ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 12, Issue, 05, pp.11342-11350, May, 2020

DOI: https://doi.org/10.24941/ijcr.38539.05.2020

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING, GC-MS AND FTIR PROFILING OF ETHANOLIC EXTRACT OF SEEDS OF *SILYBUM MARIANUM* (L.) GAERTN.

***Mugendhiran, S. and Sheeja, B.D.**

Department of Botany, Government Arts College, Udhagamandalam, The Nilgiris District, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 08th February, 2020
Received in revised form
24th March, 2020
Accepted 18th April, 2020
Published online 30th May, 2020

Key Words:
Silybum marianum,
Phytochemicals screening,
GC–MS analysis, FTIR,
Bioactive Compounds.

ABSTRACT

The Nilgiri hills consist of a well-defined plateau situated at the junction of the two great ranges of hills, Eastern and Western Ghats. Asteraceae is one of the largest families having medicinal and aromatic plants in Nilgiris. The milk thistle plant, *Silybum marianum* is an annual or biennial plant, native to the Mediterranean area and some parts of the United States; this has now spread to other warm and dry regions. To investigate the phytochemicals, FT-IR and GC-MS analysis of ethanol extracts of *Silybum marianum* was done. Seeds were shade dried and finely powdered for ethanolic extraction. Then, each of the extracts was further subjected to gas chromatography-mass spectrometry. Qualitative determination of the different biologically active compounds from crude extracts of *Silybum marianum* using gas chromatography-mass spectrometry revealed different type's chemical entities of high and low molecular weight of varying amounts present in each of the extracts. These chemical compounds are considered biologically and pharmacologically important. The study established the chemical composition and anticancer activity of the plant.

Citation: ***Mugendhiran, S.and Sheeja, B.D.*** **2020.** "Preliminary phytochemical screening, gc-ms and ftir profiling of ethanolic extract of seeds of *silybum marianum* (l.) Gaertn.", *International Journal of Current Research*, 12, (5), 11342-11350.

INTRODUCTION

The Nilgiri Biosphere Reserve is situated in the south of Western Ghats. 'Sholas' are the places of high biodiversity, which are habitats for many endemic, endangered and rare species of both flora and fauna (Pickering *et al*., 2008). The plant diversity here is a treasure house of potential drugs. There has been an increasing awareness in the recent years about the importance of medicinal plants. Medicinal plants being rich in numerous active constitutes of therapeutic value, are used as an effective source of remedy for treating human diseases. According to the World Health Organization (WHO), in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli *et al*., 2009). Drugs from the plants are easily available, safe, less expensive, efficient and rarely have any side effects. For thousands of years, in traditional treatment, medicinal plants are used to cure a variety of diseases. Humans have been using many medicinal plants as a source of medicine for different ailments and curing diseases since time immemorial. The demand for herbal medicine is increasing in both developed and developing countries since these have few or no side effects.

Corresponding author:* **Mugendhiran, S.,
Department of Botany, Government Arts College, Udhagamandalam, The Nilgiris District, Tamil Nadu, India.

A great number of medicinal plants contain some chemical constituents that exhibit antioxidant properties. Activities of antioxidants are primarily related to plant phenolic that may be found in all parts of plants (Mathew and Abraham, 2006). The Asteraceae is the richest vascular plant family in the world, with 1600–1700 genera and 24,000–30,000 species (Funk *et al*., 2005). *Silybum marianum* (L.) Gaertn. (*Carduus marianus* L.), belonging to the family Asteraceae, has been used for more than 2000 years, in particular as a remedy for hepatobiliary disorders since the 16th century (Flora *et al*., 1998; Schuppan *et al*., 1999). The plant is commonly known as the milk thistle, Our lady's thistle and St. Mary's thistle (Schuppan *et al*., 1999; Wellington *et al*., 2001). *Silybum marianum* grows 30 to 200 cm tall, having an overall conical shape. The stem is grooved and more or less cottony. With the largest specimens the stem is hollow. The leaves are oblong to lanceolate. They are either lobate or pinnate, with spiny edges. They are hairless and shiny green, with milk-white veins. The flower heads, which are 4 to 12 cm long and wide, are red-purple. The bract is hairless, with triangular, spine-edged appendages, tipped with a stout yellow spine. The achens are black, with a simple long white pappus, surrounded by a yellow basal ring. Their milk thistle contains silymarin, which is composed of the flavonolignans silybin, silydianin, and silychristine, with silybin being the most biologically active. Silymarin is found in its highest concentration in the fruit portion of the plant.

This is also found in the leaves and seeds. The seeds also contain betaine, trimethylglycine, a essential fatty acids, which may contribute to silymarin's hepatoprotective and anti-inflammatory effects (Ramasamy and Agarwal, 2008). The oil is a by-product of silymarin production. Milk thistle seeds contain a relatively high amount of oil (20–25%) (Khan *et al*.,1985). Extracted oil contains phospholipids and a high content of vitamin E, serving as a potential natural source of vitamin E (Vojtisek *et al*., 1991). Gaurav Kumar *et al*., 2010 studied the efficacy of bioactive extracts of 15 medicinal plants against multi bacteria, detected major groups of compounds as the most active fraction of four extracts by infrared spectroscopy. Iqbal Ahmad and Aqil, 2007 reported Saponins in crude dry powder of 11 plants using FTIR spectroscopy. Kareru *et al*., 2008 studied FTIR spectroscopic analysis in the powder samples of leaf, stem, and root of *Eclipta alba* and *Eclipta prostrata*. Muruganantham *et al*., 2009 recorded the functional groups in various extracts of *Aerva lanata* using spectroscopic method. Ragavendran *et al*., 2011 screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis. Oil has to be removed from seeds before the extraction of silymarin.

