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GC-MS profiling of bioactive components from aqueous extract of *Pterocarpus marsupium*

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Abstract : Medicinal plants are sources of important therapeutic aids for alleviating human ailments. *Pterocarpus marsupium* belongs to the family Fabaceae is well known in Indian system for its traditional uses. The present investigation was carried out to determine the possible bioactive components of aqueous bark extract of *Pterocarpus marsupium* using GC-MS analysis, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The results of the GC-MS analysis have provided different peaks determining the presence of 27 phytochemical compounds. The major phytocomponents present in the extractare2-Pentanone,4-hydroxy-4-methyl,Furan-2-one,3,4-dihydroxy-5-[1-hydroxy-2-fluoroethyl], Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]- trimethylsilyl ester, a-d-Mannofuranoside, methyl, Phthalic acid, di (oct-3-yl)ester, 1-Monolinoleoylglyceroltrimethylsilyl ether, Ethyl iso-allocholate and Milbemycin b,13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methyl ethyl) with different therapeutic activities. The presence of these bioactive compounds justifies *Pterocarpus marsupium* is an excellent source of phytocomponent which helps to treat various diseases and health complications in human beings. However, isolation of individual phytochemical constituents might be useful to formulate a novel drug.

Keywords : *Pterocarpus marsupium*, GC-MS analysis, bioactive components, aqueous extract, NIST library.

Introduction

Phytomedicines are the important components derived from various parts of the plants with numerous applications in pharmaceutical and herbal industry [1]. The knowledge of medicinal plant has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha[2].The medicinal values of plants are based on the presence of low-molecular weight organic compounds called secondary metabolites such as flavonoids, phenols, alkaloids, saponins and terpenoids which produce a definite physiological action in the human body[3]. During the last few decades, there has been growinginterest in the use of medicinal plants due to their easy availability, therapeutic potential, least side effects and minimum cost. Phytoconstituents isolated from medicinal plants are playing a pivotal role in development of novel compounds, which might be useful in drug discovery[4].Various plants available in the nature are still unexplored for their medicinal potential. Among them, *Pterocarpusmarsupium*Roxb.is one of the most valuable multipurpose forest trees, commonly known as Indian kino and Bijasal in Hindi. It is a large deciduous tree widely distributed in the Central, Western and Southern regions of India, having a long history of numerous traditional and ethnobotanical applications in diverse cultures[5]. The wood and bark of the tree is useful for diabetic patients. Heartwood of *P. marsupium*has anti-cataract activity, effective on glycogen content of tissue and key enzyme of carbohydrate metabolism[6,7]. Different extracts of *P. marsupium* exhibited significant anti-ulcer and antioxidant properties in rats. Methanol extract of bark showed maximum activity against *Aspergillus niger*, *Salmonella typhi* and *Enterococcus faecalis*[8].Several chemical constituents like pterostilbene, epicatechin, pterosupin, marsupin have been identified and isolated from the plant[9].

The spectrometry and chromatographic screening method could provide the necessary preliminary observations to select crude plant extracts having potentially useful properties for further chemical and pharmacological investigation[10]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC- MS that were powerful tools for separation, identification and structural determination of phytochemicals[11]. Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [12]. This is the first report for the identification of bioactive compounds from *Pterocarpus marsupium* aqueous bark extract. For this reason, the aim of this work was to investigate the bioactive constituents of *Pterocarpus marsupium* bark with the aid of GC-MS technique, which may provide an insight in its use of traditional medicine.

Materials and Methods

Plant material

Pterocarpus marsupium bark was collected from Kalaburagi district, Karnataka, in midst of June, 2015. The plant was authenticated by Dr. Imran Baig, Professor, Botanical garden, UAS, GKVK Campus, Bangalore and the voucher specimen (UASB: 4552) was deposited in the herbarium of GKVK botanical garden.

Preparation of plant extract:

Freshly collected bark of *Pterocarpus marsupium* was washed thoroughly in running water, shade dried and then powdered to required particle size. The bark powder (100g) was successively extracted by Hot Soxhlet extraction with polar solvent like water. The extract was heated at 30-40ºC in hot air oven till the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for future use.

