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Capparis sepiaria Linn - Pharmacognostical standardization and toxicity profile with chemical compounds identification (GC-MS)

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Abstract

The present study was intended to evaluate the various pharmacognostical procedures in the leaves of *Capparis sepiaria* Linn., (Capparidaceae). The various pharmacognostical parameters were carried out as per WHO guidelines procedure i.e., bitterness, fineness, microscopical sections, loss on drying, water and alcoholic extractive values, water insoluble ash, acid soluble ash, total ash, swelling index, foaming index, heavy metal analysis, phytochemical analysis and toxicity studies (acute, subacute and chronic toxicity). The study was extended with analyzing the chemical compounds identification in the EECS (ethanolic extract of *Capparis sepiaria* by using GC-MS. The presence of various phytoconstituents such as glycosides, reducing sugars, flavonoids, saponins, starch and terpenoids is evidenced in EECS & AECS. The results showed that acid insoluble ash (1.70%), total ash (8.68%), water soluble ash (3.42%), water extractive (31.55%), alcohol extractive (5.06%), foaming index (105.26 Unit), loss on drying (9.84%), swelling index (4.16%), acute toxicity (nil), sub-acute toxicity (nil), chronic toxicity (nil). The study was concluded with the plant has standardized as per the World Health Organization procedures. The result of the pharmacognostical standardization of this plant serves as a reference piece and helps in future identification and authentication of this plant specimen. Might be the plant *C. sepiaria* has potential property by the standardization and it can be included in the normal flora of the plant kingdom.

Keywords: *C. sepiaria*; Microscopical; Macroscopical standardization.

Introduction

Plant materials are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the global drug market. It is therefore essential to establish internationally recognized guidelines for assessing their quality. The World Health Assembly in resolutions has emphasized the need to ensure the quality of medicinal plant products by using modern control technique and applying suitable standards. This manual describes a series of tests for assessing the quality of medicinal plant materials. The tests are designed primarily use in national drug quality control laboratories in developing countries and complement those described in the International pharmacopoeia, which provides quality specifications only for the few plant

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materials that are included in the WHO model list of essential drugs. This manual does not constitute an herbal pharmacopoeia, but a collection of test procedures to support the development of national standards based on local market conditions, with due regard to existing, national legislation and national and regional norms [1].

Table 1. Preparation of GC-MS columns for the chemical compounds identification

GC Programme	Over temperature programme	MS Programme
Column: Elite-1(100% Dimethyl poly siloxane), 30 x 0.25 mm x 1µmdf.	110ºC-2 min hold.	**Library used:** NIST Version-Year 2005.
Equipment: GC Clarus 500 Perkin Elmer.	Up to 200ºC at the rate of 10ºC/min.	**Inlet line temperature:** 200ºC.
Carrier gas: 1 ml per min, Split: 10:1	Up to 280ºC at the rate of 5ºC/min-9 min hold.	**Source temperature:** 200ºC.
Detector: Mass detector: Turbo mass gold-Perkin Elmer.	**Injector temperature:** 250ºC.	**Electron energy:** 70eV.
Software: Turbomass 5.2.	**Total GC running time:** 45 min.	**Mass scan: (m/z):** 45-450
Sample injected: 2 µl.		**Solvent Delay:** 0-2 min.
		Total MS running time: 36 min.

Material and Methods

Plant collection and authentication

The plant *C. sepiaria* Linn., was collected from Mathur and the surrounding area, Tiruchirappalli districts of Tamil Nadu, and authenticated by Botanical Survey of India, Agriculture University, Coimbatore, India. Voucher No. BSI/5/21/04-05/Tech-7.

Figure 1. *Capparis Sepiraria* Linn. (Capparidaceae) whole plant.

Figure 2. Leaves with measurements

Figure 3. Leaves with closure view

Figure 4. Leaves with stem

Preparation of extracts

500 g of shade dried coarsely powdered leaves of *C. sepiaria* Linn., was extracted exhaustively for 72 hours in a distillation apparatus with the double quantity of ethanol and water, which was previously distilled off before extraction.

Table 2. Macroscopical (morphological) characteristics of *Capparis sepiaria* Linn.