Silybum marianum is an annual or biennial plant, native to the Mediterranean area and some parts of the United States, which has now spread to other warm and dry regions (Hadolin *et al*., 2001). It has been reported that the extracted oil from milk thistle seed contains fatty acids such as linoleic acid, oleic acid, linolenic acid, palmitic, acid and stearic acid and it has been suggested as being suitable as edible oil (El-Mallah et al., 2003; Hadolin *et al*., 2001). Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi- component mixtures such as essential oils, hydrocarbons, and solvents (Kadhim *et al*., 2016; Mohammed *et al*., 2016; Pierangeli *et al*., 2009). GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes, *etc*. It is the key tool used in sports anti-doping laboratories to test athlete's urine samples for prohibited performance - enhancing drugs like anabolic steroids. Several GC-MS have left earth for the astrochemistry studies. As a unique and powerful technology the GC-MS provides a rare opportunity to perform the analysis of new compounds for characterization and identification of synthesized or derivatized compounds (Abeer *et al*., 2017). Its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry. It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and the determination of such thermochemical constants as heats of solution and vaporization, vapour pressure, and activity coefficients (Vyas, 1998; Kaushik *et al*., 2002; Chaman Lal and Verma, 2006; De-Fatima *et al*., 2006; Milne, 1993; Andrew Marston, 2007). The present study was designed to screen the bioactive photochemical present in the plant *Silybum marianum* seeds using GCMS analysis and FTIR.

MATERIALS AND METHODS

Plant collection and extract preparation: The *Silybum marianum* seeds were collected from Udhagamandalam, Tamil Nadu, India. The voucher specimen for plant species were collected and identified and with the help of "Flora of the Presidency of Madras" (Gamble and Fischer, 1935), The Flora of the South Indian Hill Station (Fyson,1915-20), Flora of Tamil Nadu (Nair and Henry,1983), The Flora of Tamil Nadu and Carnatic (Mathew, 1983), The Flora of Nilgiris, Tamil Nadu (Sharma *et al*., 1977), and Manual of cultivated plants (Bailey,1949), Western Ghats portal was also used for easy identification and confirmation. Plant specimen is kept in Centre of Medicinal plants Research in Homoeopathy Herbarium, at Emerald Acronym SMPRGH, The Nilgiri District, Tamil Nadu under CCRH, Ministry of AYUSH Emerald (Singh, 2010). The seeds 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: The phytochemical screening of aqueous, ethanol, methanol, acetone, and ethyl acetate extracts were subjected to different chemical tests for the detection of different phytoconstituents using standard procedures. Identifying the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, phenols, carbohydrates, Amino acid, and proteins (Peach and Tracey, 1955; Raaman, 2006)

Fourier Transform Infrared Spectrophotometer (FTIR): Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of *Silybum marianum* seeds were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000cm^{-1} with a resolution of 4 cm^{-1}.

GC–MS analysis: The shade dried 50 grams powder of seeds were subjected to extraction in Soxhlet extractor with 70% ethanol for 72 hours (extract yield: 9%) and after extraction the extract was collected. The collected extract was evaporated to dryness and stored at 4 ºC until used. 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 – Perkin Elmer.

The instrument was set to an initial temperature of 110 ºC and maintained at this temperature for 2 min. At the end of this period the oven temperature rose was rose to 280 ºC, at the rate of an increase of 5 ºC per min, maintained for 9 min. Injection port temperature was ensured as 200 ºC and helium flow rate as one ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. The mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Version –The year 2011 MS data library and comparing the spectrum obtained through GC–MS, compounds present in the plant sample were identified.

RESULTS AND DISCUSSION

The results of phytochemical characterization ethanolic extracts of *Silybum marianum* is shown in Table 1. Phytochemical analysis of an ethanolic extract of the plant also revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, phenols, carbohydrates, amino acid, and proteins.

QUALITATIVE PHYTOCHEMICAL ANALYSIS: Alkaloids were detected using Mayer's reagent. The test was positive indicating the presence of a very higher concentration level of alkaloids in ethanol solution. Phenols were tested using ferric chloride. Phenols were positive for moderate-higher concentration in ethanol solution. Flavonoids were tested using a few fragments of magnesium ribbon and few drops of concentrated hydrochloric acid. Flavonoids were tested indicating the Ethanol solution present in moderate higher concentration of flavonoids. Tannin was detected using concentrated Ferric chloride test. The test was positive indicating the presence of moderate-high concentration of Ethanol solution. Saponins were tested using distilled water; observed the presence indicating to moderate. Terpenoids were detected using a concentrated Ferric chloride test. The test was positive indicating the presence of Ethanol solution. Steroids were detected by the Liebermann-Burchard test. Red color was observed which is indicative of the presence of steroids. Corbohydrates were tested using Benedict's reagent which indicates positive for Corbohydrates. Glycosides were tested using aqueous sodium hydroxide reagent. Yellow color is indicates the presence of glycosides. Amino Acid and Proteins were tested using Biuret's. Purple coloration was observed which is indicative of the presence of Amino Acid and proteins.

FT–IR ANALYSIS: FTIR spectrum was used to identify the functional group of the active compounds based on the peak value in the region of infrared radiation. The result of FTIR peak values and functional groups is represented in Table 2 and the FTIR spectrum profile is illustrated in figure 1. FTIR spectrum confirmed the presence of alcohol, phenol, alkanes, alkyl halides, amino acid, carbolic acid, aromatic, and amines in the seeds of the medicinal plant taken. The more intense band occurring at 3618.46 cm^{-1}, 3441.01 cm^{-1}, 3209.55 cm^{-1}, 2916.37 cm^{-1}, 2854.65 cm^{-1}, 2731.20 cm^{-1}, 1743.65 cm^{-1}, 1697.36 cm^{-1}, 1651.07 cm^{-1}, 1519.91 cm^{-1}, 1458.18 cm^{-1}, 1342.46 cm^{-1}, 1311.59 cm^{-1}, 1165.00 cm^{-1}, 956.69 cm^{-1}, 817.82 cm^{-1}, 756.10 cm^{-1}, 725.23 cm^{-1}, 617.22 cm^{-1}, 516.92 cm^{-1} corresponding to O-H/H/C-H/H-C=0/C=O/C-O/C=C/N-O/C-C/C-H/C-N/C-Cl/C=C-H/C-Br stretching, bending, vibration respectively indicate the presence of alcohol, amines, amides, amino acids, aromatics, alkanes, alkynes, alkyl halides, carboxylic acids, carbonyls, nitro compounds, phenols, substituted compounds in seeds of *Silybum marianum.*

