Research Article

GC-MS Analysis of Bio-active Components in Petroleum Ether Extract of *Lepidagathis scariosa* (Nees.) – Acanthaceae.

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ABSTRACT

Acacia caesia (L.) Willd., is a armed woody straggling shrub belongs to the family Mimosaceae. The local name of the species is 'Kari indu or Indamul'. It is commonly known as 'Babool' in India and ethnomedicinally have long been used for the treatment of skin, sexual problems, wound, and stomach and tooth problems. The present work was carried out to find out the bio-active compounds in the aerial plant powder of *Lepidagathis scariosa* (Nees) using Gas Chromatography- Mass Spectrometry method. This investigation aims to analyze the preliminary phytochemicals of *Lepidagathis scariosa* to understand its phytoconstituents and to characterize the phytochemical constituents of *Lepidagathis scariosa* using GC-MS analysis. *Lepidagathis scariosa* shows the existence of many active compounds. Isopropyl myristate, Isopropyl Tetradecanoate,-5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidaol,-1-Methyl-1-caprolactone compounds were present in major peak area. The result confirms that the plant has obviously presence of anti-oxidant, anti-ulcer and anti- cancerous properties.

Keywords: GC-MS, *Lepidagathis scariosa*, phytochemical analysis, petroleum ether.

INTRODUCTION

Plants are the major sources used in traditional medicine have stood up to the test of time and it reveals many novel compounds for preventive and curative medicine to modern science. India is sitting on a gold mine of well recorded and traditionally well practiced use of herbal medicine [1]. Therapeutically aromatic herbs have been recognized and used from prehistoric times. The chemical compounds of plants mainly used for biological function including defense against insects, microbes, fungi and herbivorous mammals. At the present time, over 12,000 bioactive compounds are identified in science. The recognized active compounds are screened from secondary metabolites. It is an important source with a variety of structural arrangements and properties. Pharmaceutical herbs have a wealthy source of secondary metabolites with fascinating biological activities [2].

The specific plants are used to analyse the application for particular ailments were passed down through oral tradition. Eventually information regarding medicinal plants was recorded in herbal phamacopoeias. *Lepidagathis scariosa* Nees. is the prostrate under shrub belongs to the family Acanthaceae and it has approximately 60 species distributed throughout the tropical regions [3, 4, 5]. It is used as herbal medicine in siddha, ayurveda and folk medicine for prudence various ailments.

Extraction methods are highly used in pharmaceutical research, which involves separation of medicinally active compounds from plant tissues by using selective solvents. During extraction time, solvents diffuse into solid plant material and solubilize compounds with similar polarity. More than 2,000 phytochemical compounds have been identified from plants [6]. Pharmacognostic studies are pivotal in herbal technology as it ensures plant identify, lays down standardization parameters which will help and prevent adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.

The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Such screening experiments form a primary platform for further phytochemicals and pharmacological studies that may open the possibility of finding new clinically effective compounds. For phytochemical fractionation assays GC-MS and FT-IR chromatography techniques are highly used nowadays.

Gas chromatography (GC) is recognized as the most suitable technique to find out how many components and in what proportion they are in a complex mixture of volatile compounds. When it is coupled to mass spectrometry (GC-MS), additional information arises about each separated compound molecular mass, elemental composition, functional groups, molecular geometry and spatial isomerism [7, 8].

With the above back ground, present study has been initiated with a view to screen the pharmacognostical profile for the selected species *Lepidagathis scariosa* for standardization.

MATERIALS AND METHODS

Plant collection and authentication

Fresh leaves of *Lepidagathis scariosa* were collected from Chennimalai hill, Erode district, Tamil Nadu. The plants were collected in their flowering and fruiting seasons from the natural habitat. While collecting the study plant, a thorough observation was made regarding the location, natural habitat, distribution pattern, habit, floral and fruit characteristics etc.

The collected study plant was identified with the help of the existing Floras [9, 10] and compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu (voucher specimen number – BSI/SRC/5/23/ 2017/Tech/1691). The collected plant specimens were pressed properly following the method of Jain and Rao [11]. Dried specimens were poisoned with 0.1 % HgCl2 dissolved in absolute alcohol and mounted with glue on standard herbarium sheet (42 x 28 cm). The herbaria were deposited in Department of Botany, Vellalar College for Woman, Thindal, Erode, Tamil Nadu. Photographs were also taken to supplement the herbarium. The medicinal properties of the plant were checked by the earlier reports [12, 13]. Secondary information were collected from numerous research papers, reports, records, documents, articles, books and journals related to the present study.

