Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2017; 8(3); 104-110
doi: 10.25258/ijpqa.v8i03.9571

ISSN 0975 9506

Research Article

Bioactive Compounds Investigation From Methanol Bark Extract of *Pterocarpus marsupium* Using GC-MS Analysis

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Received: 8th June, 17; Revised:17th August, 17; Accepted:25th August, 17; Available Online:25th September, 2017

ABSTRACT

Introduction: Medicinal plants still represent untapped sources of novel compounds with potential therapeutic effects for mitigating human maladies. *Pterocarpus marsupium*, commonly known as Bijasal, is a well-known drug in ayurvedic system of medicine. Materials and methods: The dried bark powder of *P.marsupium* was extracted by Soxhlet with methanol and analyzed by gas chromatography, while the mass spectra data of the compounds found in the extract was matched with the data available at National Institute of Standards and Technology (NIST) library. Results: The results of the GC-MS analysis provide different peaks determining the presence of 10 major phytocomponents, that are 1-Penten-3-one,1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-; Geranyl isovalerate; 2-Pentadecanone,6, 10, 14-trimethyl; Cyclopropaneoctanal, 2-octyl-; Octadecanoic acid; 10-Octadecanoic acid, methyl ester; Heptadecanoic acid, 16-methyl,-methyl ester; Phytol; 4,8,12,16-Tetramethyl heptadecan-4-olide; Dasycarpidan-1-methanol, acetate with different therapeutic activities. Conclusion: The presence of these bioactive compounds justifies that the *Pterocarpus marsupium* can offer a base for using this compound as herbal alternative for the synthesis and development of new drug to treat various infectious diseases. However, isolation of individual phytochemical constituents might be useful to formulate a new novel drug.

Keywords: *Pterocarpus marsupium*, GC-MS analysis, bioactive components, methanol extract, NIST Library.

INTRODUCTION

Herbal medicines occupy distinct position right from the primitive period to present day due to their therapeutic potential, which signifies a major part of habitual treatments for different maladies[1,2]. Complementary medicine has a long history of serving people all over the world and has taken an important place especially in developing countries where limited health services are available[3,4]. The medicinal value of the plant lies in the bioactive phytochemical constituents that produce definite physiological effect on human body[5]. The most important bioactive secondary metabolites found in plants are alkaloids, flavonoids, tannins and phenolic compounds. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity[6]. Therefore, the present study was aimed to identify the phytoconstituents which may have therapeutic value present in the *Pterocarpus marsupium* bark using GC-MS analysis. *Pterocarpus marsupium* (Fabaceae) is commonly known as Vijayasar is a large deciduous tree that is native to India, Sri Lanka and Nepal[7]. The heart wood, leaves, flowers and bark of the plant have useful medicinal properties. Traditionally, bark is useful in vitiated condition of kapha, pitta, elephantiasis, erysipelas, urethrorrhea, rectalgia, opthalmopathy, hemorrhages, dysentery, cough, grayness of hair, urinary discharge and piles[8]. The powdered bark is mixed with *Schleichera oleosa* and taken with cold water to treat dysentery[9]. It has been scientifically reported for antihyperlipidaemic[10], hepatoprotective[11], anti-ulcer[12], anti-inflammatory[13] and anti-diabetic activity[14]. A variety of flavonoids and their derivatives have been isolated from various parts of the plant[15]. Methanol extract of bark has showed maximum activity against *Aspergillus niger*, *Salmonella typhi* and *Enterococcus faecalis*[16]. The selective COX-2 inhibitory activity of *P.marsupium* extract, attributed to its pterostilbene content has also been reported[17]. In recent years, Gas Chromatography - Mass Spectroscopy (GC-MS) studies are being increasingly applied for the analysis of medicinal plants as this technique will proved a valuable and important method in analyzing fatty acids, non-polar compounds, lipids, volatile essential oil and alkaloids[18]. Taking into consideration the medicinal importance of *Pterocarpus marsupium*, the methanol extract of the plant bark was analyzed for the first time using GC-MS.

