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Determination of phytochemicals by GC-MS in methanol extract of *Elephantopus scaber* L.

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Abstract
Medicinal plants constitute a natural reservoir for drugs; have been found to be useful in the treatment of various diseases. *Elephantopus scaber* Linn is a scabrescent aromatic herb, in which roots were used as an antipyretic, cardiotonic, dysuria, diarrhoea, dysentery, stomach pain and diuretic. The leaf extract had already been evaluated for diuretic, anti inflammatory hepato protective effect and used for the treatment of eczema and ulcers. Here qualitative phytochemical estimation of *Elephantopus scaber* was carried out for active ingredients. Preliminary phytochemical study revealed that extracts of *E. scaber* contains alkaloids, flavonoids, phenols, reducing sugars, saponins, tannins, terpenoids, volatile oils, carbohydrates, and lactones. Phytochemical estimation of bioactive components in methanolic extract of *E. Scaber* was done by GC-MS analysis. Further studies are needed to isolate active principle of the extract as well as to elucidate their exact mechanism of action in various disorders.

Keywords: Phytochemistry, GC-MS, *Elephantopus scaber*.

Introduction
Since time immemorial plants have been used for the treatment of various ailments. Quinine obtained from the bark of several spices of Cinchona has long been used as anti-malarial drugs. Numerous other drugs of plant origin have been found to be useful in the treatment of various disorders. Among these are *Rauwolfia, Belladonna, Nux-vomica, Ephedrine, Ergot, Aconit, Podophyllum, Ginseng, Cuscara*, and *curare* (Kochhar, 1981) [15]. Even today several important drugs used in the modern system of medicine are obtained from plants. The monumental ayurvedic work like *Charaksamhitha* and *Sushrutasamhitha* followed by other ayurvedic and sidha treaties have incorporated nearly 700 plant drugs entering in to several medicinal preparations used in the management of health care (Arvind Kumar *et al*., 2011) [17]. Plants provide the basic raw material for some of the most important drugs (Evans and Williams, 1996). The medicinal importance of a plant is due to the presence of complex secondary metabolites like alkaloids, glycosides, resins, volatile oil, gums, tannins, flavanoids, fixed oil, etc. These active principles usually remain concentrated in the storage organs of the plants leaves, roots, seeds (Arvind Kumar *et al.*, 2011) [17]. These secondary metabolites can be isolated by using techniques such as column, thin layer, paper, gas liquids, and HPLC. Phytochemical investigations carried out during the 1970s and 1980s have discovered a number of alkaloids and other pharmacologically active substances. Alkaloids have been associated with medicinal uses includes analgesic, antispasmodic and antibacterial properties and glycosides known to lower the blood pressure. Phytochemical compounds may be the bioactive constituents and plants are an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

There are several reports on the antimicrobial activity of plants from different regions of the world, because of the side effects and the resistance that pathogenic microorganisms guild against antibiotics. Recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. In the present day scenario, herbal medicines are gaining popularity in day to day life. India is a veritable emporium of medicinal plants so it is called the botanical garden of the world (Handa and Kapoor, 1989) [12]. Phytochemical screening of the plant extract was carried out by various methods to detect the presence or absence of certain bioactive compounds (Amarasingham *et al.*, 1964) [3]. The leaves of the plant are commonly used in Ayurvedic medicine for the improvement of memory deficit. The major phytochemical constitutes of the plant are elephantopin, triterpenes, stigmasterol, epifriedelinol and lupeol (Rajkapoor *et al*., 2002) [26]. Both plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Prusti *et al.*, 2008) [24]. Isodeoxyelephantopin from *E. scaber* induces cell cycle arrest and caspase -3-mediated apotopsis in breast carcinoma T47D cell and lung carcinoma