Gas Chromatography-Mass Spectrum Analysis:

2µl of aqueous bark extract from *Pterocarpus marsupium* was used for GC-MS analysis[13]. This extract was dissolved in HPLC grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier, JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50m x 0.25mm I.D. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Runtime was 30minutes[14].

Identification of components:

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns[15]. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library. The compound bioactivityprediction is done based on Dr. Duke’s Phytochemical and Ethnobotanical Databases (Dr. Duke Database, 2017). The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the totalarea.The name, molecular weight, molecular formula and the structure of the components of test materials were recorded.

Results And Discussion

Gas Chromatography–Mass Spectrometry (GC - MS) is a valuable tool for reliable identification of bioactive compounds[16]. In the present study, 27 bioactive compounds have been identified from the aqueous extract of *Pterocarpu smarsupium* bark by GC - MS analysis as shown in Figure-1.The active principles with their Retention Time (RT), molecular formula, molecular weight and peak area in percentage are presented in Table-1.

Table-1: Bioactive compounds detected in aqueous bark extract of *Pterocarpus marsupium*

Sl. No:	RT	Compound Name	Molecular formula	Molecular weight	Peak area%
1	4.36	2-Pentanone,4-hydroxy-4-methyl	$C_6H_{12}O_2$	116.15	1.68
2	4.57	Furan-2-one,3,4-dihydroxy-5-[1-hydroxy-2-fluoroethyl]	$C_6H_7FO_5$	178.11	62.54
3	4.57	Hydrazine, 1-methyl-1-(2-propynyl)	$C_4H_8N_2$	-	62.54
4	9.85	Cyclopentasiloxane,decamethyl	$C_{10}H_{30}O_5Si_5$	370.77	0.96
5	9.85	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	0.96
6	16.22	a-d-Mannofuranoside,methyl	$C_7H_{14}O_6$	194.18	2.23
7	24.37	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1, 1',4,4'-tetrone	$C_{28}H_{25}NO_7$	487.51	3.94
8	24.37	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene	$C_{30}H_{42}C_{l2}N_4O_3$	577.59	3.94
9	24.37	Phthalic acid, di(oct-3-yl)ester	$C_{24}H_{38}O_4$	390.55	3.94
10	24.62	1-Monolinoleoylglyceroltrimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498.89	2.23
11	24.62	Rhodopin	$C_{40}H_{5}8_{O}$	558.49	2.23
12	26.60	Bis(cis-13-docosenamido)methane	$C_{45}H_{86}N_2O_2$	687.19	2.89
13	26.60	9-Octadecenamide,(Z)	$C_{18}H_{35}NO$	281.47	2.89
14	32.07	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.62	11.30
15	32.07	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl) oxy]propyl ester,(Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	496	11.30
16	32.52	Milbemycin b,13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)	$C_{33}H_{46}ClNO_7$	604.18	12.22

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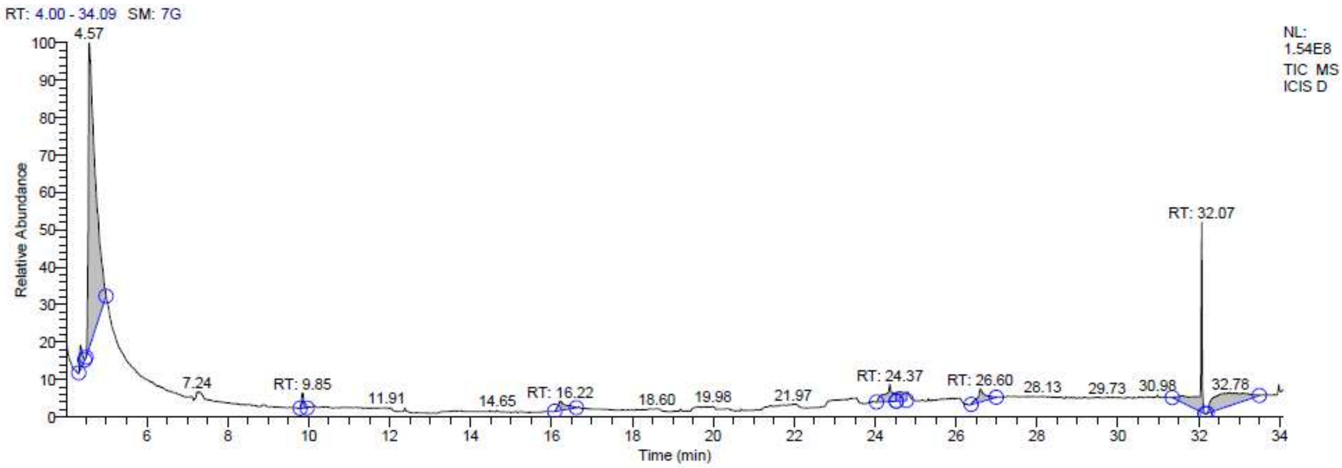