MATERIALS AND METHODS

Plant material

The bark of *Pterocarpus marsupium* was collected from in and around Kalaburagi district, Karnataka, in the month of June, 2015. The plant was authenticated by Dr. Imran Baig, Professor, Botanical garden, UAS, GKVK Campus,

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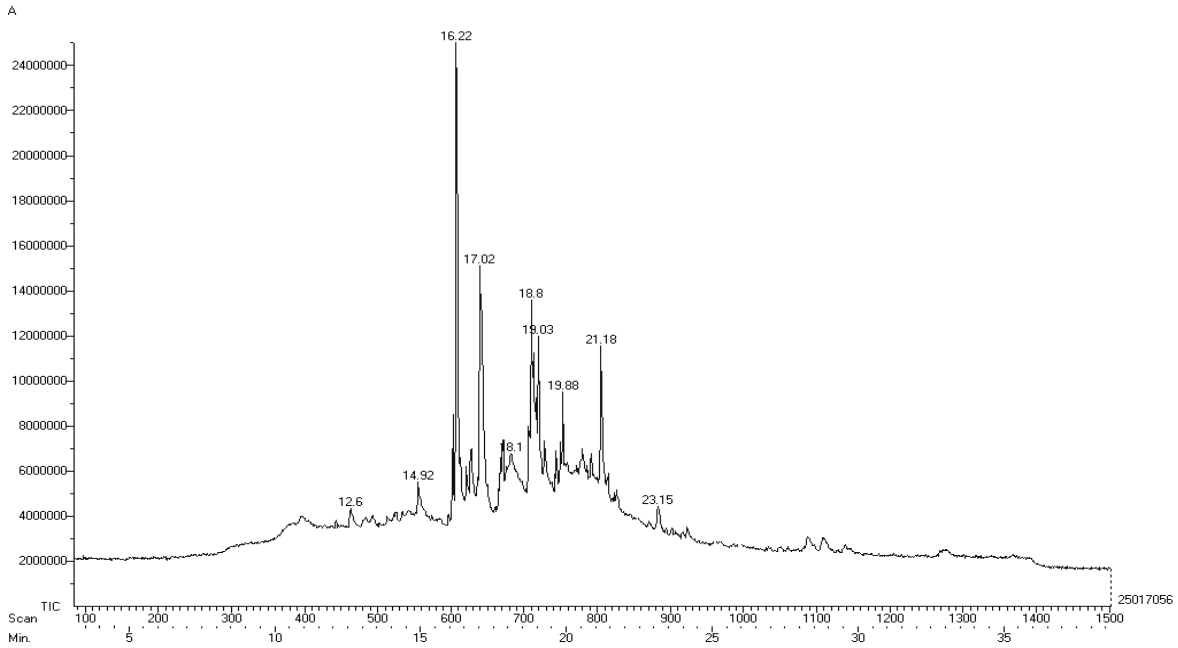

Figure 1: GC-MS Chromatogram of the methanol extract of *Pterocarpus marsupium* bark.

Table 1: Bioactive compounds detected in methanol extract of *Pterocarpus marsupium* bark.

Sl. No:	RT	Compound Name	Compound nature	Molecular formula	Molecular weight	Biological activity
1	12.6	1-Penten-3-one,1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	Alkane	$C_{14}H_{22}O$	206.32	-
2	14.92	Geranyl isovalerate	Monoterpene	$C_{15}H_{26}O_2$	238.37	Anti-inflammatory, antioxidant and anti-viral activities
3	16.22	2-Pentadecanone,6,10,14-trimethyl	Diterpenoids	$C_{18}H_{36}O$	268.48	-
4	17.02	Cyclopropaneoctanal,2-octyl-		$C_{19}H_{36}O$	280.49	-
5	18.1	Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$	284.48	Antibacterial, antiviral, anti-inflammatory, soap lubricant, cosmetics
6	18.8	10-Octadecanoic acid, methyl ester	Unsaturated fatty Acid methyl ester	$C_{19}H_{36}O_2$	296.49	Antibacterial, antifungal, antioxidant, decrease blood cholesterol
7	19.03	Heptadecanoic acid,16-methyl,-methyl ester	Stearic acid	$C_{19}H_{38}O_2$	298.50	Used against skin cancer protein.
8	19.88	Phytol	Triterpene	$C_{20}H_{40}O$	296	Antimicrobial,anticancer,antiinflammatory,diuretic,immunostimulatory,antidi-abetic
9	21.18	4,8,12,16-Tetramethylheptadecan-4-olide	Isoprenoid	$C_{21}H_{40}O_2$	324.54	-
10	23.17	Dasycarpidan-1-methanol,acetate	Ester	$C_{20}H_{26}N_2O_2$	326.44	-

