it. A dark brown residue was obtained. The residue was kept separately in airtight containers and stored in a deep freezer.

Phytochemical analysis tests

Phytochemical analysis of an ethanolic extract of *Silybum marianum* leaves for secondary metabolites such as alkaloids, flavonoids, carbohydrates, proteins, phenols, saponins, tannins, terpenoids, phytosterols, and phlobatannins was done using standard methods [18].

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (MHz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from the relative peak area of each component in the chromatogram.

Identification of Compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1: Phytochemical Analysis of *S.m* (L)

S.No	Test	Result
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	-
4	Tannins	+
5	Terpenoids	-
6	Phenol	+
7	Cardiac glycosides	+
8	Anthroquinones	+

- = Absence; + = Presence

The results of phytochemical characterization ethanolic extracts of *S. marianum* are shown in Table 1. Phytochemical analysis of an ethanolic extract of the plant also revealed the presence of alkaloids, flavonoids, tannins, phenol, cardiac glycosides, anthraquinones. Phytochemical analysis of an ethanolic extract of the plant also revealed the absence of saponins, terpenoids.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

Phytochemical components in ethanolic extract of *S. marianum* by GC-MS report. The GC-MS analysis revealed the presence of 10 compounds (Table 2 and 3) from the ethanolic leaves extract of *S. marianum* (Figure 1). From the results, it was observed that presence of (+)-2-Born alone, Isobornyl thiocyanoacetate, a-Santoline alcohol, Dodecane, Dodecane, 2,6,11-trimethyl-, Hexadecane, d-Mannose, Undecanoic Acid, 9-Octadecanoic acid, Oleic Acid. Above these compounds were identified based on the RT value, molecular weight, molecular formula, etc (Fig 1) and table 2, 3.

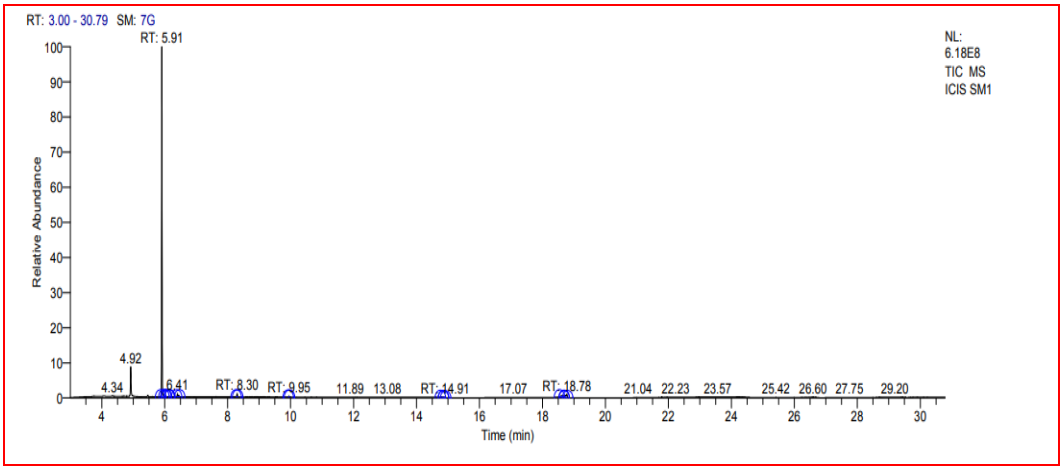


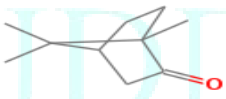
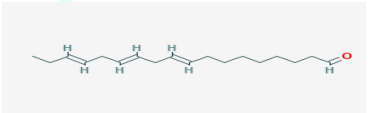
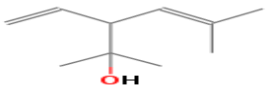
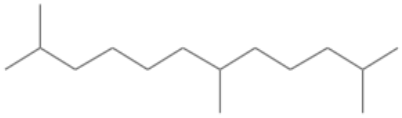
Figure 1: GC-MS CHROMATOGRAM OF *Silybum marianum* (L).