Figure- 1: GC-MS Chromatogram of the aqueous extract of *Pterocarpus marsupium* bark

*P.marsupium*is an ayurvedic plant, but thereare no sufficient phytochemicalanalyses has been carried out onit.There is growing awareness incorrelating the phytochemical components andtheir biological activities[17,18,19]. Table-2 lists the major phytocompounds and their biological activities obtained through Dr. Duke's Phytochemical and Ethnobotanical Databases. Thus this type of GC-MS analysis is the first steptowards understanding the nature of activeprinciples in this medicinal plant and will be helpful for further detailed study.The mass spectra and structuresof these bioactive compounds are presented in Figures 2, 3, 4, 5,6,7,8 and 9.

Table-2: Biological activity of phyto-components identified in the aqueous bark extract of *Pterocarp s marsupium*

Compound name	Compound nature	Biological activity
2-Pentanone,4-hydroxy-4-methyl	Tertiary alcohol	Antimicrobial
Furan-2-one,3,4-dihydroxy-5-[1-hydroxy-2-fluoroethyl]	nucleoside derivative	Anti-viral
Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-trimethylsilyl ester	Phenolic	Anti-fungal, anti-bacterial
a-d-Mannofuranoside, methyl	Carbohydrate	Anti-bacterial, anti-fungal
Phthalic acid, di(oct-3-yl)ester	Ester	Anti-hypercholesterol, anti-inflammatory
1-Monolinoleoylglyceroltrimethylsilyl ether	Steroid	Anti-microbial, anti-oxidant, anti-inflammatory, anti-arthritic, anti-asthma, Diuretic
Ethyl iso-allocholate	Steroid	Anti-microbial, anti-cancer, anti-arthritic, anti-asthma, diuretic, anti-inflammatory

Milbemycin b,13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)	macrolides	Anti-parasitic