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, later with distilled 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 methanol. 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 methanol bark extract from *Pterocarpus marsupium* was used for GC-MS analysis[19]. 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 with silica 50m x 0.25mm I.D. Analysis conditions were 20 minutes

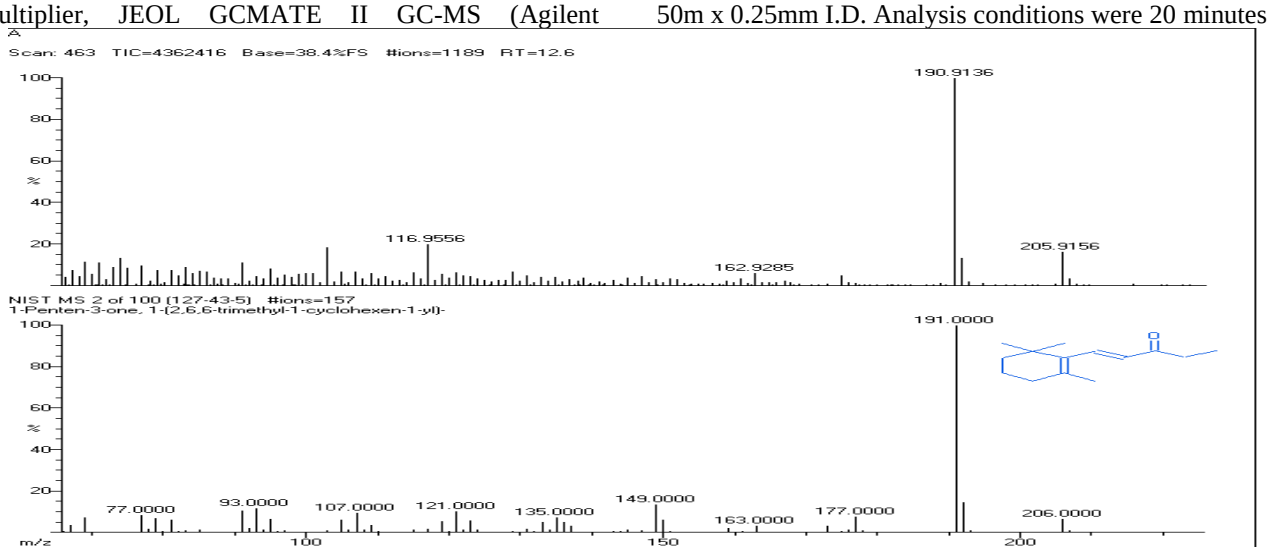

Figure 2: Mass spectrum and structure of 1-Penten-3-one, 1-(2,6, 6-trimethyl-1-cyclohexen-1-yl)-.