Shade drying and powdering of the collected plant material

Freshly collected aerial plant parts were cleaned to remove adhering dust and then shade dried at 31˚C for 15 days. The shade dried plant materials were mechanically ground to coarse powder and passes through a Willy Mill to get 60-Mesh size and used for phytochemical and GC/MS quantification. Samples were stored in the good grade plastic containers which are maintained at room temperature until analysis [14].

Soxhlet extraction

The air dried aerial plant powder (50 g) of *Lepidagathis scariosa* was extracted in Soxhlet apparatus successively with different solvents in the increasing order of polarity (Petroleum ether (60-80°C) and Ethanol (78.5°C) (50g/250 ml)) for 6-7 hrs. Each time, before extracting with the next solvent, the powdered material was dried in a hot air oven at 40°C. Finally, the material was macerated using hot water (99.98°C) with occasional stirring for 16 hrs and the water extract was filtered. The different solvent extracts were concentrated, vacuum dried and weighed. The extracts were dried over anhydrous sodium sulphate, stored in sealed vials in refrigerator (5-8°C) until analysis[15]. The prepared extracts were subjected to phytochemical analysis and GC/MS studies.

Qualitative phytochemical analysis

Phytochemical screening of different successive solvent extracts were carried out following the methods of Horborne [16] and Kokate et al., [17]. Carbohydrate, proteins and aminoacids, alkaloids, tannins andphenols, flavonoids, terpenoids, saponins, glycosides, anthroquinone, quinone, coumarin, gum and mucilage and fixed oil were qualitatively analyzed.

Gas Chromatography – Mass Spectrometry (GC/MS) studies

The analysis of unidentified constituents, the GC-MS play a major role in plant origin. The crude petroleum ether (1 µl) extract containing different phytochemical compounds of *Lepidagathis scariosa* was subjected for (GC-MS) analysis.

GC/MS conditions

The crude petroleum ether extract was subjected to GC-MS analysis on the instrument - THERMO GC – TRACE ULTRA VER: 5.0, THERMO MS DSQ II, DB 35 - MS CAPILLARY STANDARD NON - POLAR COLUMN and the GC-MS trace ultra-version 5.0 software employing the following conditions: RT x 5 MS column (30 Mts x 0.25 mm ID x 0.25 µM df, composed of 100 % Dimethyl poly diloxane). Initially oven temperature was maintained at 70ºC for 2 minutes and the temperature was gradually increased up to 260ºC for 6 minutes. One µL of sample was injected for analysis. Helium gas 99.995% of purity was used as a carrier gas as well as an eluent. The flow rate of helium gas was set to 1 mL/min. The sample injector temperature was maintained at 260ºC and the split ratio is 10 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectrum was recorded for the mass range 40-1000 m/z for about 37.50 minutes.

Identification of components was based on comparison of their mass spectra. As the compounds separated on elusion through the column were detected in electronic signals. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008 WILEY8, FAME. The total GC running time was 37.51 minutes [18].

Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Qualitative phytochemical evaluation

To investigate the chemical constituents of *Lepidagathis scariosa* plant powder, the successive solvent extracts were subjected to qualitative phytochemical screening. The results of the preliminary phytochemical screening showed the presence of various phytochemicals (Table 1). The petroleum ether and ethanol extracts revealed the presence of proteins, alkaloid, phenols, flavonoid, terpenoids, saponin, quinone, coumarin, gum and mucilage and fixed oil. In the aqueous extract showed the presence of carbohydrate, alkaloids, phenol, terpenoids, anthroquinone, flavonoids, quinone, coumarins, gum and mucilages and fixed oil. Tannins and glycosides are totally absent in all the three extracts tested.