A549 cells. Isodeoxyelephantopin (IDOE) isolated from *Elephantopus scaber* is used in Chinese medicine for the treatment of some types of cancer. Bioactivity screening of the extracts and their compounds had shown that *E. scaber* possess wound healing, anti-venom, anti-microbial, anti-inflammatory, anti-diabetic, cytotoxic and anti-tumour activities (Ho *et al*., 2009) [13]. Isolated a new sesquiterpene lactones from *Elephantopus scaber* L., which can inhibits human lymphocyte proliferation and growth of tumour cell lines and also can induces apoptosis *in vitro*. The cytotoxic properties of South Indian *Elephantopus scaber* L. Indicated that the ant proliferative property of deoxyelephantopin and isodeoxyelephantopin could be used in regimens for treating tumors with extensive proliferative potencies (Geetha *et al*., 2013). Ethanolic extract and isolated deoxyelephantopin from *E.scaber* showed more significant wound healing activity in wound model animal (Singh *et al*., 2005) [28]. Methanol and aqueous extract of *E. scaber* L. leaf on selected five bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* and four fungal strains *Aspergillus niger, Aspergillus flavus, Rhizopus indicus* and *Mucor indicus* showed phytochemical and antimicrobial potential of methanolic and aqueous extracts of aerial parts of E.scaber L (Kamalakannan *et al*., 2011). A new elemanolide sesquiterpene named elescaberin together with two known compounds namely isodeoxyelephantopin and deoxyelephantopin isolated from the whole plant of *E. scaber* (Qiao *et al*., 2008) [19]. GC-MS analysis of methanol extracts of *Vernonia cinerea* revealed two major compounds such as n-hexadecanoic acid and 1,2 benzenedicarboxylic acid disoocty ester (Abirami *et al*., 2012) [1]. Genotoxicity was studied using pUC19 with different concentration of Isolongifolene and the activity analysed using gel electrophoresis shows that Isolongifolene protect the genomic DNA from radical damage and it does not produce any toxicity to the pUC19 (Kowsalya *et al*., 2013) [16]. Ethanol extract, ether extract and methanol extract of rhizomes of the plant Nervilia aragoana using Gas Chromatography and Mass Spectroscopy identified wide range of fatty acids, heterocyclic compounds which are having anti fungal, anti inflammatory, antibiotic and skin conditioning property. So that it can be recommended as a plant of phytopharmaceutical importance (Thomas *et al*., 2013) [29]. GC-MS analysis in the methanolic extract of *Justicia wynaadensis* evaluated twenty four compounds. The major constituents are Dihydrocoumarin, Phytol and Palmitic acid. Significant quantities of Linoleic acid, Stearic acid, Squalene and phytosterols such as Campesterol and Stigmasterol were also present (Ponnamma *et al*., 2012) [23]. GC-MS analysis in methanolic extract of leaves of *Cassia italica* reveals the presence of 17 compounds such as Phytol, Squalene and n-Hexadecanoic acid (Sermakkani *et al*., 2012) [27]. Fifteen chemical constituents have been identified from methanolic leaf extract of *Kedrostis foetidissima* through GC-MS analysis, among that the major constituents are 7, 10- hexadecadienoic acid and docosanoic acid.

Elephantopus scaber Linn. commonly known as Elephant's foot, (*Asteraceae*) is a scabrescent aromatic herb distributed in the moist deciduous forests of central Western Ghats. According to the traditional claims, the roots were used as an antipyretic, cardiotonic, dysuria, diarrhea, dysentery, stomachic pain, and diuretic. The leaf extract had already been evaluated for diuretic, anti-inflammatory, hepatoprotective effect, and also treatment for eczema and ulcers. The biological actions of flavonoids within the nervous system are to enhance neuronal function, stimulate neuronal regeneration, control neuronal resistance to neurotoxins, including oxidants and inflammatory mediators and also responsible for neuronal differentiation, long-term potentiation, and memory.

2. Materials and Methods

2.1 Collection and Processing of Plant

The plants were selected on the basis of their medicinal properties. Fresh root and leaves of *Elephantopus scaber* were collected from local areas of Varandarappilly, Thrissur in the month of January 2014 for the experimental purpose. The plants were brought to the laboratory after proper identification. The collected material was identified by referring Flora (Gamble, 1967) [10]. The roots and leaves were cleaned, shade dried, coarsely powdered and stored in well closed containers till further used.

2.2 Preparation of Crude Extracts

50 gm of the plant powder weighed and extracted serially by the solvents of increasing polarity such as Petroleum ether, Chloroform, Acetone, and Methanol in a Soxhlet apparatus. The plant extracts obtained after extraction are concentrated by distillation. After distillation, solution was stored in well closed containers.