Characteristics	Results
Tree Height	5-11 Feet
Color of the Plant Leaves	Pale Green
Color of the bark	Pale Green
Thorns	Up to 3cm length
Fineness	Moderate course
Bitterness	Mild
Moisturizes	Moderately dried
Odour	Mild Chilly like smell crush

The excess ethanol from the crude extract was distilled off under reduced pressure and the concentrated crude extract was stored in a desiccators for further analysis was reported by Harborne [2], Kokate [3] and Wagner and Roth [4].

Phytochemical screening

The methods of Harborne [5], Trease and Evans [6], Ikhiri et al., [7] and Dahou et al., [8] were used to screen the chemical constituents the EECS.

Table 3. Preliminary phytochemical screening on EECS & AECS

Chemicals/Plant Name	*EECS*	*AECS*
Alkaloids	+	-
Amino Acids	-	-
Anthraquinones	-	-
Flavonoids	+	+
Glycosides	+	-
Proteins	+	-
Reducing sugars	+	+
Saponins	+	+
Starch	+	+
Steroids	+	-
Tannins	+	-
Terpenoids	-	-
Gums	+	+
Resin	-	-
Mucilages	+	+
Volatile Oil	-	-

The presence of alkaloid (Dragendroff reagent and Mayer's reagent), flavonoids (Shinoda test), steroids (Liberman Burchard test) and terpenes (Vanillin–sulfuric acid reagent) were assessed.

Table 4. Standardization of *Capparis sepiaria* Linn.

Standardization Procedures	*C. sepiaria*
Bitterness	Mild
Fineness	Moderately fine
Microbial content	Nil
Waste materials (Foreign matter)	Nil
Acid insoluble ash	1.70 %
Water soluble ash	3.42 %
Total ash	8.68 %
Water extractive value	31.55 %
Alcohol extractive value	5.06 %
Foaming index	105.26 unit
Loss on drying	9.84 %
Swelling index	4.16 %
Acute toxicity	Nil/5000mg/kg/i.p.,
Sub-acute toxicity	Nil/5000mg/kg/i.p.,
Chronic toxicity	Nil/5000mg/kg/i.p.,
Arsenic	Not Detected
Cadmium	0.04 ppm
Lead	0.07 ppm
Mercury	Not Detected

The dry ethanolic extracts of *C. sepiaria* were separately tested for the presence of alkaloids, amino acids, glycosides, proteins, saponins, starch, tannins and terpenoids.

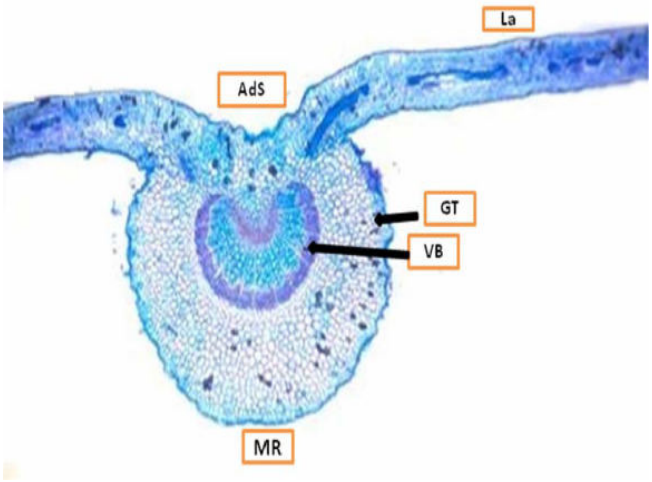


Figure 5. T.S. of leaf through midrib with lamina: Ads – Adaxial side; GT – Ground tissue; La – Lamina; MR- Midrib; VB – Vascular bundle.

Quality control methods for medicinal plant materials (WHO-Geneva)

The pharmacognostical standardization i.e., quality control methods (WHO-World Health Organization-Geneva) is comprises the various analytical and phytochemical procedures.