GAS CHROMATOGRAPHY MASS SPECTROSCOPY: From GCMS analysis, 24 active components were detected from the ethanolic Seed extract of *Silybum marianum.* The identification of phytochemical compounds was based on retention time, molecular formula, peak area; molecular weight and medicinal activity are presented in Table 3. Among the identified compounds, Tricyclo[5.2.2.0 (2,6)]undecan-11-one-8,9 or Silane, Trimethylpropoxy is found to be the major compound attained the largest peak (62.96%) with the retention time (2.748 min) which is followed by Disiloxane, Pentamethyl- (5.94%). Another compound Ethane, 1, 1'-oxybis [2, 2-dimethoxy- showed the peak area of 4.51%. The compound 1- Benzene, 1, 2-Dimethyl- and Kauran-18-AL, 17-(Acetyloxy)- showed the peak area of 3.97%. Benzene, 1,2-Dimethyl- showed the peak area of 3.30%. Propane, 2, 2-Bis(Ethylthio)- showed the peak area of 3.19%. Beta.-Sitosterol - showed the peak area of 2.91%. Benzene, Ethyl- showed the peak area of 1.93%. Stigmasterol- showed a peak area of 1.31%. 1, 2-Benzenedicarboxylic Acid- showed the peak area of 0.80%. Methyl 3, 3-dimethoxypropionate- showed the peak area of 0.79%. Phytol showed a peak area of 0.72%. Bromobenzene P627 showed the peak area of 0.66% Acetic Acid, Butyl Ester- showed the peak area of 0.62%. Benzene, 1, 3-bis (1-methylethyl) - showed the peak area of 0.38%. Benzene, 1, 3-bis(1-methylethyl)- showed the peak area of 0.30%. 1-Hexanol, 2-Ethyl-, 1,1,3,3,5,5,7,7,9,9 ,11,11,13, 13,15, 15-Hex, and Octasiloxane, 1,1,3,3,5, 5,7,7,9,9,1 1,11,13,1 showed the peak area of 0.25% with the retention time 46.23 min. The other compounds showing less prominent peaks are presented in Fig. 2.

Table: 2. Tricyclo [5.2.2.0(2,6)]undecan-11-one-8,9 or Silane, Trimethylpropoxy compound. It has 2.748 RT value, $C_6H_{16}OS_i$ molecular formula, a 132 molecular weight. It was used as anti-microbial, antioxidant, and anticancer activities reported by (Abdul- Aziz *et al*., 2019). Disiloxane, Pentamethyl- compound. It has 11.496 RT value, $C_{16}H_{34}O_5Si_2$ molecular formula, a 362 molecular weight, reported by (Christoph Grondal and Dieter Enders, 2007). Ethane, 1, 1'-oxybis[2,2-dimethoxy- compound. It has 7.447 RT value, $C_7H_{18}OS_i$ molecular formula, 146 molecular weight, reported by (Yuvaraj *et al*.,2019). Benzene, 1, 2-Dimethyl-compound. It has 7.447 RT value, $C_7H_{18}OS_i$ molecular formula, and 146 molecular weight, reported by (Yuvaraj *et al*., 2019). Kauran-18-Al, 17-(Acetyloxy)- Or Hdroxydehydrostevic acid - compound. It has 42.678 RT value, $C_{20}H_{30}O_3$ molecular formula, a 318 molecular weight, reported by (Baby Shalini and Sriman Narayanan, 2015; Neeranjana Mehra, Navin Kumar Jain, 2019). Benzene, 1,2-Dimethyl- or O-Xylene - compound. It has 3.741 RT value, C_8H_{10} molecular formula, and 106 molecular weight, reported by (Neeranjana Mehra, Navin Kumar Jain, 2019; Aja *et al*., 2016). Propane, 2,2-Bis (Ethylthio)- compound. It has 3.324 RT value, $C_5H_{14}OS_i$ molecular formula, a 106 molecular weight, reported by (Ki-Hyun Kim *et al*.,2019). Beta-Sitosterol - compound. It has 52.778 RT values, reported by (Peng, 1992). Stigmasterol - compound. It has 51.436 RT value, $C_{29}H_{50}O$ molecular formula, a 414 molecular weight, reported by (Awad *et al*., 1996; Mohan *et al*., 2012).

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* (seeds) 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. The elements and functional groups in the ethanol extract of the whole plant of *Ichnocarpus frutescens* using FTIR spectroscopic method (Jayapriya and Gricilda Shoba, 2015). The FTIR spectroscopic analysis of methanolic leaf extract of *Ampelocissus latifolia* for antimicrobial compounds. A survey of literature revealed that the FTIR analysis of functional groups was not done so far with the medicinal plants such as *Phyllanthus amarus, Senna*

Table 1. Qualitative phytochemical analysis of seeds of *Silybum marianum* (L.)Gaertn

Phytochemicals	Aqueous	Ethanol	Methanol	Ethyl acetate	Chloroform
Alkaloids	+	+++	++	+	+
Phenols	+++	++	++	+	+
Flavonoids	+++	++	++	+	++
Tannins	++	++	+	++	+
Saponins	-	++	+	-	+
Terpenoids	-	+++	+	-	-
Steroids	-	++	+	-	-
Carbohydrates	++	+++	++	+	+
Glycosides	++	+++	++	+	+
Amino acids	++	++	+	++	+
Proteins	++	++	+	+	+

+ → present in small concentration; ++ → present inmoderately high concentration; +++ → present in very high concentration;-- → absent.