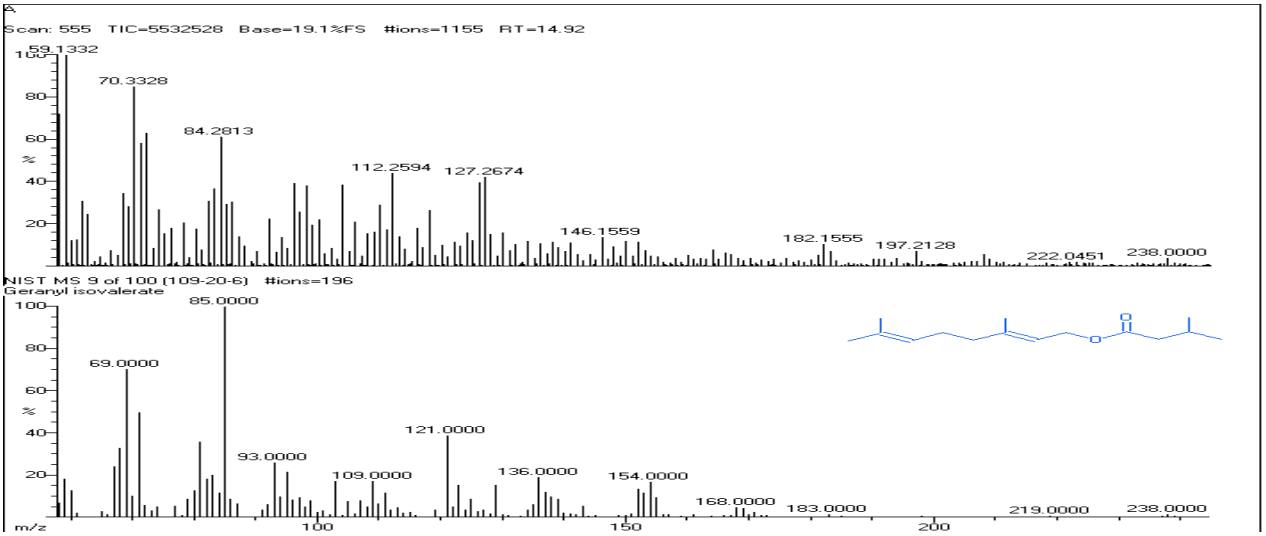

Figure 3: Mass spectrum and structure of Geranyl isovalerate.

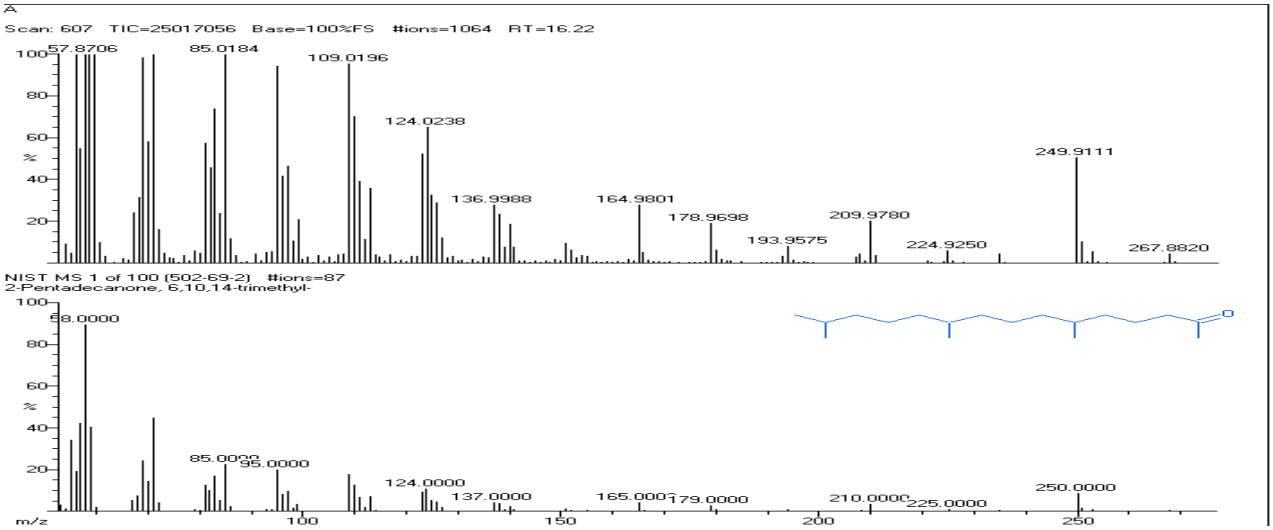

Figure 4: Mass spectrum and structure of 2-Pentadecanone,6,10,14-trimethyl.