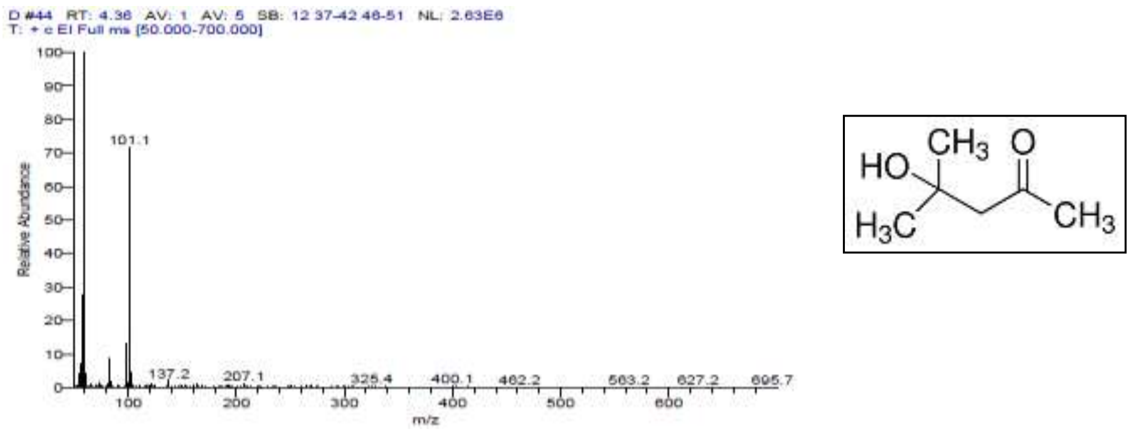

Figure-2: Mass spectrum and structure of 2-Pentanone,4-hydroxy-4-methyl

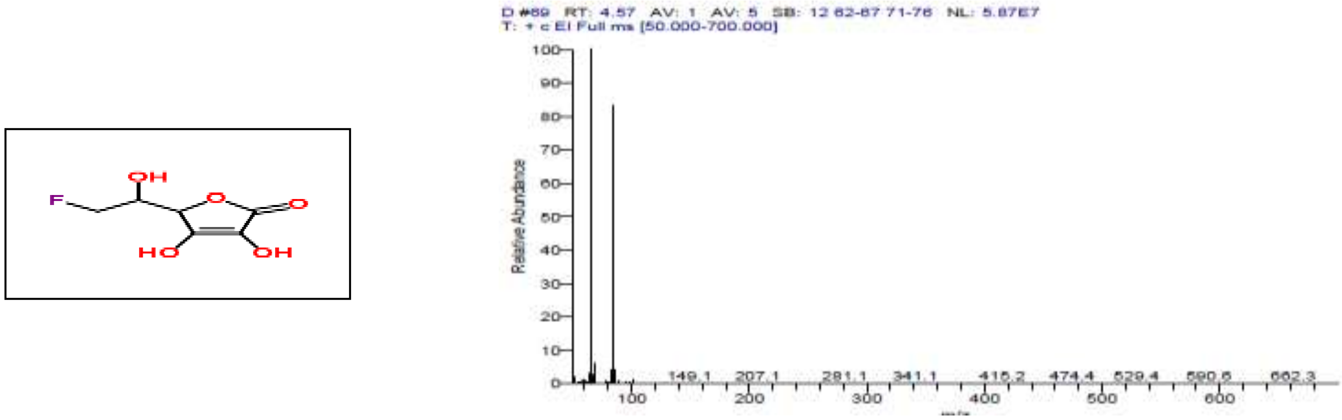

Figure-3: Mass spectrum and structure of Furan-2-one, 3, 4-dihydroxy-5-[1-hydroxy-2-fluoroethyl]

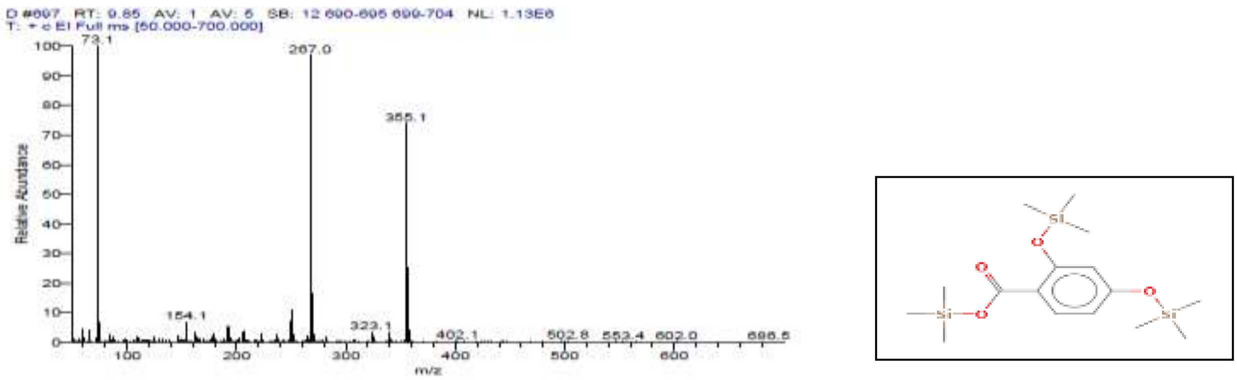

Figure-4: Mass spectrum and structure of Benzoic acid, 2, 6-bis [(trimethylsilyl)oxy]- trimethylsilylester