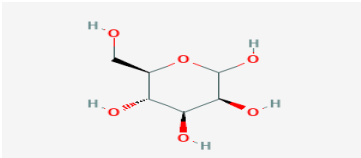
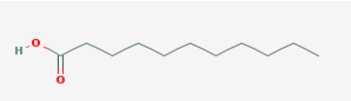
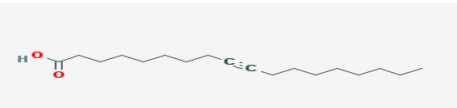
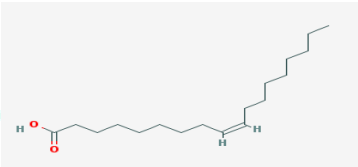
Table 2: Physical properties of bioactive compounds in *S.m* (L)

S.No	Name of the Compound	RT	Molecular Formula	CAS Registry Number
1	(+)-2-Bornanone	5.91	$C_{10}H_{16}O$	464-49-3
2	9,12,15-Octadecatrienal	6.05	$C_{13}H_{30}O$	26537-71-3
3	α-Santoline alcohol	6.15	$C_{10}H_{18}O$	90823-36-2
4	Dodecane	6.41	$C_{12}H_{26}$	112-40-3
5	Dodecane, 2,6,11-trimethyl-	8.30	$C_{15}H_{32}$	31295-56-4
6	Hexadecane	9.95	$C_{16}H_{34}$	544-76-3
7	d-Mannose	14.79	$C_6H_{12}O_6$	3458-28-4
8	Undecanoic Acid	14.91	$C_{11}H_{22}O_2$	112-37-8
9	9-Octadecenoic acid	18.65	$C_{18}H_{32}O_2$	26537-70-2
10	Oleic Acid	18.78	$C_{18}H_{34}O_2$	112-80-1

RT= Retention Time

Table 3: GC-MS Analysis of *S.m* (L)

Name of the Compound	Nature of the Compound	Structure	Mol. Wt (g/mol)	Activity
(+)-2-Bornanone	Monoterpene oxide		152.2334	Antitumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide, Anticancer.
9,12,15-Octadecatrienal	Aldehyde		262.437	Antibacterial and Antioxidant activity
α-Santoline alcohol	alcohol		154.253	Anticancer effect
Dodecane	Alkane		170.34	Antibacterial activity
Dodecane, 2,6,11-trimethyl-	Alkane		212.41	No activity

Hexadecane	Alkane		226.448	Antimicrobial and Antioxidant Activity
d-Mannose	Sugar		180.156	Antibacterial Activity, Antimalarials Agents, Antiprotozoal Agents, Antiparasitic Agents.
Undecanoic Acid	Carboxilic Acid		186.295	Antimycotic activity
9-Octadecynoic acid	Carboxilic Acid		280.452	A novel DNA binding agent
Oleic Acid	Carboxylic Acid		282.468	Antimicrobial activity, Antibacterial activity, Antitumour activity.

(+)-2-Bornanone is an aromatic compound. It has 5.91 RT value, $C_{10}H_{16}O$ molecular formula and 152.2334 molecular weight. It has anti-tumour, analgesic anti-bacterial, anti-inflammatory sedative, fungicide, anticancer activities. It was used as an anti-cancer agent reported by Mariat George *et al.,* (2015) [19].

9,12,15-Octadecatrienal is an aldehyde compound. It is an aliphatic compound. It has 6.05 RT value, $C_{13}H_{30}O$ molecular formula and 262.437 molecular weight. This compound present in most of the fruit essential oils. This compound has antimicrobial and antioxidant activity [20].

α-Santoline alcohol is an organic and alcohol compound. It has 6.15 RT value, $C_{10}H_{18}O$ molecular formula and 154.253 molecular weight. It has an alcohol-antimicrobial, insecticidal activity [21].