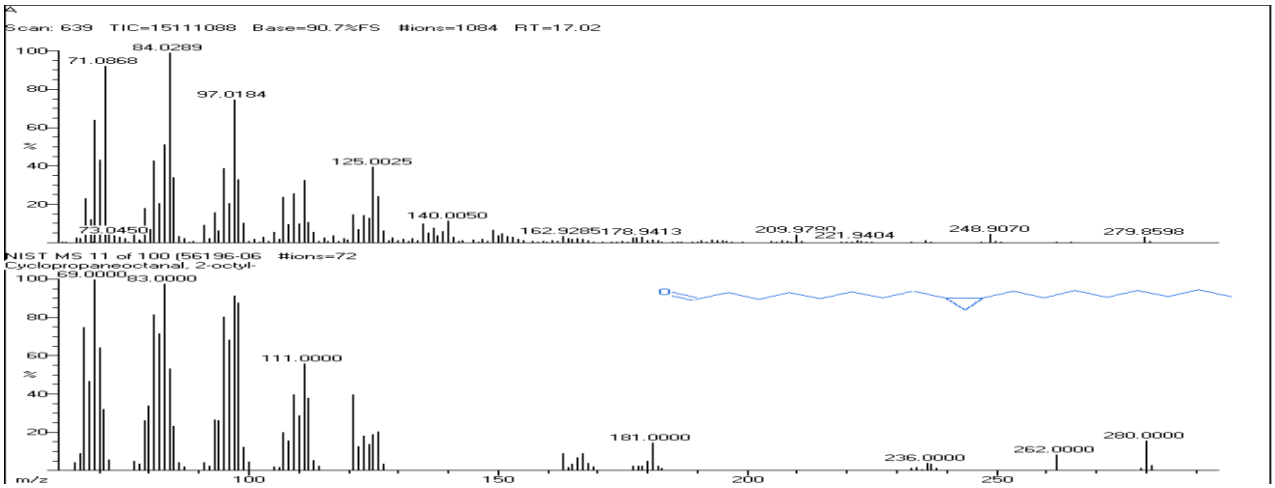

Figure 5: Mass spectrum and structure of Cyclopropaneoctanal, 2-octyl-.

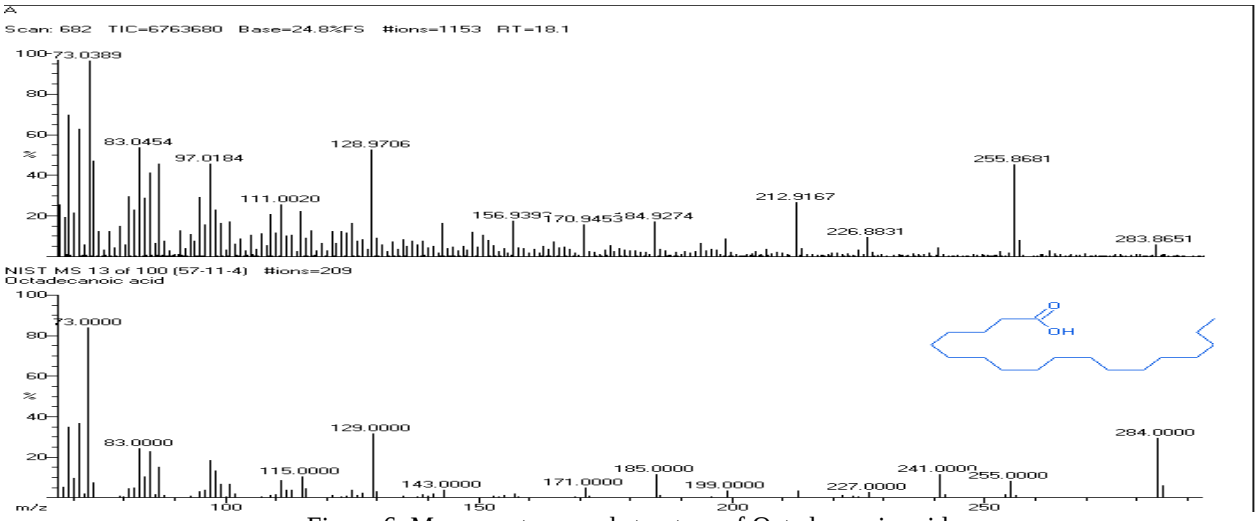

Figure 6: Mass spectrum and structure of Octadecanoic acid.