Table 1: Qualitative phytochemical screening of different extracts of *Lepidagathis scariosa*

S. No.	Constituents	Petroleum ether extract	Ethanol extract	Aqueous extract
1.	Carbohydrate	-	-	+
2.	Proteins and aminoacids	-	+	-
3.	Alkaloid	+	+	+
4.	Tannins	-	-	-
5.	Phenols	+	+	+
6.	Flavonoid	+	+	-
7.	Terpenoides	+	+	+
8.	Saponin	+	+	-
9.	Glycosides	-	-	-
10.	Anthroquinone	-	-	+
11.	Quinone	+	+	+
12.	Coumarin	+	+	+
13.	Gum and mucilage	+	+	+
14.	Fixed oil	+	+	+

GC/MS analysis

The components present in petroleum ether extract of *Lepidagathis scariosa* were identified by GC-MS analysis. This analysis reveals the presence of phytoconstituents belonging to the type-acids, esters, alcohols, aldehyde, glucoside, methoxy etc. The identified compounds of the petroleum ether leaf extract of *Lepidagathis scariosa* their retention indices, percentage composition, molecular formula, molecular weight, probability, hit spectrum, nature of compound and activities of identified compounds were given in Table 2 and 3. The GC-MS Chromatogram of the number of peaks of the compounds detected was shown in Figure.1.

The GC-MS characterization of petroleum ether extract of *Lepidagathis scariosa* showed 14 chemical compounds. The results showed the presence of 1-Methyl-1-caprolactone ($C_7H_{14}O_2$), 1-Tetradecanol (CAS) ($C_{14}H_{30}O$), 1-Nonanol (CAS) ($C_9H_{20}O$), Isopropyl myristate ($C_{17}H_{34}O_2$), Isopropyl Tetradecanoate ($C_{17}H_{34}O_2$), 5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidaole ($C_{12}H_{12}N_4O$), Hexadecanoic acid, ethyl ester (CAS) ($C_{18}H_{36}O_2$), Phytol (C20H40O), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl - [R-[R*,R*-(E)]]- (CAS) ($C_{20}H_{40}O$), Octadecanoic acid, ethyl ester (CAS) ($C_{20}H_{40}O_2$), Methyl 2-(4,8-Diacetoxy-3-bromo-6-methoxy-9,10-dioxo-9,10-di-hydroanthraquinon-2-yl methyl)-4-(2-methyl[1,3]dioxolane-2-yl)-3-oxobutanoat ($C_{29}H_{27}BrO_{12}$), Lucenin 2 ($C_{27}H_{30}O_{16}$), Quassin ($C_{22}H_{28}O_6$) and Dimethoxyglycerol Docosyl Ether ($C_{27}H_{56}O_5$). From the above chemical compounds Methyl 2-(4,8-Diacetoxy-3-bromo-6-methoxy-9,10-dioxo-9,10-dihydroanthra quinon -2-ylmethyl)-4-(2-methyl [1,3]dioxolane-2-yl)-3-oxobutanoat expressed the high peak area percentage (91.27) with a retention time 31.42. The spectrum profile of GC – MS confirmed the presence of 3 major components Isopropyl myristate ($C_{17}H_{34}O_2$), Isopropyl Tetradecanoate ($C_{17}H_{34}O_2$), 5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidaole ($C_{12}H_{12}N_4O$) with retention time 20.25, 20.26 and 20.28 (Table 2).

Table 3 shows the major phytocomponents and their chemical structure and biological activities. In terms of percentage amounts Isopropyl myristate ($C_{17}H_{34}O_2$), Isopropyl Tetradecanoate(C17H34O2), 5-(Hydroxy methyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidaole ($C_{12}H_{12}N_4O$) were predominant in the extract and have the property of antioxidant, antiulcer, skin enhancer and pesticide. Among the other identified phytochemicals 1-Methyl-1-caprolactone, 1-Tetradecanol (CAS), 1-Nonanol (CAS), Hexadecanoic acid, ethyl ester (CAS), Phytol, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl - [R-[R*,R*-(E)]]- (CAS), Octadecanoic acid, ethyl ester (CAS), Methyl 2-(4,8-Diacetoxy-3-bromo-6-methoxy-9,10-dioxo-9,10-dihydro anthraquinon-2-ylmethyl)-4-(2- methyl [1,3] dioxolane-2-yl)-3-oxobutanoat,Lucenin2, Quassin a and Dimethoxy glycerol Docosyl Ether have the property of anticancerous, emollient, flavoring agent, antioxidant, skin enhancer, pesticide, cardiovascular diseases, central nervous system, control of insulin secretion, hypocholesterolemic, nematicide, haemolytic, antibacterial activity, antinociceptive, anti-inflammatory, hypocholesterolemic, nematicide, dermatitis, mouthwash, vaginal douche and veterinary activities, anti-ulcer, antilarval activity, aniti-worms, anorexia, indigestion, constipation, fever, purgative and mouthwash. The mass spectra are fingerprint of that compound which can be identified from the matching of compounds with Wiley 9.0 and National Institute of Standards and Technology Libraries. The biological activities listed are based on Dr. Duke's phytochemical and ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