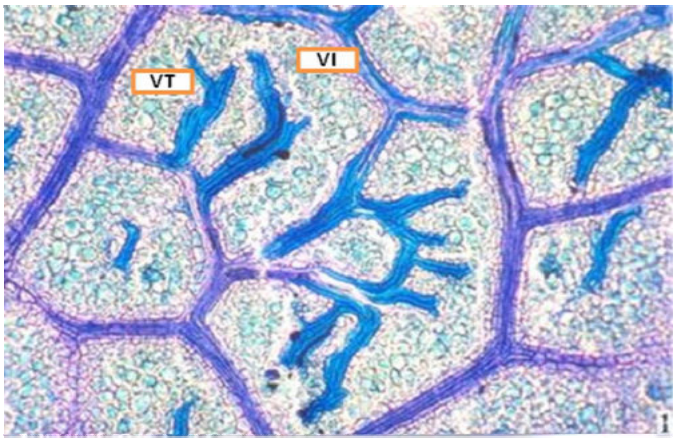


Figure 6. Venation pattern and Epidermal Morphology: EC – Epidermal cells; St – Stomata; Vi – Vein islets; VT – Vein – termination.

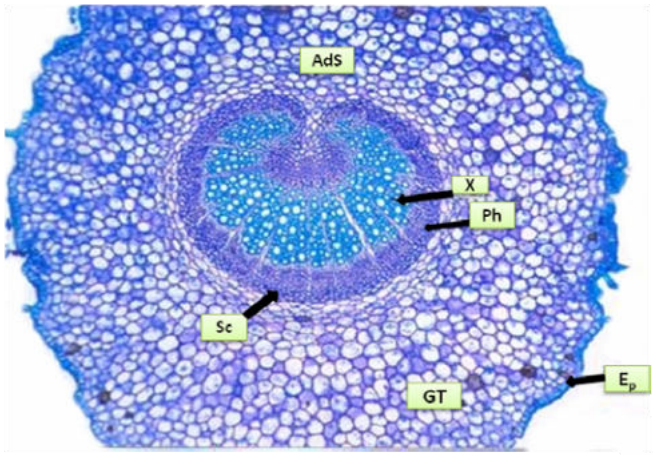


Figure 7. T.S of petiole ground plan: AdS – Adaxial side; Ep – Epidermis; Fi – Fibers; GT – Ground tissue; Ph – Phloem; Sc – Sclerenchyma; Ve – Vessel; X – Xylem.

They are: powder fineness, foreign matter, macroscopic and microscopic examination (i.e.,morphological and microscopic microtome sections of the parts), determination of ash values, determination of water and ethanol extractive values, volatile matter, bitterness, swelling index, foaming index, microbial content, phytochemical analysis and finally heavy metal analysis such, arsenic, cadmium, lead and mercury. The all procedures were followed by WHO, 2002. [1]

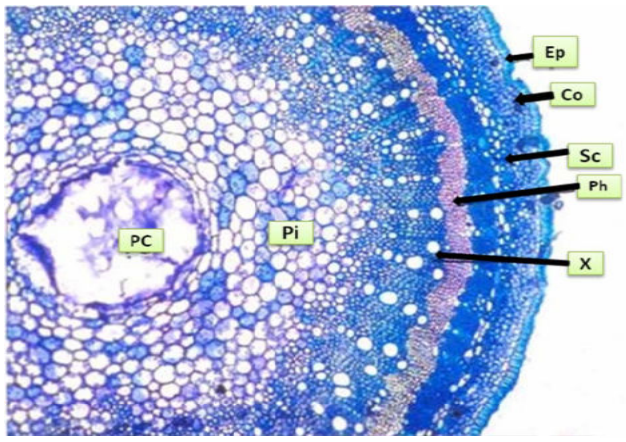
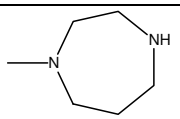
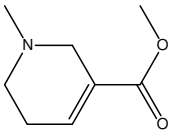
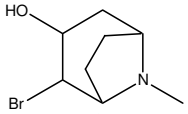
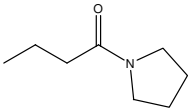
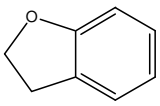
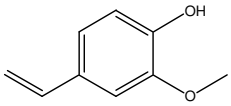
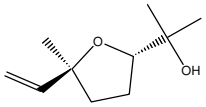
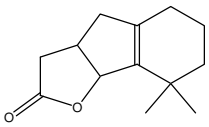
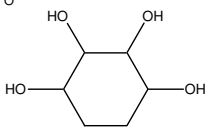
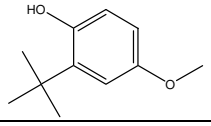


