



**IDENTIFICATION OF LONG CHAIN FATTY ACIDS, TERPENOIDS AND
GLUCOSINOLATE (DESULPHOSINIGRIN) IN *MICHELIA CHAMPACA* BY
GAS CHROMATOGRAPHY: MASS SPECTROSCOPY (GC-MS) ANALYSIS**

PAWAR MK^{1*}, DWIVEDI JA² AND DWIVEDI UK³

- 1:** Registered PhD Scholar, Department of Pharmaceutical Sciences, Pacific Academy of Higher Education and Research University, Udaipur-313024, Rajasthan, India
- 2:** Professor and HOD, Department of Pharmaceutics, Pacific Academy of Higher Education and Research University, Udaipur-313024, Rajasthan, India
- 3:** Professor, Department of Pharmaceutics, Geetanjali Institute of Pharmacy, Udaipur-313001, Rajasthan, India

*Corresponding Author: Ms. Manjushree Kundlik Pawar: E Mail: mkpawar230@gmail.com

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ABSTRACT

Background: Phytochemical analysis is very crucial for drug discovery, development of novel therapeutic agents and to screen presence of any new bioactive compounds in plant derived substances. Also, globally, majority of population still depends on herbal remedies and *Michelia champaca* (MC) is a well-known ethnopharmacological herb in Ayurveda and exhibits a wide range of therapeutic potential. **Objective:** The aim and objective of this study was to study the phytochemical profile of ethanolic extract of MC by Gas Chromatography Liquid Chromatography (GC-MS) analysis. **Materials and Methods:** Ethanolic extract of MC was prepared by soxhlet extraction method and the extract was subjected to GC-MS analysis (Agilent 7890A GC system) for chemical characterization of the extract. The constituents were analysed by matching mass spectra with MS libraries. **Results:** Total 21 compounds were identified in the extract. The important constituents belonging to phytochemical class - sugar alcohol (Erythritol), sesquiterpenes (caryophyllene oxide), long chain fatty acids (hexadecanoic acid; octadecanoic acid; 8,11-14-eicosatrienoic acid; docosanoic acid, ethyl ester etc.), glucosinolate (Desulphosinigrin), acyclic diterpenoid (phytol), phytosterol (γ -

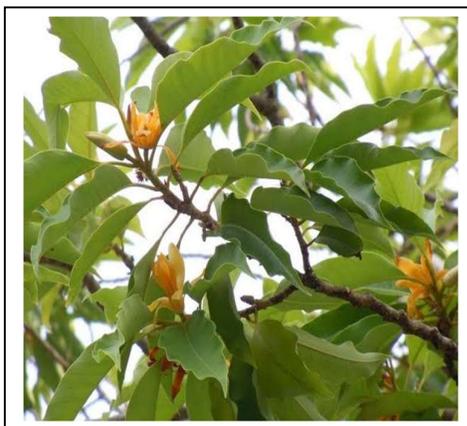
sitosterol), sesquiterpene alcohol (2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-(E,E) etc. were identified in this analysis. **Conclusion:** Results concluded that the plant has shown to be a potential source of bioactive compounds of significant importance in pharmacology. The researchers would isolate the constituent/s from this plant and can work with different animal or *in-vitro* models.

Keywords: GC-MS, *Michelia champaca*, Phytoconstituents, Pharmacological, Desulphosinigrin

INTRODUCTION

Phytochemicals are plant derived secondary metabolites which are important in investigation of various groups of chemical compounds which may further help in drug discovery and development of novel therapeutic agents. GC-MS technique helps in the identification of new bioactive compounds, present if any, in the samples. Previous phytochemical screening of this plant has led to identification of many compounds and to continue the search for novel agents from magnoliaceous plants, whole plant of *Michelia champaca* was

chosen by us. Findings of this research study revealed the presence of bioactive compounds in ethanolic extract of *Michelia champaca* – a well know ethnobotanical herb., the phytochemicals belonging to different groups like sugar alcohol, long chain fatty acids, terpenoids and glucosinolate (Desulphosinigrin) were identified. The identification of Desulphosinigrin forms the first report of the occurrence in the ethanolic extract of *michelia champaca* by GC-MS analysis in this research.