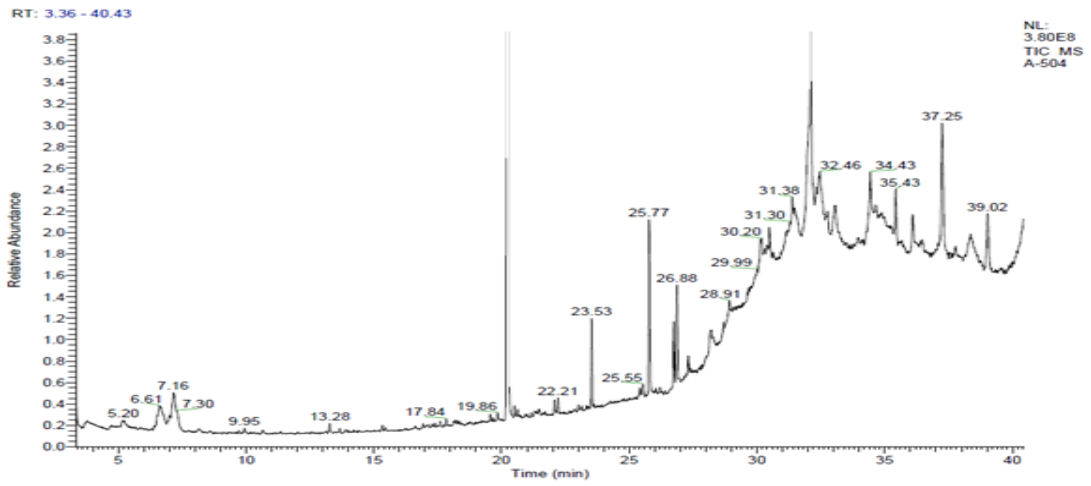
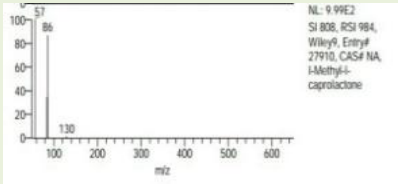
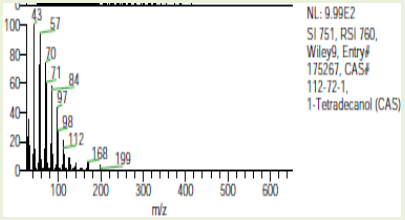
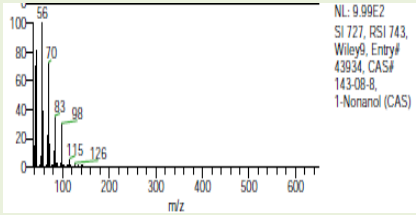
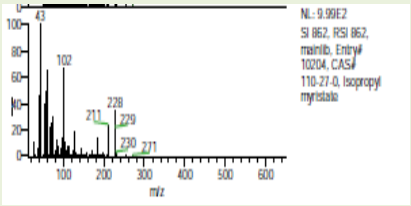


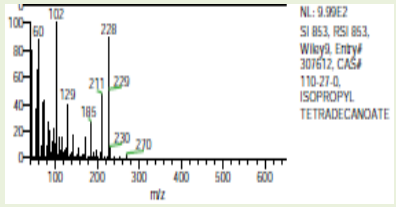
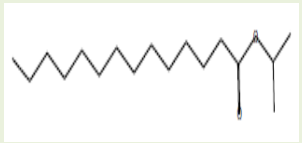
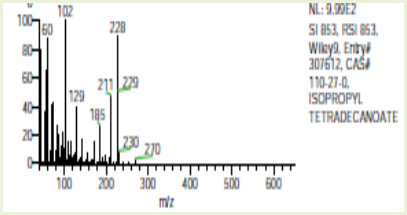
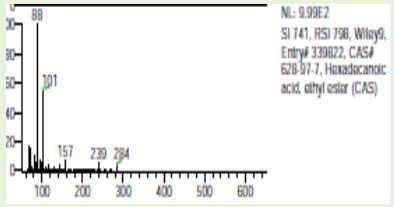
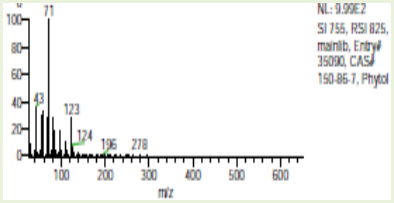
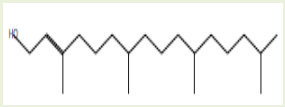
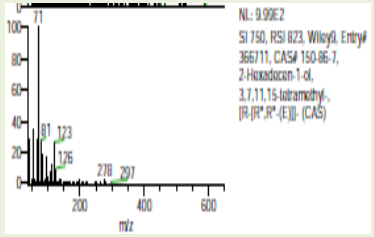
Figure 1: GC-MS Chromatogram of the petroleum ether extract of *Lepidagathis scariosa*