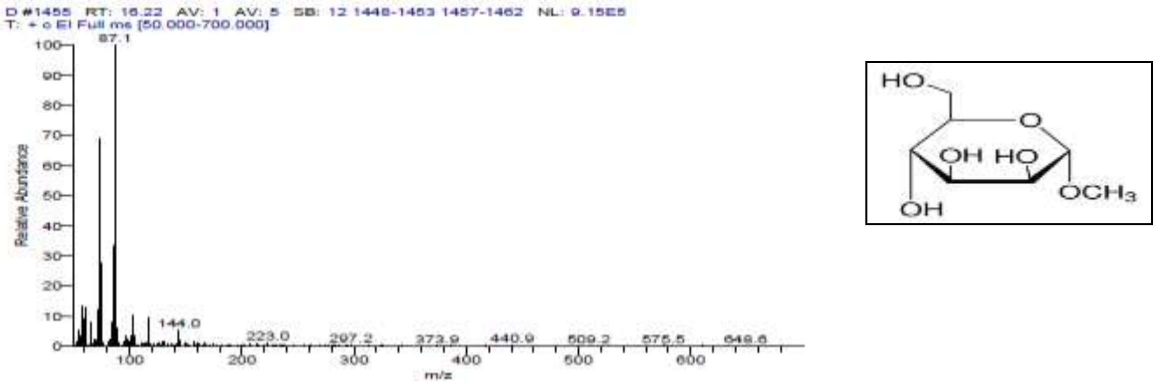

Figure-5: Mass spectrum and structure ofa-d-Mannofuranoside, methyl

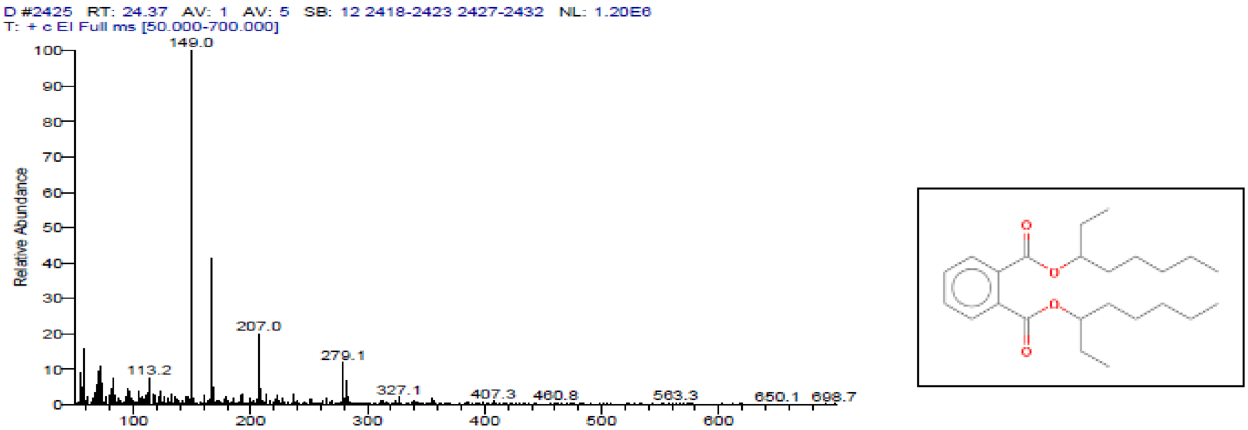

Figure-6: Mass spectrum and structure of Phthalic acid, di(oct-3-yl)ester

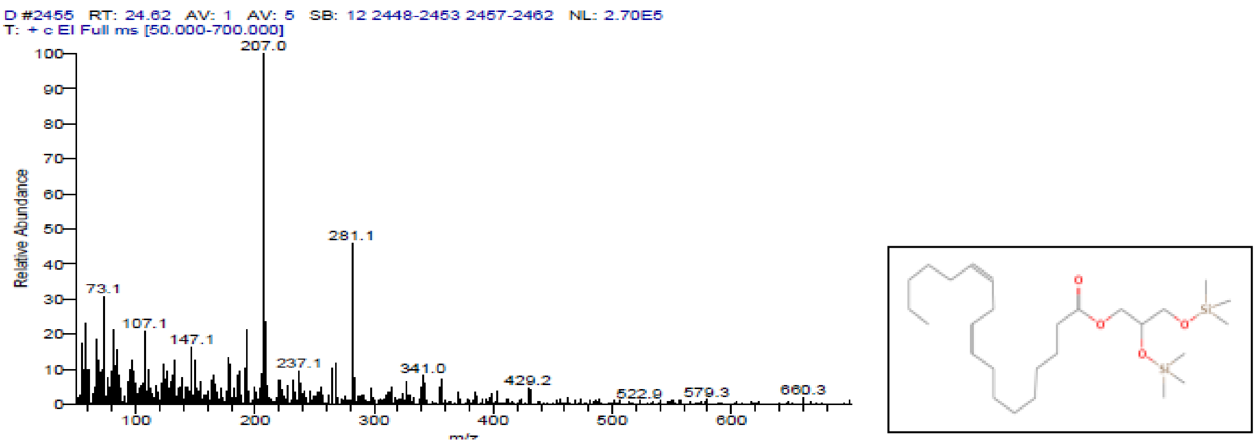

Figure-7: Mass spectrum and structure of1-Monolinoleoylglyceroltrimethylsilyl ether