Table 2. FTIR spectrum analysis of Seeds of *Silybum marianum* (L.)Gaertn

S.No	Frequency (cm^{-1})	Intensity	Assignment	Characterization
1.	3618.46	Strong, Sharp	O-H stretch, free hydroxyl	Alcohol, phenols.
2.	3441.01	Strong Broad	O–H stretch, H–bonded	Alcohol, phenols
3.	3209.55	Strong Broad Medium	O–H stretch, H–bonded, O–H stretch	Alcohols, phenols, carboxylic acids
4.	2916.37	Medium Medium	C–H stretch	Alkanes
5.	2854.65	Medium	C–H stretch	Alkanes
6.	2731.20	Strong	H–C=O: C–H stretch	Aldehydes
7.	1743.65	Strong Strong	C=O stretch, C=O stretch	Carbonyls (general) esters, saturated aliphatic
8.	1697.36	Strong	C=O stretch	α,β–unsaturated aldehydes, ketones
9.	1651.07	Medium	–C=C– stretch	Alkenes
10.	1519.91	Strong	N–O asymmetric stretch	Nitro compounds
11.	1458.18	Medium Medium	C–C stretch (in–ring), C–H bend	Aromatics, alkanes
12.	1342.46	Medium	N–O symmetric stretch	Nitro compounds
13.	1311.59	Strong	C–N stretch	Aromatic amines
14.	1165.00	Strong, Medium, Medium	C–O stretch , C–H wag (–CH_2 X), C–N stretch	Alcohols, carboxylic acids, esters, ethers alkyl halides, aliphatic amines
15.	956.69	Strong	=C–H bend	Alkenes
16.	817.82	Strong Medium	C–H “oop”, C–Cl stretch	Aromatics, alkyl halides
17.	756.10	Medium	C–Cl stretch	Alkyl halides
18.	725.23	Medium	C–Cl stretch	Alkyl halides
19.	617.22	Broad Strong	–C=C–H: C–H bend	Alkynes
20.	516.92	Medium	C–Br stretch	Alkyl halides

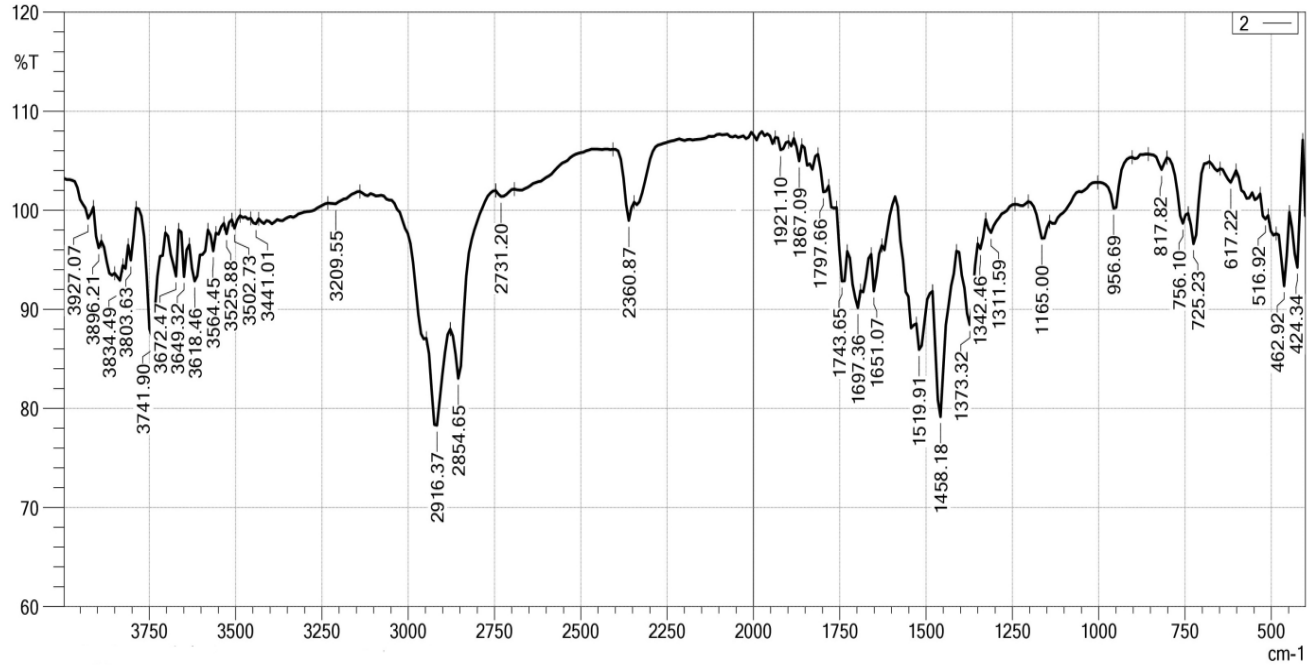
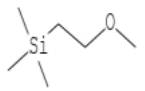
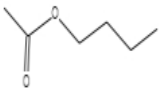
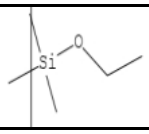
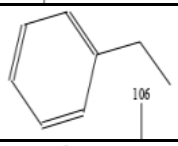
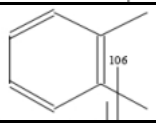
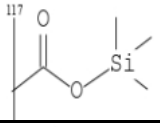
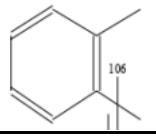
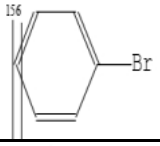
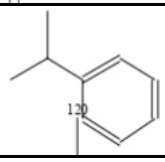
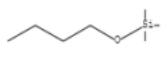
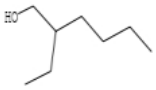
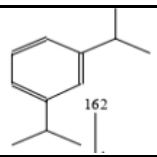
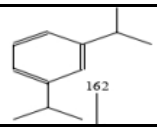
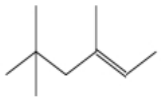
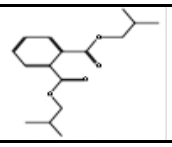
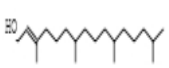
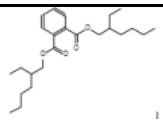
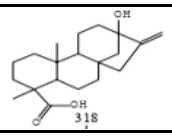
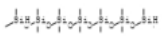
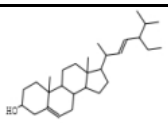
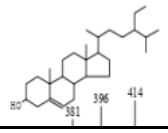