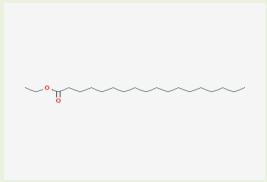
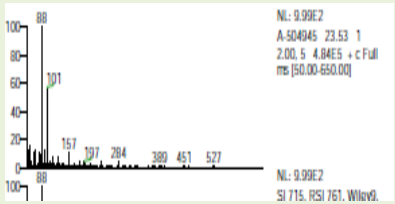
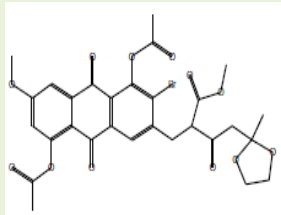
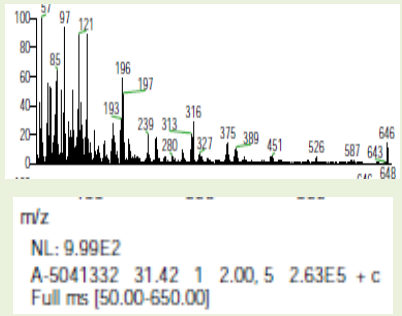
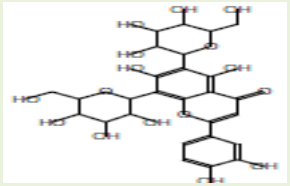
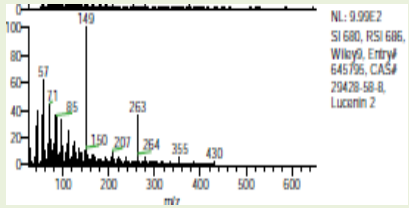
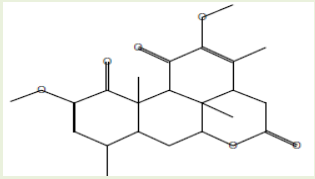
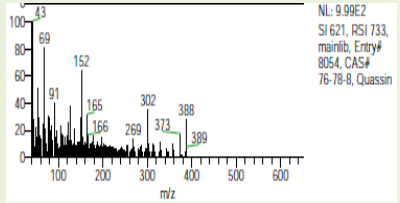
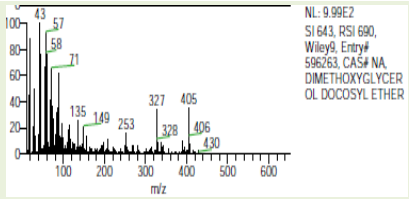
Table 2: Phytocomponents identified from the petroleum ether extract of *Lepidagathis scariosa* by GC-MS analysis

S. NO.	RT (MIN)	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	PROBABILITY	PEAK AREA %
1.	3.03	1-Methyl-1-caprolactone	$C_7H_{14}O_2$	130	74.08	12.63
2.	6.65	1-Tetradecanol (CAS)	$C_{14}H_{30}O$	214	60.02	0.43
3.	7.18	1-Nonanol (CAS)	$C_9H_{20}O$	144	55.96	0.57
4.	20.25	Isopropyl myristate	$C_{17}H_{34}O_2$	270	50.96	71.42
5.	20.26	Isopropyl Tetradecanoate	$C_{17}H_{34}O_2$	270	50.96	71.42
6.	20.28	5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidaole	$C_{12}H_{12}N_4O$	228	34.96	71.42
7.	23.53	Hexadecanoic acid, ethyl ester (CAS)	$C_{18}H_{36}O_2$	284	49.64	0.48
8.	25.77	Phytol	$C_{20}H_{40}O$	296	30.70	1.25
9.	25.77	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl - [R-[R*,R*-(E)]]- (CAS)	$C_{20}H_{40}O$	296	30.70	25.77
10.	27.32	Octadecanoic acid, ethyl ester (CAS)	$C_{20}H_{40}O_2$	312	27.75	0.17
11.	31.42	Methyl 2-(4,8-Diacetoxy-3-bromo-6-methoxy-9,10-dioxo-9,10-di hydroanthraquinon-2-ylmethyl)-4-(2-methyl[1,3]dioxolane-2-yl)-3-oxobutanoat	$C_{29}H_{27}BrO_{12}$	646	91.27	0.66
12.	32.76	Lucenin 2	$C_{27}H_{30}O_{16}$	610	41.67	0.07
13.	33.95	Quassin	$C_{22}H_{28}O_6$	388	33.96	0.11
14.	38.37	Dimethoxyglycerol Docosyl Ether	$C_{27}H_{56}O_5$	460	35.11	0.44