Dodecane is an aliphatic compound. It has 6.41 RT value, $C_{12}H_{26}$ molecular formula and 170.34 molecular weight. Dodecane has antibacterial activity and antifungal activity. It enhances antifungal activity [22].

Dodecane,2,6,11-trimethyl-, is an aliphatic alkane compound. It has 8.30 RT value, $C_{15}H_{32}$ molecular formula and 212.41 molecular weight. It has no activity [23].

Hexadecane is an aliphatic compound. It has 9.95 RT value, $C_{16}H_{34}$ molecular formula and 226.448 molecular weight. It has antimicrobial, antifungal and antioxidant Activity [24, 25].

d-Mannose is a C-2 epimer of glucose and occurs naturally in lots of plants and fruits, especially cranberries. It has 14.79 RT value, $C_6H_{12}O_6$ molecular formula and 180.156 molecular weight. D-mannose plays a role in T cell activation; we cultured native murine CD4+CD25- T cells in medium supplemented with mannose or other sugars in the presence of T cell receptor (TCR) stimulation. It is used as an antibacterial activity, antimalarials agents, antiprotozoal agents and antiparasitic agents [26].

Undecanoic acid (UDA) is a fatty acid have a significant amount of antimycotic activity. It has 14.91 RT value, $C_{11}H_{22}O_2$ molecular formula and 186.295 molecular weight. Undecanoic acid (UDA) is one of the most effective fatty acid compounds. It has been suggested that its antimycotic properties are linked to the ability to inhibit the production of exocellular keratinase, lipase and several phospholipids (Das & Banerjee, 1982) [27].

9-Octadecenoic acid is an aliphatic carboxylic acid. It has 18.65 RT value, $C_{18}H_{32}O$ molecular formula and 280.452 molecular weight. The high concentration of 9-Octadecenoic acid (8.16%), in leaf oil, make it potentially useful in the medicines because they exhibit antitumor and antioxidant activities (Dr Duke online database). However, further study has to be conducted for its confirmation. It is worth noting that the methanol extract of *H. suaveolens* L, (Point) has been reported to be used in folk medicine in the treatment of asthma and malaria, cereals conservation and to repel, larvicidal, adulticidal activities of mosquitoes. This compound also has anti-preventive, favour, fungicide, pesticide, perfumery, anti-inflammatory, hypocholesterolemic, cancer preventive effect [28].

Oleic Acid is an aliphatic carboxylic acid. It has 18.78 RT value, $C_{18}H_{34}O_2$ molecular formula and 282.468 molecular weight. It has antimicrobial activity, antibacterial activity and antitumour activity. It has more amount of antibacterial activity [29].

The plant-based compounds have an effective dosage response and minimum side effects when compared to the synthetic compounds. The studies conducted on *Silybum marianum* (L) for in vitro biological activities are validated. The presence of most common phytochemicals might be

responsible for their therapeutic effects. We report the presence of some of the significant components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

CONCLUSION

In the present study 10 compounds from the ethanolic leaves extract of *Silybum marianum* (L). were identified by Gas-chromatography– Mass spectrometry (GC-MS) analysis. The biological activities of each of the identified phytocomponents used for antimicrobial, antifungal, antioxidant, anti-tumour and anti-cancer activities. Chemical identification of the plant constituents was conducted based on their retention time (RT), molecular formula, molecular weight and mass spectral data, as well as by computer search mass spectral databases. The chemical structures and medicinal properties also identified. The results revealed the presence of medicinally significant constituents in the plants studied. Therefore, ethanolic extracts from these plants could be seen as a good source for using drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify and characterize the active constituents responsible for the biological activity of the organism. Also, additional work is encouraged to see whether these plants have said health benefits, especially as anti-cancer drugs, and elucidate the possible mechanism of action of these extracts. The presence of phytochemicals (secondary metabolites) is responsible for their therapeutic effects. It further reflects hope for the development of many more novel therapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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