2.3 Phytochemical Analysis

The powdered drugs were subjected to various qualitative tests for the identification of phytoconstituents (Kokate, 1990). Extraction was done by hot Soxhlet method. The solvents are removed by distillation over water bath. Last traces of the solvent were removed by distilled under vaccum. The extract thus obtained was subjected to preliminary phytochemical screening for the determination of major chemical groups by standard procedures (Harborne *et al*., 1998).

2.4 GC-MS Analysis

2 µl of the methanol extract of *Elephantopus scaber L.* was employed for GC- MS analysis. The phytochemical investigation of methanolic extract was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral library search programme.

2.4.1 Identification of Phytochemicals by GC-MS

Interpretation on mass spectrum of GC-MS was done using the database of Cochin University of Science and Technology (CUSAT) in Kochi, The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the CUSAT Library. The compound name, probability, molecular formula, molecular weight, & structure of the components of the test materials were ascertained.

3. Results

3.1 Phytochemical Analysis

After the successful conventional hot soxhlet extraction of the whole part of the plant in investigation, the preliminary phytochemical study revealed that extracts of *E.scaber* contains alkaloids, flavonoids, phenols, reducing sugars, saponins, tannins, terpenoids, volatile oils, carbohydrates, and lactones as summarized in table (1).

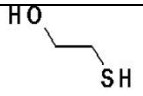
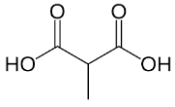
Table 1: Phytochemical Analysis of *Elephantopus scaber*.

Chemical constituents	Test	Petroleum Ether	Chloroform	Aceton	Methanol
Phytosterol	Salkowski test	_	_	+	_
Triterpenoids	Libermann-Burchard test	_	+	_	_
Saponins	Foam test	+	+	_	_
Alkaloids	Wagner's test	+	+	+	_
Flavanoids	Lead –Acetate test	_	_	_	+
	Alkaline test	_	_	_	+
Lactones	Legal's test	_	_	+	_
Tannins	Lead –Acetate test	_	_	_	+
	Braemer's test	_	_	_	+
	Catechin test	_	_	_	_
Sterol	L-B test	_	_	_	_
Resin	General test	_	_	_	_
Protein	Ninhydrin test	_	_	_	_
	Biuret test	_	_	_	_
Glycosides	Keller-Killani test	_	+	_	_
Coumarins	General test	_	_	_	+
Monosaccharides	Benedict's test	_	_	_	_
	Molish's test	_	_	_	_
	Selivanoff's test	_	_	_	_
Polysaccharides	Iodine test	_	_	_	_
	Molish's test	+	+	+	+
	Benedict's test	_	_	_	_
	Selivanoff's test	_	_	_	_
Volatile oils	General test	_	+	_	_
Phenols	General test	_	_	_	+
Aleuron grains	General test	_	_	_	+
Xanthoproteins	General test	_	_	+	_

3.2 GC-MS Analysis

GC-MS method was used for testing the presence and specific nature of active principles in *E.scaber which can be* used in cosmetic, drugs, pharmaceutical or food industry. The present study has been found useful in the identification of several constituents present in the methanolic extract of the *E.scaber.* Lavandulol, an oxygenated terpenoid, well known as preservative in food, drugs and cosmetics, has been tested *in vitro* for antibacterial, antifungal activity. Isolongifolol, a suggested as potential anticarcinogenic agent exhibit cytotoxic activity against several solid tumor cell lines was also identified by the GC-MS analysis of the plant extract. The presence of various bioactive compounds (identified as sesquiterpenoids, aldehydes, alcohols, terpinolene) justifies the use of the whole plant for various ailments by traditional practitioners. These findings support the traditional use of *E. scaber* in various disorders. The studies on the active principles in the whole plant methanolic extract by GC-MS analysis clearly showed the presence of 14 compounds. The active principles with their retention time (RT), molecular formula and molecular weight (MW) are presented in Table (2). The activity of the compounds identified is presented in the Table (3). The results revealed that undecanoic acid, Hexadecanoic acid, Tetradecanoic acid, Isolongifolene, Arabinose lavandulol, 2-Mercaptoethanol, Sorbose, 9, 12, 15-Octadecatrienoic acid methyl ester, Butanedioic acid, and Bicyclo [4.1.0.] heptanes 7-pentyl, was found as the major components in the methanol extract. The GC-MS chromatogram of the 12 peak of the compounds detected is shown in Figure-1. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library.