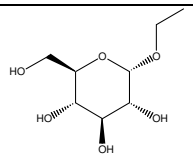
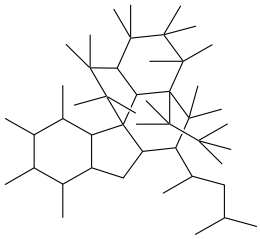
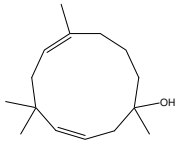
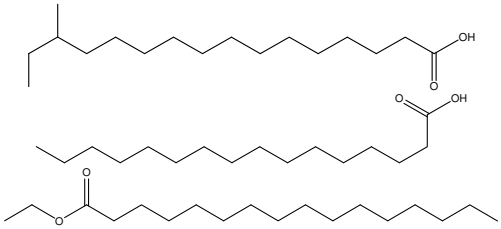
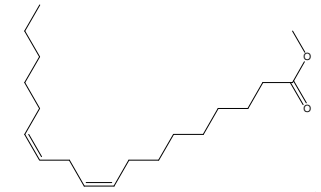
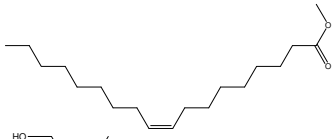
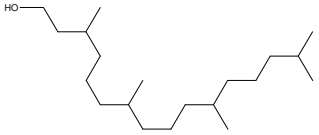
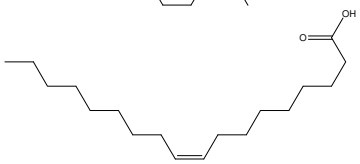
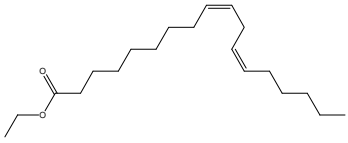
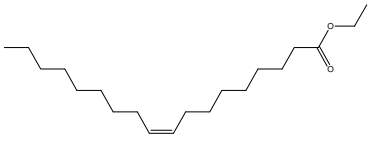
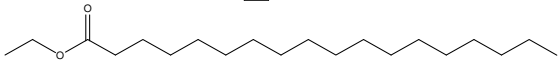
Figure 8. T.S of the stem half – portion enlarged: Co – Cortex; Ep – Epidermis; Pc – Pith cavity; Ph – Phloem; Pi – Pith; Sc – Sclerenchyma; SG – Starch grains; X – Xylem.

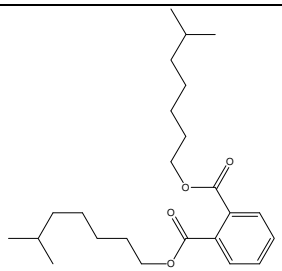
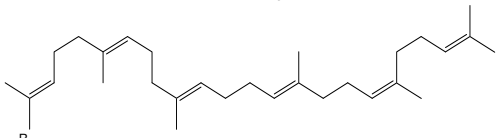
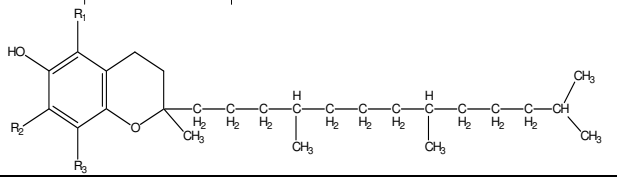
Experimental animals

The toxicity study was performed in Swiss albino mice (25-30 g). The animals were purchased from Kings Institute, Guindy, Chennai. They were housed in large spacious polypropylene cages and supplied with pellet feed and water *ad libitum*. The animals were acclimatized for at least one week in lab condition before commencement of the experiment in standard laboratory conditions 12±1 h day and night rhythm, maintained at 25±2^{o}C and 35-60 % humidity. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Committee for the purpose of control and supervision of Experiment on Animals (CPCSEA).