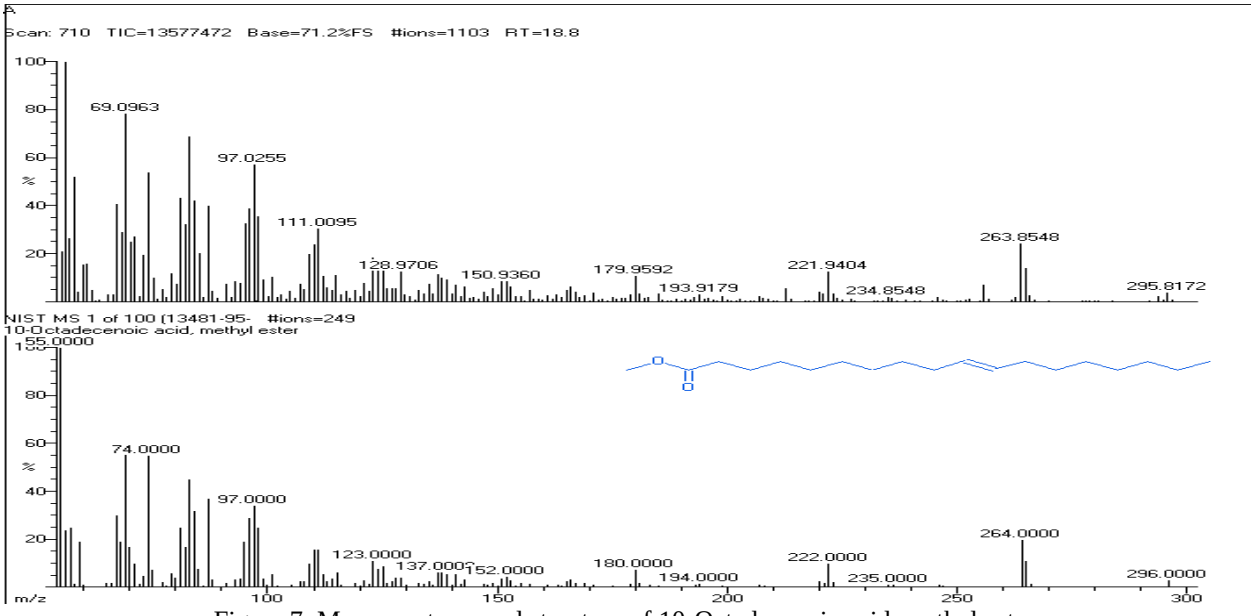

Figure 7: Mass spectrum and structure of 10-Octadecanoic acid, methyl ester.

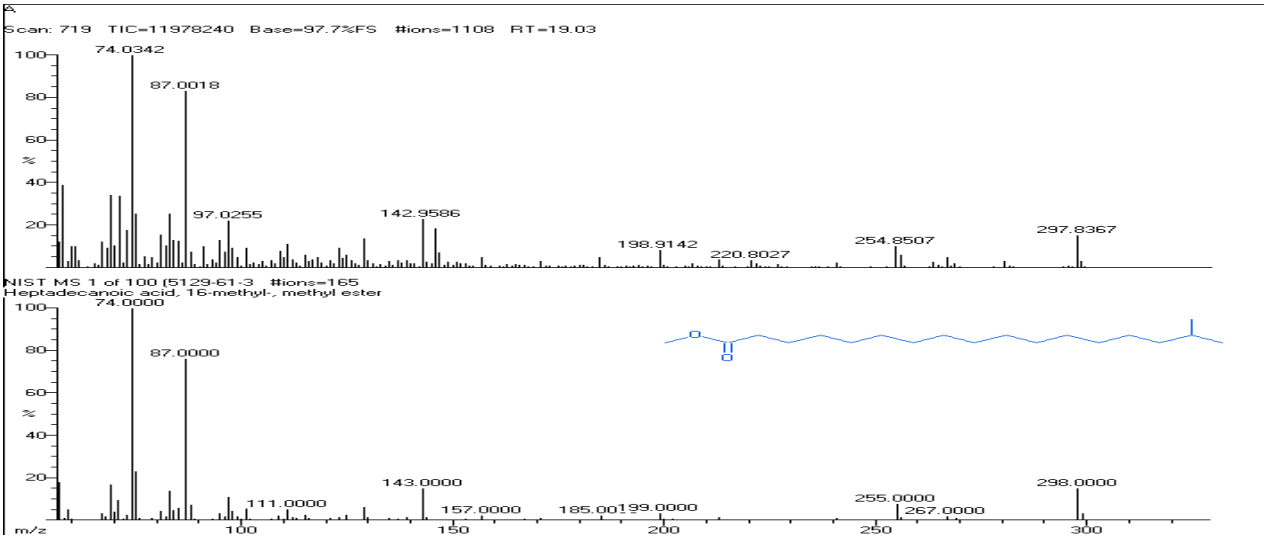

Figure 8: Mass spectrum and structure of Heptadecanoic acid, 16-methyl,-methyl ester.