Table 2: Various components in the methanolic extract of *E.scaber*

RT (mnt)	Name of the compound	Molecular formula	Molecular weight	Compound nature	Structure of the compound
5.98	2- Mercaptoethanol	C_2H_6OS	78.01	Alcohol	
5.98	Methyl malonic acid	$C_4H_6O_4$	118.02	carboxylic acid	

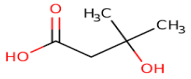
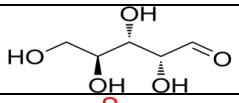
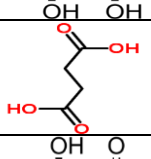
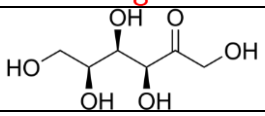
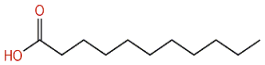
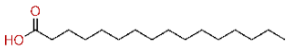
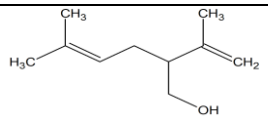
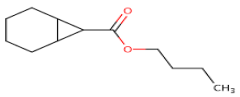
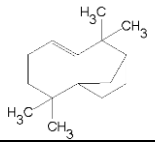
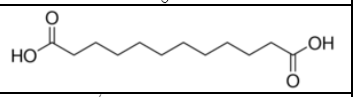
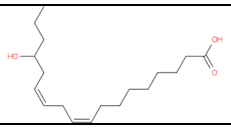
5.98	β –hydroxyisovaleric acid	$C_5H_{10}O_3$	118.06	Metabolite of leucine	
23.33	Arabinose	$C_5H_{10}O_5$	150.05	Sugar	
5.98	Butanedioic acid	$C_8H_{14}O_4$	174.08	carboxylic acid	
23.33	Sorbose	$C_6H_{12}O_6$	180.06	Sugar	
23.33	Undecanoic acid	$C_{11}H_{22}O_2$	186.16	Fatty acid	
23.33	Tridecanoic acid	$C_{13}H_{26}O_2$	214.19	Fatty acid	
23.33	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.20	Fatty acid	
23.33	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	Fatty acid	
26.51	Lavandulol	$C_{10}H_{18}O$	154.13	Primary alcohol	
26.51	Bicyclo [4.1.0.] heptanes 7-pentyl	$C_{12}H_{22}$	166.17	Hydrocarbon	
26.63	Isolongifolene	$C_{15}H_{26}O$	222.19	Hydroxy compound	
26.63	Dodecanedioic acid	$C_{12}H_{22}O_4$	230.15	Fatty acid	
26.63	9,12,15- octadecatrienoic acid methyl ester	$C_{19}H_{32}O_2$	292.24	Linolenic acid	

Table 3: Activity of compounds present in methanolic extract of *E.scaber*.

SI.no	Name of the compound	Molecular formula	Activity	References
1	Hexadecanoic acid	$C_{16}H_{32}O_2$	Anti- inflammatory	Della Loggia *et al.*(1994)
2	Tetradecanoic acid	$C_{14}H_{28}O_2$	Antioxidant , Lubricant Hypercholesterolemic, Cancer preventive	Kumar *et al.* (2010) [18]
3	Undecanoic acid	$C_{11}H_{22}O_2$	Antimicrobial , Pesticide, Antifungal, Nematicide.	Rahman *et al.* (2005) [25]
4	Lavandulol	$C_{10}H_{18}O$	Preservative in food, drugs and cosmetics, antibacterial, antifungal activity.	Angioni *et al.* (2006) [4].