Table 5. GC-MS analysis of EECS chemical compound identification

No	RT	Name of the Compound	Molecular formula	Molecular weight	Peak area %	Structures
1.	2.94	N-Methylhomopiperazine	$C_6H_{14}N_2$	114	0.72	
2.	4.81	3-Pyridinecarboxylic acid, 1,2,5,6-tetrahydro-1-methyl-, methyl ester	$C_8H_{13}NO_2$	155	6.55	
3.	5.95	8-Azabicyclo[3.2.1]octan-3-ol, 2-bromo-8-methyl-, (exo,exo)-	$C_8H_{14}BrNO$	219	0.81	
4.	6.40	Pyrrolidine, 1-(1-oxobutyl)-	$C_8H_{15}NO$	141	1.23	
5.	6.89	Benzofuran, 2,3-dihydro-	C_8H_8O	120	4.03	
6.	8.11	2-Methoxy-4-vinylphenol	$C^9H_{10}O_2$	150	0.54	
7.	8.99	2-Furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis-	$C_{10}H_{18}O_2$	170	0.14	
8.	9.26	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}O_2$	206	0.16	
9.	10.18	1,2,3,4-Cyclohexanetetrol	$C_6H_{12}O_4$	148	1.96	
10.	11.21	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180	12.21	

11.	12.39	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	208	10.76	
12.	14.26	Aspidospermidine-3-carboxylic acid, 2,3-didehydro-, methyl ester, (5α, 12β, 19α)- (Synonym:Vincadifformine))	$C_{21}H_{26}N_2O_2$	338	0.51	
13.	14.89	3,7-Cycloundecadien-1-ol, 1,5,5,8-tetramethyl-	$C_{15}H_{26}O$	222	4.06	
14.	15.96	Pentadecanoic acid 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	2.13	
15.	16.68	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.32	
16.	16.96	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3.91	
17.	18.53	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	0.60	
18.	18.62	9-Octadecenoic acid(Z)-,methyl ester	$C_{20}H_{36}O$	296	2.47	
19.	18.96	Phytol	$C_{18}H_{40}O_2$	296	3.86	
20.	19.39	Oleic Acid	$C_{20}H_{34}O_2$	282	6.49	
21.	19.54	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	1.01	
22.	19.64	Ethyl Oleate	$C_{20}H_{38}O_2$	310	5.23	
23.	20.01	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.88	

24.	25.33	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	17.77	
25.	29.76	Squalene	$C_{30}H_{50}$	410	3.63	
26.	34.70	T-Tocopherol	$C_{28}H_{48}O_2$	416	1.02	

Acute, sub-acute and chronic toxicity studies

The acute, sub-acute and chronic oral toxicity study was carried out in Swiss Albino mice as per OECD guidelines [9]. The LD_{50} cut-off dose was found to be in EECS 100 mg to 5000 mg/kg/body weight. The animals were checked the ethanolic extracts for the acute, sub-acute and chronical toxicity for toxic for one day, one month and three months respectively., as per the OECD guidelines.

Chemical compounds identification by GC-MS analysis

The EECS was dissolved in ethanol and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components.

Results and discussions

To ensure reproducible quality of herbal products, proper control of starting material is ulmost essential. Thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [1]. The plant *C. sepiaria* showed in (Figure 1-4), macroscopical as morphological characteristics were shown in (Table 2). Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs [3]. The organoleptic or macroscopic studies yielded important characteristics, such as the fractured surfaces of fresh and dried (leaves, stem, petiole), typical tongue sensitizing aromatic taste and characteristic odour of the plant parts; which are useful diagnostic characters. Similarly the microscopic or histological features, e.g. presence of pericyclic sclerenchyma, absence of scleroids etc., may be useful for this purpose. Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. This is especially important for materials that absorb moisture easily or deteriorate quickly in presence of water. The test for loss on drying determines both water and volatile matter [1,3]. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a

form of adulteration. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash, and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the water soluble portion of the total ash [3,10]; these ash values are important quantitative standards. The extracts obtained by exhausting plant materials with specific ethanol are indicative of approximate measures of their chemical constituents extracted from a specific amount of extract.