Figure 1. FTIR spectrum analysis of Seeds of *Silybum marianum* (L.) Gaertn

Table 3. Physical properties of bioactive compounds in *Silybum marianum* (L.)Gaertn

S. No	R. No	Name of Compound	Molecular formula	Molecular weight	CAS Registry No.	Peak Area %	Structure
1.	2.748	Tricyclo[5.2.2.0(2,6)]Undecan-11-One-8,9 Or Silane, Trimethylpropoxy	$C_6H_{16}OSi$	132	18173-63-2	62.96	
2.	2.876	Acetic Acid, Butyl Ester	$C_6H_{12}O_2$	116	123-86-4	0.62	
3.	3.324	Propane, 2,2-Bis(Ethylthio)-	$C_5H_{14}OSi$	118	1825-62-3	3.19	
4.	3.588	Benzene, Ethyl-	C_8H_{10}	106	100-41-4	1.93	
5.	3.741	Benzene, 1,2-Dimethyl- Or O-Xylene	C_8H_{10}	106	:95-47-6	3.30	
6.	3.895	Methyl 3,3-Dimethoxypropionate	$C_5H_{12}O_2Si$	132	2345-38-2	0.79	
7.	4.164	Benzene, 1,2-Dimethyl-	C_8H_{10}	106	95-47-6	3.97	
8.	4.858	Bromobenzene P627	C_6H_5Br	156	108-86-1	0.66	
9.	6.592	Benzene, (1-Methylethyl)-	C_9H_{12}	120	98-82-8	0.44	
10.	7.447	Ethane, 1,1'-Oxybis[2,2-Dimethoxy-	$C_7H_{18}OSi$	146	1825-65-6	4.51	
11.	9.721	1-Hexanol, 2-Ethyl-	$C_8H_{18}O$	130	104-76-7	0.25	
12.	11.290	Benzene, 1,3-Bis(1-Methylethyl)-	$C_{12}H_{18}$	162	99-62-7	0.30	
13.	11.496	Disiloxane, Pentamethyl-	$C_{16}H_{34}O_5Si_2$	362	126-80-7	5.94	
14.	11.851	Benzene, 1,3-Bis(1-Methylethyl)-	$C_{12}H_{18}$	162	99-62-7	0.38	

Continue ……

15.	12.055	3,5,5-Trimethyl-Hexanethiol	C_9H_{18}	126	26456-76-8	0.16	
16.	29.215	1,2-Benzenedicarboxylic Acid, B	$C_{16}H_{22}O_4$	278	84-74-2	0.20	
17.	33.574	Phytol	$C_{20}H_{40}O$	296	150-86-7	0.72	
18.	40.335	1,2-Benzenedicarboxylic Acid, D	$C_{24}H_{38}O_4$	390	117-81-7	0.80	
19.	42.678	Kauran-18-Al, 17-(Acetyloxy)-, (4	$C_{20}H_{30}O_3$	318	471-80-7	3.97	
20.	44.254	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexa	$C_{14}H_{44}O_6Si_7$	504	19095-23-9	0.25	
21.	45.738	1-Octadecanol	$C_{15}H_{32}O$	228	629-76-5	0.17	
22.	46.233	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,1	$C_{29}H_{48}O$	412	83-48-7	0.25	
23.	51.436	Stigmasterol	$C_{29}H_{50}O$	414	83-47-6	1.31	
24.	52.778	Beta.-Sitosterol				2.91	

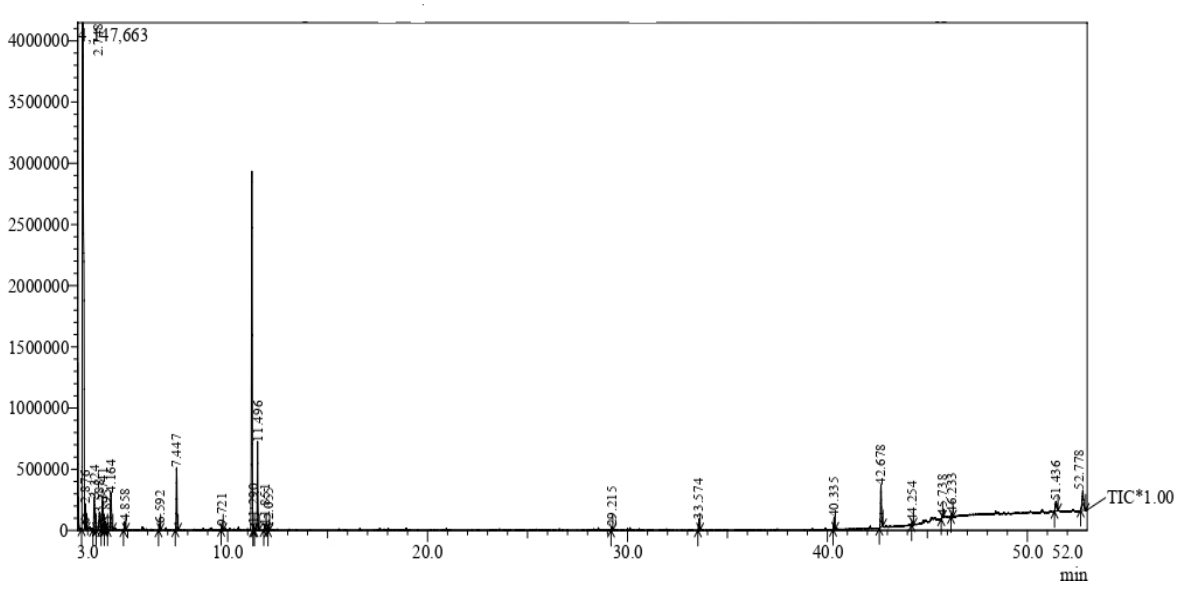


Figure 2. GC-MS chromatogram of *Silybum marianum* (L.)Gaertn.