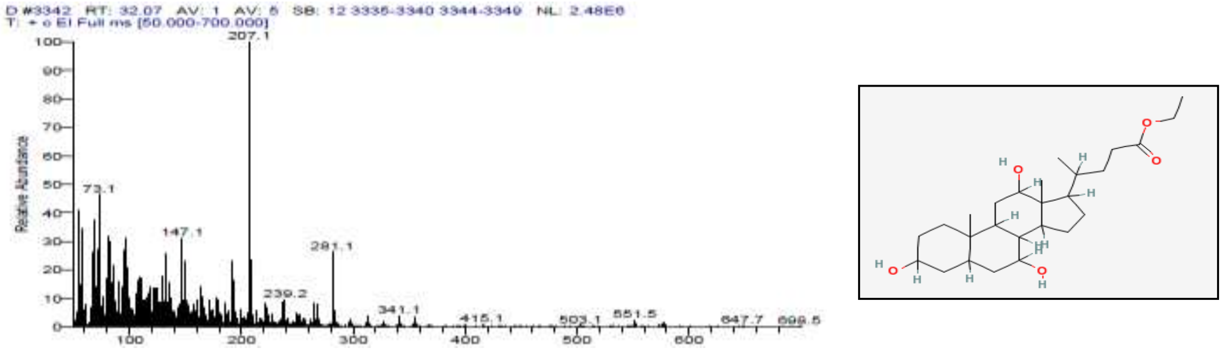

Figure-8: Mass spectrum and structure of Ethyl iso-allocholate

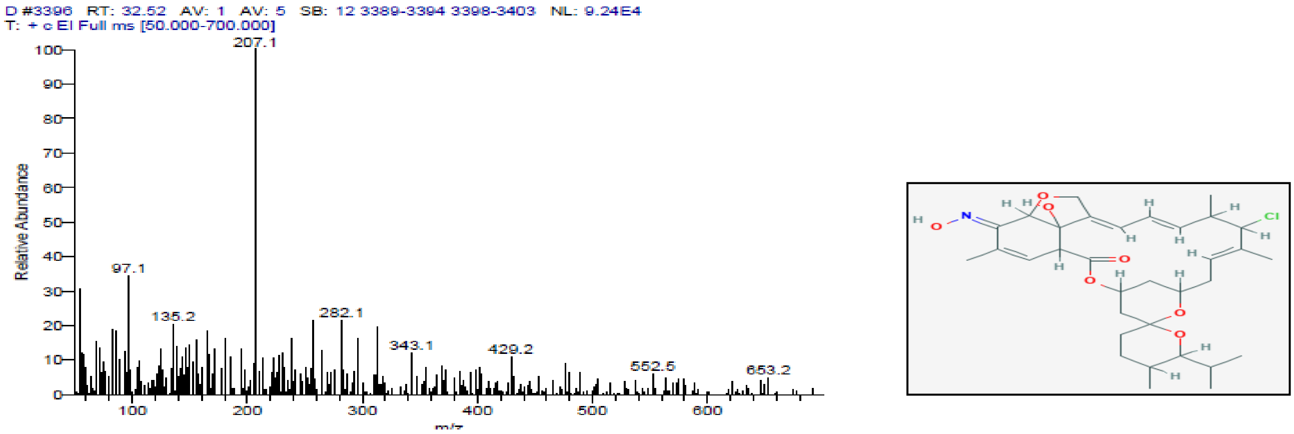

Figure-9: Mass spectrum and structure of Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)

Conclusion

Pterocarpus marsupium is traditionally used for the treatment of various ailments and its wider application can easily be noticed by the presence of different bioactive molecules.So it is recommended as a plant of phytopharmaceutical importance. Itpaves the way for the development of several treatmentregimens based on this extract. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity.

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