5	Arabinose	$C_5H_{10}O_5$	Culture medium for certain bacteria	De Man *et al*.(1960) [7]
6	Methyl malonic acid	$C_4H_6O_4$	It's excess con. indicate vit.B_{12} deficiency	Cox *et al.*(1962) [6]
7	Butanedioic acid	$C_8H_{14}O_4$	Antimicrobial , Skin irritant Anti- inflammatory.	Mohammadzadeh *et al*.(2007) [20-21]
8	Isolongifolene	$C_{15}H_{26}O$	Anti- proliferative activity Antioxidant	Afoulous *et al*.(2013) [2]
9	9,12,15- Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	Anti- inflammatory, Hypocholesterolemic Cancer preventive Nematicide, Hepatoprotective	Malathi, K.,(2017) [20]
10	β –hydroxyisovaleric acid	$C_5H_{10}O_3$	Antimicrobial	Zhu *et al.*(2007) [31]
11	Sorbose	$C_6H_{12}O_6$	Commercial production of vit. C often begin with it.	Myers *et al.* (2007) [22].
12	2- Mercaptoethanol	C_2H_6OS	Toxic, Anti –inflammatory Skin allergens	Wang *et al.* (2007) [30].
13	Bicyclo [4.1.0] heptanes,7 pentyl	$C_{12}H_{22}$	Activity not reported	
14	Tridecanoic acid	$C_{13}H_{26}O_2$	Flavoring agent Antimicrobial, antifungal	Avrahami *et al.* (2003) [5].

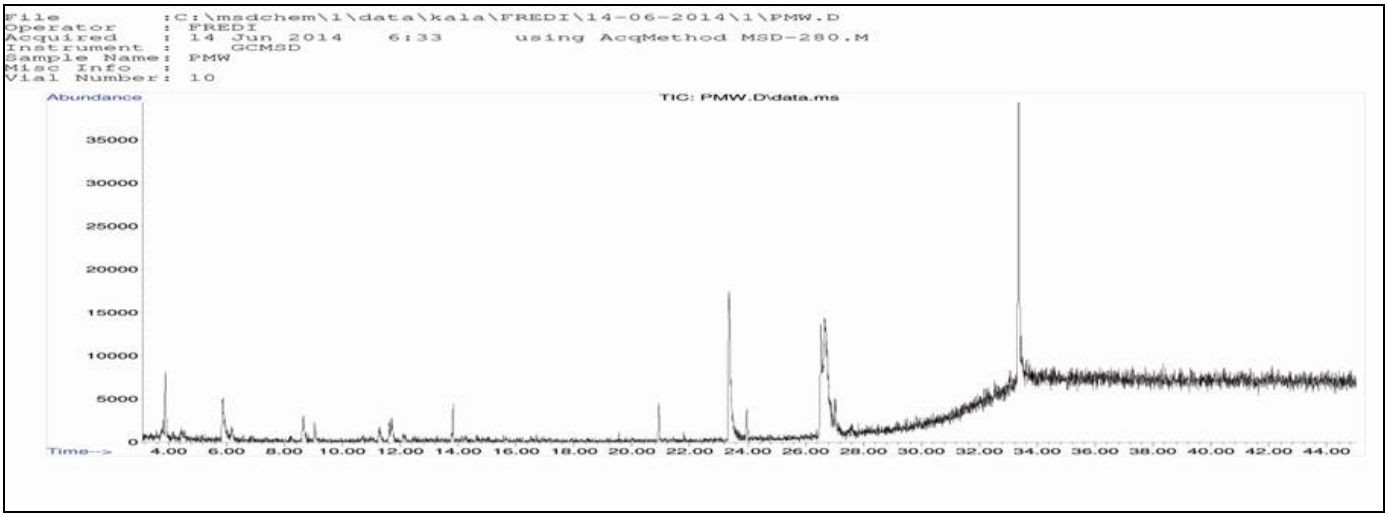


Fig 1: The GC-MS spectrum confirmed the presence of various components with different retention times