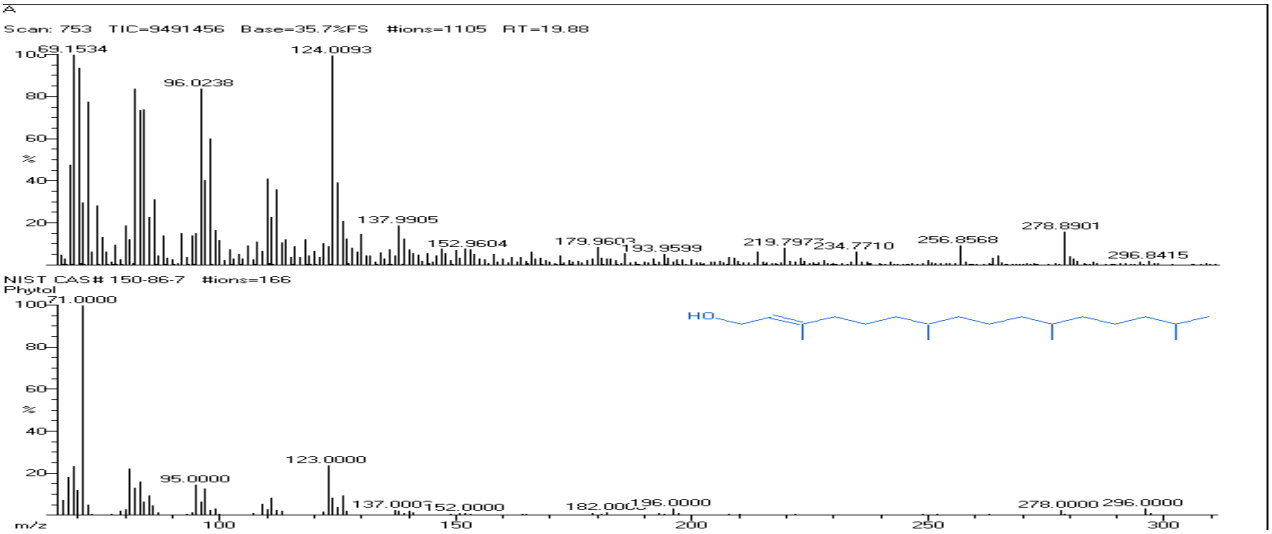

Figure 9: Mass spectrum and structure of Phytol.

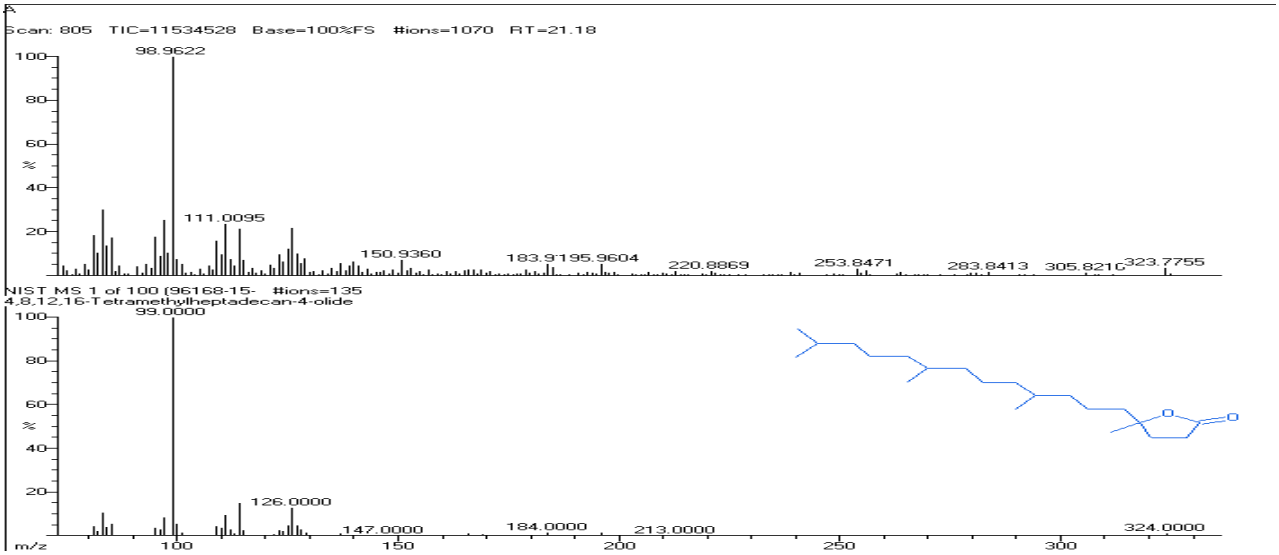

Figure 10: Mass spectrum and structure of 4,8,12,16-Tetramethylheptadecan-4-olide.