auriculata, Phyllanthus maderaspatensis, and *Solanum torvum*. Hence, an attempt is made in the present study to analyze the functional groups of phytoactive compounds present in the leaf extracts (in different solvents such as petroleum ether, chloroform, ethyl acetate and methanol) of the four Indian medicinal plants, *Phyllanthus amarus, Senna auriculata, Phyllanthus maderaspatensis* and *Solanum torvum* by FTIR spectroscopic analysis (Ramamurthy and Kennan, 2007). Traditional medicine also known as indigenous or folk medicine comprises of medical knowledge systems that had been developed over generations within various societies before the era of modern medicine.

Traditional medicines are prepared from a single plant or a combination of more than one plant. Indian contribution to the herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for the medicinal activity of plant species (Parag, 2013). This plant also grows in many regions in Iran. Extracts from the mature milk thistle seeds are used as a medical remedy for liver disease, liver cirrhosis and to prevent liver cancer (Raaman, 2006; Angeles *et al*., 2005; Ramasamy and Agarwal, 2008; Baya Mhamdi *et al*., 2016; Bahram *et al*., 2009). Achachlouei and Damirchi, determine the oil composition of some varieties of milk thistle seeds grown in different parts of Iran. In this paper, qualitative and quantitative characterization of 4-desmethyl-, 4-monomethyl-, and 4, 40-dimethylsterols was also carried out by the saponification of oil samples and then fractionation of the total sterols by preparative-TLC followed by GC and GC–MS analyses.

These data can help to introduce the milk thistle seeds oil as a valuable by-product of silymarin production and its potential application in food preparation. Baya Mhamdi *et al*., 2016 determination of *Silybum marianum* seeds composition through their fatty acids, essential oil, phenolic analysis the study of the antioxidant activity of their methanolic extracts. The seeds have been used as a coffee substitute. The fruits were traditionally employed to stimulate milk production. *Silybum marianum* has been shown to have clinical applications in the treatment of toxic hepatitis, fatty liver, cirrhosis, ischemic injury, radiation toxicity, and viral hepatitis. Silymarin (a mixture of at least 4 closely related flavonolignans extracted from *Silybum marianum),* offers good protection in various toxic models of experimental liver diseases in laboratory animals. It acts by antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory, and liver regenerating mechanisms (Amiridumari *et al*., 2013).

Silybum has been used medicinally throughout Europe as a remedy for depression and liver problems for hundreds and perhaps thousands of years. The ancient Greeks described its medicinal properties in their herbals and Roman legionnaires carried the plants and seeds with them to Europe as food and medicine. Recent studies have validated this time-honoured herbal knowledge, proving *Silybum's* ability to protect the liver from alcohol and other forms of toxic damage. A study by Flora *et al*., 1996 revealed that 31% of patients were taking OTC milk thistle as an alternative agent for liver diseases. Dioscorides, the famous Greek herbalist, wrote about the use of tea from milk thistle seed against snake poison/bite (Greive, 1981). The use of milk thistle against hyperbilirubinemia was described by Pliny, The Elder (AD 23–79) (Foster, 1991). Later, in 1596, Gerarde considered milk thistle as the best remedy against black bile or melancholy (Hobbs, 1987).
Furthermore, in 1787 the seeds and the roots of the plant were noted as an excellent remedy to treat liver and spleen obstruction and to cure jaundice along with expelling stones (Greive, 1981).

Identification of Compounds

Interpretation of the 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.

CONCLUSION

Constituents in the plants studied. In the present study 24 compounds from the ethanolic Seed 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 were studied. 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 were also identified. 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 the future may serve the production of synthetically improved therapeutic agents.

REFERENCES

Abeer Fauzi Al-Rubaye., Imad Hadi Hameed and Mohanad Jawad Kadhim., 2017. A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants. *International Journal of Toxicological and Pharmacological Research*, 9(1): 81-85.

Aja, P. M., Ugwuokechukwu, P.C., Okoro, C. O., Nweke, O. L., Ali Ikechukwu, A. and Ogbu Amiridumari, H., Sarir, H., Afzali, N., and Fanimakki, O., 2013. Effects of milk thistle seed against aflatoxin B1 in broiler model. *J Res Med Sci.*, 18(9): 786-790.

Andrew, M., 2007. Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry*, 68: 2785-2797.

Angeles, S.M., Fernandez-Tarrago, J., Purificacion, C., 2005. Yeast extract and methyl jasmonate-induced silymarin production in cell cultures of *Silybummarianum* (L.) Gaertner. *J Biotechnol* ., 119:60–69.

Awad, A.B., Ying-Chen., Fink, C.S., and Hennessey, T. 1996. beta –Sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids. *Anticancer Research,* 16(15): 2797- 2804.

Baby Shalini, V., Sriman Narayanan, J. 2015. Characterization studies on medicinal plants of Andrographis paniculata (Nees). *Journal of Medicinal Plants Studies,* 3(5): 96-102.

Bahram Fathi-Achachlouei and SodeifAzadmard-Damirchi., 2009. Milk Thistle Seed Oil Constituents from Different Varieties Grown in Iran. *J Am Oil Chem Soc*., 86: 643–649.