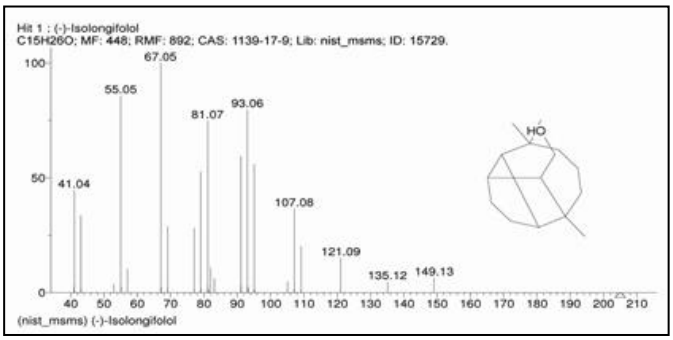


Fig 2: Isolongifolen

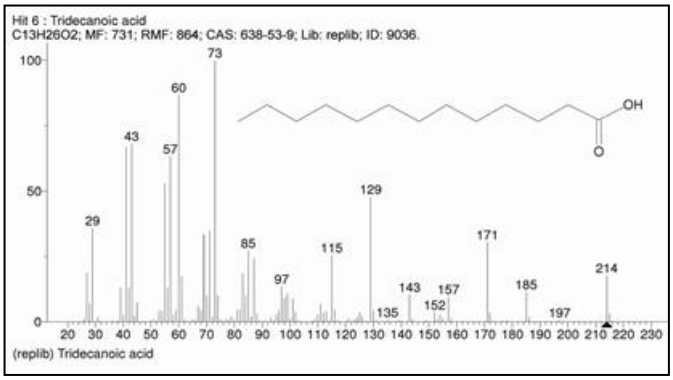


Fig 3: Tridecanoic acid

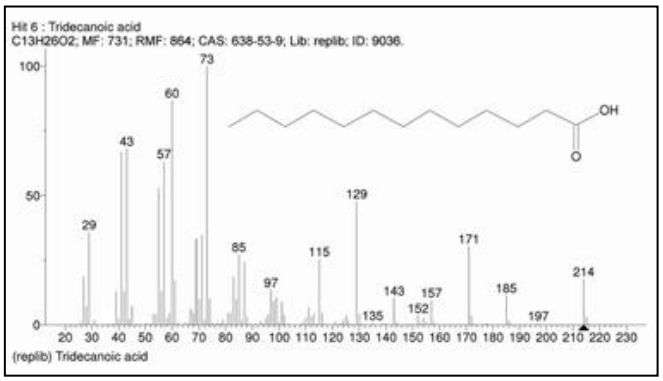


Fig 4: n-Hexadecanoic acid

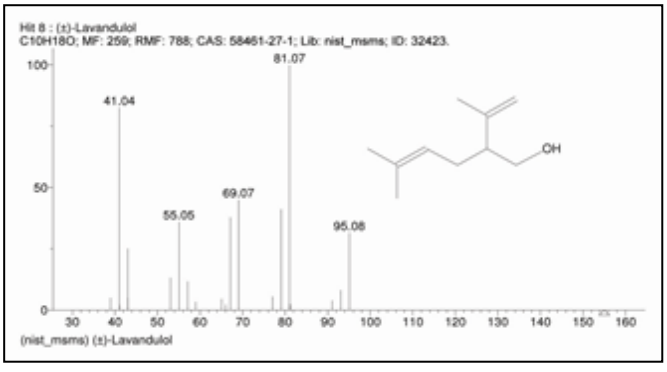


Fig 5: Lavandulol

4. Discussion

Medicinal plants have curative properties due to the presence of various complex chemical substances. In recent years, screening of such plants for biological activities has resulted in the development of therapeutics used in the treatment of many diseases.

4.1 Phytochemical Analysis

Phytochemical analysis conducted on the plant extract revealed the presence of flavanoids, tannins, coumarins, phytosterols, saponins, xanthoprotein, aleurone grains, triterpenoids, alkaloids, lactones, glycosides, polysaccharides, volatile oils and phenols. The constituents present in *E. scaber* are known to exhibit medicinal as well as physiological activities. The secondary metabolites such as flavanoids, tannins, coumarins, are present in methanol extract of *Elephantopus scaber*. Flavanoids have antioxidant powers that may provide important health benefits. Diets rich in flavonoids reduces the risk of a variety of diseases and may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogen compounds. Flavanoids are found to be useful in the treatment of immune system disorders which are responsible for 5-10 % of recurrent miscarriages.

Coumarin has appetite-suppressing properties, which in plants may reduce the impact of grazing animals. Though the compound has a pleasant sweet odor, it has a bitter taste, and animals tend to avoid it. Alkaloids are present in the all extracts except in methanol. Alkaloids have been associated with medicinal uses like analgesic, antispasmodic and antibacterial properties and also used as psychoactive substances. Phytosterols are present in acetone extract. Phytosterols reduce cholesterol levels and may inhibit lung, stomach, ovarian and breast cancers. Tannins serve as defence mechanism against predation. Glycosides are present in the chloroform extract of *Elephantopus scaber*. Glycosides are known to lower the blood pressure. But resin is absent in *Elephantopus.* Volatile oils are present in the chloroform extract. Lactones are present in acetone extract. Saponins are present in both petroleum ether and chloroform extract. In plants, saponins may serve as anti-feedants and protect the plant against microbes and fungi and some saponins are toxic to cold-blooded organisms and insects at particular concentrations. However, saponins are often bitter to taste, and so can reduce plant palatability (e.g., in livestock feeds). Macromolecules such as polysaccharides are present in all extracts of *Elephantopus*. Aleurone grains are present in Methanol extract. Xanthoprotein present in acetone extract of the *Elephantopus.* Triterpenoids present in the chloroform extract and phenols present in the methanolic extract.