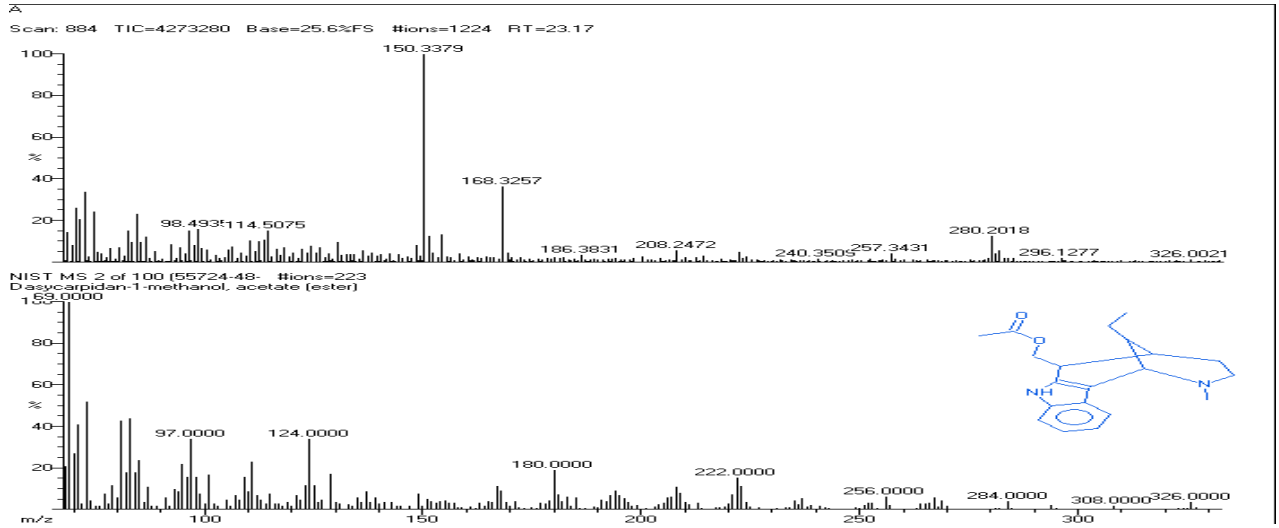

Figure 11: Mass spectrum and structure of Dasycarpidan-1-methanol, acetate.

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. Run time was 30 minutes[20].

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[21]. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library. The compound bioactivity prediction 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 total area. The name, molecular weight, molecular formula and the structure of the components of test materials were recorded.

RESULTS AND DISCUSSION

GC-MS is a combined technique which is used to identify different substances within the sample. It works on separation of the individual compound by GC according to their RT and the separated compounds were further analyzed at a molecular level by MS[22]. In the present study, 10 bioactive compounds have been identified from the methanol extract of *Pterocarpus marsupium* bark by GC - MS analysis as shown in Figure-1. The active principles with their Retention Time (RT), molecular formula, molecular weight and their biological activities obtained through Dr. Duke's Phytochemical and Ethnobotanical Databases are presented in Table-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[23]. This report is the first of its kind to analyze the chemical constituents of *P.marsupium* methanol bark extract using GC-MS. The mass spectra and structures of identified bioactive compounds are presented in Figures 2-11. The presence of various bioactive compounds confirms the application of *P.marsupium* for various ailments by traditional practitioners.

CONCLUSION

In developing countries, providing modern healthcare facilities is still in infancy. Due to economic constraint, it is prudent to look for options in herbal medicines. *Pterocarpus marsupium* carried out an importance from the ancient times to cure mankind against various disease conditions. The medicinal applications of this plant are countless and more investigations must be carried out to evaluate the mechanism of action of active principles so that its potential can be fully utilized. By the isolation of various phytochemicals it enables to exploit its therapeutic value which plays a major role in modern system of medicine.

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