Bailey L H. 1949. Manual cultivated plants: most commonly grown in the Continental United States and Canada. *The Macmillan Company New York.*

BayaMhamdi, FetenAbbassi, AbderrazakSmaoui, Chedly Abdelly, and Brahim Marzouk, 2016. Fatty acids, essential oil and phenolics composition of Silybummarianum seeds and their antioxidant activities. *Pak. J. Pharm. Sci*., 29(3): 951-959.

Chaman Lal and Verma, L.R. 2006. Use of certain bio-products for insect-pest control. *Indian Journal of Traditional Knowledge*, 5(1): 79- 82.

Christoph Grondal and Dieter Enders., 2007. A Direct Organocatalytic Entry to Selectively Protected Aldopntose and Derivatives. *Adv. Synth. Catal*., 349(4-5), 694-702.

De-Fatima, A., Modolo, L.V., Conegero, L.S., Pilli, R.A., Ferreira, C.V., Kohn, L.K., and de-Carvalho, J.E. 2006. Lactones and their derivatives: biological activities, mechanisms of action, and potential leads for drug design. *Curr. Med. Chem*., 13: 3371-3384.

El-Mallah, M.H., El-Shami, S.M. and Hassanein, M.M. 2003. Detailed studies on some lipids of *Silybummarianum* (L.) seed oil. *Grasasy- Aceites*., 54:397–402.

Flora, K. D., Rosen, H. R., and Benner, K. G., 1996. The use ofnaturopathic remedies for chronic liver disease. *Am. J. Gastroenterol*. 91: 2654–2655.

Flora, K., Hahn, M., Rosen, H., and Benner, K. 1998. Milk thistle (*Silybummarianum*) for the therapy of liver disease. *Am. J. Gastroenterol*., 93: 139–143.

Foster, S., 1991. Milk thistle: *Silybummarianum.* Botanical Series, (305). Austin, TX: American Botanical Council.

Funk, V.A., Bayer, R., and Keeley, S., 2005. Everywhere but Antarctica: using a supertree to understand the diversity and distribution ofthe Compositae. In: Friis I, Balslev H (Eds.) Proceedings of a symposium on plant diversity and complexity pattems – local, regional and global dimensions, *Copenhagen*. 343-374.

Fyson, P. F., 1915-20. The Flora of the Nilgiri and Pulney Hill Tops. *Superintendent, Government Press, Madras*, **3.**

Gamble, J. S., and Fischer, C. E. C., 1935. Flora of the Presidency of Madras. *BotanicalSurvey of India, Calcutta*, **3**.

Gaurav Kumar, L., Karthik and Bhaskara Rao, K.V. 2010. Phytochemical composition and in vitro Antimicrobial activity of *Bauhinia racemosa*Lamk. (CAESALPINIACEAE). *International Journal of Pharmaceutical Sciences and Research*. 1(11): 51-58.

Greive, M., 1981. A Modern Herbal, Vol. 2. New York, NY: Dover Publications.

Hadolin, M., Skerget, M., Knez, Z. and Bauman, D., 2001. High-pressure extraction of vitamin E-rich oil from *Silybummarianum*L. *Food Chem*., 74:355–364

Hobbs, C., 1987. Milk Thistle: The Liver Herb. Capitola, CA: Botanica Press.

Iqbal Ahmad., Farrukh Aqil., 2007. In vitro efficacy of bioactive extracts of 15medicinal plants against ESbL producing multidrug-resistant enteric bacteria. *Microbiological Research*, 62: 264 -275.

Jayapriya, G., Gricilda Shoba, F., 2015. GC-MS analysis of bio-active compounds in methanolic leaf extracts of *Justicia adhatoda* L. *Journal of Pharmacognosy and Phytochemistry*, 4(1): 113-117.

Kadhim, M.J., Sosa, A.A. and Hameed, I.H., 2016. Evaluation of antibacterial activity and bioactive chemical analysis of *Ocimumbasilicum*using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. *International Journal of Pharmacognosy and Phytochemical Research*, 8(6): 127-146.

Kareru, P.G., Keriko., Gachanja, J.M., and Kenji, A.N., 2008. Direct detection of triterpenoid saponins in medicinal plants. *African Journal of Traditional, Complementary, and Alternative Medicines*, 5 (1): 56 60.

Kaushik, J.C., Arya Sanjay., Tripathi, N.N. and Arya, S., 2002. Antifungal properties of some plant extracts against the damping off fungi of forest nurseries. *Indian Journal of Forestry*, 25: 359-361.

Khan, S.A., Khan, K.H., Zak, A.S., Waheed, I., Raie, M.Y., and Bhatty, M.K., 1985. Fatty acids from indigenous resources for possible industrial applications. VIII. Investigations ofsome species of Compositae family. *Pak J Sci Ind Res*., 28:400–402.

Ki-Hyun Kim., Ji-Won Ahn. and Sudhir Kumar Pandey., 2019. Comparison ofGC-MS calibration properties of Volatile Organic Compounds and Relative Quantification without Calibration Standards. *Journal of Chromatographic Science*, 49: 19-28.

Mathew K M. 1983. The Flora of the Tamil Nadu, and Carnatic. *The Rapinat Herbarium, St. Joseph's College, Tiruchirapalli*, 1969, 3.

Mathew, S., Abraham, E.T. 2006. *In vitro* antioxidant activity and scavenging effects of *Cinnamomumverum*leaves extract assayed by different methodologies. *Food Chem Toxic*., 44:198-206.

Milne, A. 1993. Inhalational and local anesthetics reduce tactile and thermal responses in *Mimosa pudica* L. *Masui,*: 1190-1193.