Phenols play important roles in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants. Phenolic compounds can act as protective agents, inhibitors, natural animal toxicants and pesticides against invading organisms, i.e. herbivores, nematodes, phytophagous insects, and fungal and bacterial pathogens. The scent and pigmentation conferred by other phenolics can attract symbiotic microbes, pollinators and animals that disperse fruits.

4.2 GC-MS Analysis

The presence of various bio-active compounds detected after GC-MS analysis using the methanolic extract of *E. scaber* justifies the use of whole plant for various elements by traditional practitioner. The present study helps to predict the formula and structure of 14 biomolecules. Further investigation may lead to isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be helpful for further drug development. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will be definitely giving fruitful results and will open a new area of investigation of individual components and their pharmacological potency.

The components may be grouped in to main classes:The major constituents was found to be whose retention time (mnt) are found at undecanoic acid (23.33), Hexadecanoic acid (23.33), Tetradecanoic acid (23.33), Isolongifolene (26.63), Arabinose (23.33), lavandulol (26.52), 2-Mercaptoethanol (5.98), Sorbose (23.33), 9,12,15-Octadecatrienoic acid methyl ester (26.63), Butanedioic acid (5.98), and Bicyclo [4.1.0.] heptanes 7-pentyl (26.51), respectively. Terpenoids are an important part of volatiles from plants. Lavandulol, an oxygenated terpenoid, well known as preservative in food, drugs and cosmetics, has been tested *in vitro* for antibacterial, antifungal activity. Isolongifolol is suggested as potential anticarcinogenic agent exhibit cytotoxic activity against several solid tumor cell lines. Alcohols are known to possess bactericidal rather than bacteriostatic activity against vegetative cells. It has been proposed that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement, which may explain their activity, suggesting an increase in electronegativity increases the antibacterial activity. Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms.

Hence, the *E. scaber* may have chemopreventive, anticancer, anti-microbial activity, antioxidant and preservative activity due to the presence of secondary metabolites in the methanolic extract. Due to the presence of Isolongifolene which is a known anticancer compound it can be used as potent anticancer medicine. These findings support the traditional use of *E. scaber* in various disorders. Further studies are needed to isolate active principle of the extract as well as to elucidate their exact mechanism of action in various disorders.

5. Conclusion

The present study has been found to be useful in the identification of several constituents present in the methanolic extract of *E. scaber.* Phytochemical analysis conducted on petroleum ether, chloroform, acetone and methanolic extract of *E. scaber* revealed the presence of flavanoids, tannins, coumarins, phytosterols, saponins, xanthoprotein, aleurone grains, triterpenoids, alkaloids, lactones, glycosides, polysaccharides, volatile oils and phenols. Flavanoids have antioxidant powers that may provide important health benefits. Phytosterols reduce cholesterol levels and may inhibit lung, stomach, ovarian and breast cancers. Glycosides are known to lower the blood pressure. Phytochemicals which were identified in the whole plant extracts of *E. scaber* indicated their potential as a source of principles that may supply novel medicines. GC-MS analysis revealed the presence of various bioactive compounds (identified as sesquiterpenoids, aldehydes, alcohols, terpinolene) which justifies the use of the whole plant for treating various ailments by traditional practitioners. Lavandulol, an

oxygenated terpenoid, well known as preservative in food, drugs and cosmetics, has been tested *in vitro* for antibacterial, antifungal activity. Hence compounds present in *E. scaber* can be exploited as a nature friendly, strong anti-microbial agent having agricultural importance and also its great pharmaceutical values are to be analyzed.

6. Conflict of Interest

The authors confirm that this article content has no conflict of interest.

7. Acknowledgement

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