Table 3: Mass spectrum and Compound structure of phytocomponents identified by GC-MS in petroleum ether extract of *Lepidagathis scariosa*

S. NO.	NAME OF THE COMPOUND	STRUCTURE	HIT SPECTRUM	NATURE OF COMPOUND	ACTIVITY
1.	1-Methyl-1-caprolactone	-		Cinnamic acid	Anticancerous activity
2.	1-Tetradecanol (CAS)			Fatty alcohol	Emollient property
3.	1-Nonanol (CAS)			Fatty alcohol	Flavouring agent
4.	Isopropyl myristate			Ester	Antioxidant, skin enhancer and pesticide

5.	**Isopropyl Tetradecanoate**			Ester	Anti ulcer, skin enhancer and pesticide
6.	**5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidaole**			Methoxy group	-
7.	**Hexadecanoic acid, ethyl ester (CAS)**			Fatty acid	Cardiovascular diseases, antioxidant,-central nervous system, control of insulin-secretion, hypocholesterolemic, nematicide and hemolytic
8.	**Phytol**			Hydroxyl group	Antibacterial activity, antinociceptive, antioxidant activities and anti-inflammatory activities
9.	**2-Hexadecen-1-ol, 3,7,11,15-tetramethyl - [R-[R*,R*-(E)]]-(CAS)**			Aldehyde	Antioxidant and antimicrobial activities

10.	**Octadecanoic acid, ethyl ester (CAS)**			Acid	Hypocholesterolemic, nematicide
11.	**Methyl 2-(4,8-Diacetoxy-3-bromo-6-methoxy-9,10-dioxo-9,10-di hydroanthraquinon-2-ylmethyl)-4-(2-methyl[1,3]dioxolane-2-yl)-3-oxobutanoat**			Methoxy group	-
12.	**Lucenin 2**			Glucoside	Dermatitis, mouthwash, vaginal-douche-and veterinary activities
13.	**Quassin**			Crystall	Anti-ulcer,-antilarval activity,-aniti-worms, anorexia,-indigestion, constipation,-fever, purgative and mouthwash
14.	**Dimethoxyglycerol Docosyl Ether**			Methoxy group	-

DISCUSSION

The present study has authenticated the usefulness of the *Lepidagathis scariosa* plants for medicinal purposes. These species could also be seen as potential sources of useful drugs due to their rich contents of phytochemicals.

GC-MS chromatograms of the petroleum ether extract of *Lepidagathis scariosa* showed 14 peaks and have been identified after comparison of the mass spectra. Pentadecanol is the active ingredient of the topical composition for treating acne Vulgaris [19]. Silane is the inorganic compound has the property of anti-microbial activity. Docosane, alkane group have the property of anti-inflammatory, analgesic, antimicrobial activities and infertility. The squalene has antioxidant and chemo-preventive activity against the colon carcinogenesis. Hexadecanoic acid (palmitic acid) is a fatty acid and it may be an active antimicrobial, cytotoxic and antidiarrhoeal agent [20, 21].

The results of GC-MS profile can be used as pharmacognostical tool for identification of the plant. The presence of various bioactive compounds confirms the application of *Lepidagathis scariosa* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug. The outcome of study strongly supports the usage of this straggling shrub to human society. Further the isolation, identification, purification, characterization and structural elucidation of bioactive compounds from this species are taken under investigation. The current study may be of great use and interest to the researchers, pharmaceutical industries and medical practitioners. The plant species selected for the study can be used as a potential source of useful drugs. It also justifies the folklore medicinal use and claims about therapeutic values of study plant as a curative agent. All characters obtained from the results are also required for database preparation in this digital world by which further experiments or research can be preceded.

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