Mohamad S Abdul- Aziz., Amal, S., Hathout., Aziza A El-Neleety., Ahmed A Hamed., Bassem A Sabry., Soher E Aly., and Mosaad Attia Abdul- Wahhab. 2019. Molecular identification of actinomycetes with antimicrobial, antioxidant, and anticancer properties *Biological and Environmental Sciences*, 10(2): 218-231.

Mohammed, G.J., Kadhim, M.J., Hussein, H.M., 2016. Characterization of bioactive chemical compounds from *Aspergillus terreus*and evaluation of antibacterial and antifungal activity. *International Journal of Pharmacognosy and Phytochemical Research*, 8(6): 889-905.

Mohan, V.R., Jegadeswari, P., Nishanthini, A., Muthukumarasamy, S. 2012. GC-MS Analysis of Bioactive Compounds of Aristolochiakrysagathra (Aristolochiaceae). *Journal of Current Chemical and Pharmaceutical Sciences*, 2(4): 226-236.

Muruganantham, S., Anbalagan, G., Ramamurthy, N. 2009. FT-IR and SEM-EDS Comparative Analysis of Medicinal Plants, *Eclipta alba* Hassk, and *Ecliptaprostrata* Linn. *Romanian Journal of Biophysics*, 19(4): 285-294.

Nair, N. C., and Henry, A. N., 1983. Flora of Tamil NaduSeries 1: Analysis Vol. 1. *Botanical Survey of India, Howrah. Nature*, 446: 1079–1081.

NeeranjanaMehra and Navin Kumar Jain, 2019. Comparative phytochemical screening of *Curcuma angustifolia, Curcuma decipiens*, and *Curcuma longa* by using GC-MS. *Journal of Pharmacognosy and Phytochemistry*; 8(2): 1227-1234.

Parag, A., Pednekar., Bhanu, R., 2013. Antimicrobial and Antioxidant potential with FTIR analysis of *Ampelocissuslatifolia* (roxb.) Planch. Leaves. *Asian Journal of Pharmaceutical and Clinical Research*, 6(1): 67-73.

Patience, N. 2016. The Gas Chromatography- Mass spectrometry (GC-MS) Analysis of Ethanol leaf-extract of Vigna unguiculata (Cowpea). *International Journal of Research and Review in Pharmacy and Applied Science*, 6(1): 1284-1289.

Peach, D., and Tracey, M.V. 1955. Modern methods of plant analysis. 4th edn., *Springer Berlin, Verlag*., 373 - 374.

Peng, C., 1992. A Method of tentative identification of unknown gas chromatographic peaks by retention index. *Journal of Radioanalytical and Nuclear Chemistry*, 160(2): 449-460.

Pickering, C., Wendy, H., and Ken, G. 2008. Vascular plant diversity and climate change in the alpine zone of the Snowy Mountains, Australia. *Biodiversity and Conservation*, 17: 1627–1644.

Pierangeli, G., Vital, G., and Rivera, W. 2009. Antimicrobial activity and cytotoxicity of *Chromolaenaodorata* (L. f) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr Extracts. *Journal of Medicinal Plants Research*., 3(7): 511-518.

Pierangeli, G., Vital, G., and Rivera, W. 2009. Antimicrobial activity and cytotoxicity of *Chromolaenaodorata* (L. f) King and Robinson and *Uncariaperrottetii* (A. Rich) Merr Extracts. *J. Medicinal Plants Res*., 3(7): 511-518.

Raaman, N., 2006. *Phytochemical Techniques*, 1-275.

Raaman, N., 2006. Phytochemicals techniques. New India publishing agency, New Delhi., 19-25.

Ragavendran, P., Sophia, D., Arul Raj, C., Gopalakrishnan, V.K. 2011. Functional group analysis of various extracts of *Aervalanata*L. by FTIR spectrum. *Pharmacology online*,1: 358-364.

Ramamurthy, Kennan. S. 2007. Fourier transforms infrared spectroscopic analysis of a plant (*Calotropisgigantean* Linn) from an Industrial Village, Cuddalore DT, Tamil Nadu, India. *Romanian Journal of Biophysics*, 17 (4): 269-276

Ramasamy, K., Agarwal, R. 2008. Multitargeted therapy of cancer by silymarin, *Cancer letters*, 269:354–362.

Ramasamy, K., Agarwal, R. 2008. Multitargeted therapy of cancer by silymarin, *Cancer letters*, 269: 354–362.

Schuppan, D., Jia, J., Brinkhaus, B. and Hahn, E.G., 1999. Herbal products for liver diseases: a therapeutic challenge for the new millennium. *Hepatology,* 30: 1099–1104.

Sharma B. D, Shetty B V., Vajravelu E., Kumari G. R., Vivekanathan K. *et al*, Studies on the Flora of Nilgiris, Tamil Nadu. *Biol. Mem.,* 1977, **2** (1&2), 1-186.

Singh H. Hand Book on Herbaria in India and Neighbouring Countries. New Delhi: National Institute of Science Communication and Information Resources, CSIR; 2010.

The Western Ghats Portal. https:// thewesternghats. indiabiodiversity.org/

Vojtisek, B., Hronova, B., Hamrik, J., Jankova, B. 1991. Milk thistle (*Silybummarianum*) in feed given to ketonic cows. *Vet Med*., 36:31–33.

Vyas, G.D. 1999. Soil Fertility Deterioration in Crop Land Due to Pesticide. *Journal of Indian Botanical Society*. 78: 177-178.

Wellington, K., and Jarvis, B. 2001. Silymarin: a review ofits clinical properties in the management of hepatic disorders. *Biodrugs*., 15, 465–489.

Yuvaraj, R.,Mudiganti Ram Krishna Rao., Prabhu, K., Lakshmi Sundram,R.,SampadShil,, Sathish KumarM., and Vijayalakshmi N. 2019. The Gas Chromatography-Mass spectrometry study of one Medicinal plant, Stchytarphetaindica. *Drug Invention Today*. 12(8): 1665-1669.

****