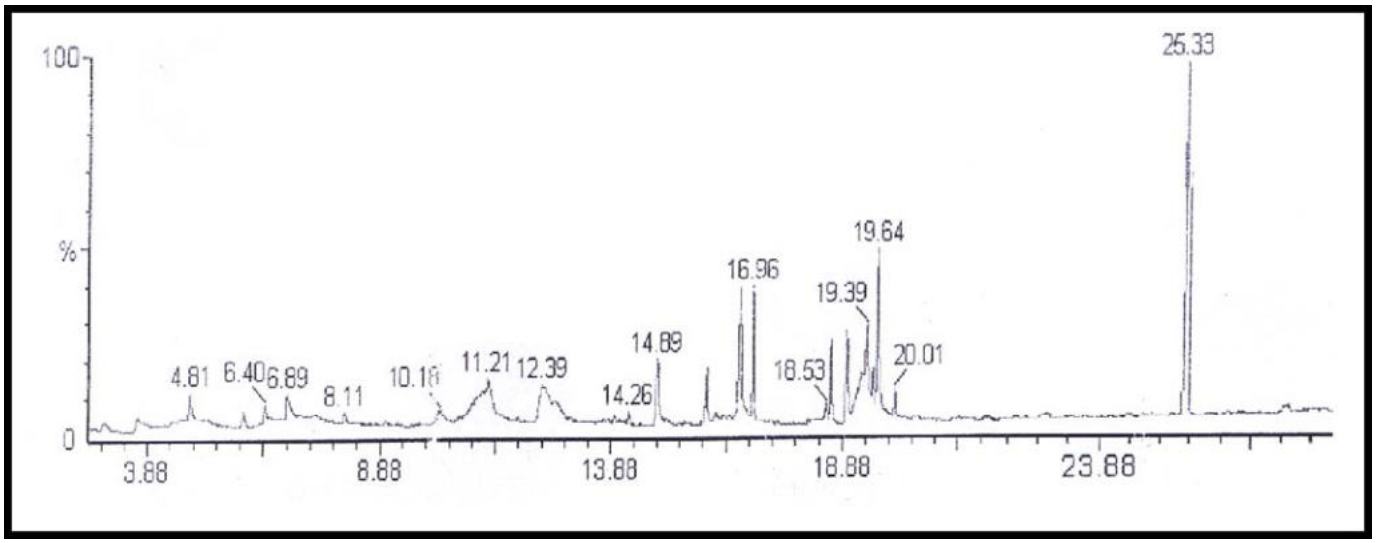


Figure 9. GC-MS spectrum of ethanolic extract of *Capparis sepiaria* Linn.

The results showed greater extractive values (almost double) in water extraction, indicating the effect of chemical compounds present in the plant. In another method alcohol formerly ethanol yielded lesser extractives when compared with water extract, some screening and insolubility is done there. The all pharmacognostical standardization procedures were shown in (Table 4). The plant material was subjected to preliminary phytochemical screening involving in ethanolic and aqueous extract phytoconstituents possessing different solubility pattern, followed by various chemical tests for qualitative detection of various chemical constituents. And it was found that true alkaloids, flavonoids, glycosides, proteins, reducing sugars, saponins, starch, steroids, tannins, gums and mucilage are present in ethanol extract further flavonoids, reducing sugars, saponins, starch, gums, mucilage was found to be in aqueous extract shown in (Table 3). The percent extractives in to two different solvents indicate the quantity and nature of constituents in the extract. The fresh plant leaves, stem, petiole of the sections were shown in (Figure 5-8). The EECS was analyzed the chemical compounds present in the extract, even the extract showed that the most potential chemicals such squalene, τ-tocopherol, ethyl oleate, oleic acid, phytol, ethyl α-d-glucopyranoside, n-methyl-homopiperazine, vincadifformine etc., The chemical compounds shown in (Table 5) and the corresponding chemical shift peaks of the spectrum were shown in (Figure 9). The plant extracts of *C. sepiaria* did not show any sign of toxicity to animals and also in behavioral change from 100 mg/kg/b.w. up to

5000 mg/kg/b.w. The present investigation of *Capparis sepiaria* Linn. can be concluded that the pharmacognostical study and yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to as certain the identity and to determine the quality and purity of the plant material in future studies for the first-rate pharmacological activity.

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