Sugar alcohols also referred as ‘polyols’ are chemical compounds containing three or more hydroxyl groups and are derivatives of mono- and disaccharides that have been

hydrogenated. Polyols can be acyclic compounds such as erythritol, xylitol and sorbitol or cyclic such as myo-inositol [1]. Long chain fatty acids (LCFA) are

molecules that act as metabolic intermediates and constituents of membranes and these fatty acids play an important role in metabolic disorders and in chronic diseases where inflammation is involved. LCFAs are saturated or unsaturated fatty acids containing 13-21 carbons. The role of fatty acids (FAs) in some diseases such as cancer, inflammation and autoimmune diseases has been well-discussed in several reviews. Fatty acids are classified according to their carbon (C) chain-length and the number of double bonds. LCFAs have chain-lengths of C11-20 and FAs longer than C20 ($C > 20$) are called very long-chain FAs (VLCFAs). Based on double bonds, FAs are classified into saturated FAs (no double bond), monounsaturated FAs (one double bond) and polyunsaturated FAs (two or more double bonds). Polyunsaturated fatty acids are further subdivided into n-3 (or $\omega 3$) and n-6 (or $\omega 6$) series depending on the position of the terminal double bond (the double bond most distant from the carboxyl group). In the $n-x$ series, x indicates the ordinal number of carbon atom with a double bond from the end of the carbon chain [2-4].

Terpenoids have common biosynthetic origin based on isoprene units [$\text{CH}_2=\text{C}(\text{CH}_3)\text{-CH}=\text{CH}_2$]. Carbon skeletons in them are built from the union of two or more of C_5 units. The classification is based on number of units as follows: C_{10} -

Monoterpenoids (2 carbon atoms), C_{15} -Sesquiterpenoids (3 carbon atoms), C_{20} -Diterpenoids (4 carbon atoms), C_{30} -Triterpenoids (6 carbon atoms) from squalene parent molecule and C_{40} -Tetraterpenoids (8 carbon atoms) [4]. Sesquiterpenes are well known for their therapeutic potential in cancer treatment [5]. Stigmasterol and its analogues have shown potentiality of acting as chemopreventive agent in cancer by inhibiting the cells in colon and breast cancer [6-14]. Triterpenoids are divided to four groups of compounds: true triterpenes, steroids, saponins and cardiac glycosides or cardenolides [4].

Glucosinolates are sulphur containing organic compounds, biosynthesized from amino acids, having acrid taste or an obnoxious smell. They are divided into three groups based on the structure of different amino acid precursors: 1. Aliphatic glucosinolates derived from methionine, isoleucine, leucine, or valine. 2. Aromatic glucosinolates derived from phenylalanine or tyrosine, and 3. Indole glucosinolates derived from tryptophan. Glucosinolates are biologically active compounds and are known for their fungicidal, bactericidal, nematocidal, allelopathic properties and because of their cancer chemo-protective attributes, they have been recently reviewed and researched intensely [4, 15].

Gas Chromatography Mass-Spectrometry (GC-MS), a hyphenated system has become a technological platform for metabolite profiling in plant and the medicinal herbs having numerous bioactive compounds can be identified at less than 1ng by using this technique. This method has proved to be a valuable method for analysis of non-polar components and volatile essential oils, fatty acids, lipids, and alkaloids [16-20]. Thus, in the current research work, the researchers have interpreted the results based on mass elucidation with structural identification of different classes of compounds and identified the phytoconstituents belonging to sugar alcohol, long chain fatty acids, terpenoids and glucosinolates chemical groups by subjecting the ethanolic extract of MC to GC-MS analysis which may be of therapeutic potential.

MATERIALS AND METHODS

Ethanolic extract of *Michelia champaca*:

The whole plant was collected from Sanjay Gandhi National Park (SGNP), Mumbai. The plant was authenticated at Agharkar Research Institute, Pune, by Dr. R.K. Chaudhary. The plant was shade dried, crushed and finely powdered passing through sieve no. 80 to remove the twigs and associated materials. The powder was then extracted with ethanol (50 g in 500 ml) and subjected to soxhlet extraction and dry form was obtained on rotary evaporator. The extract was stored at 8-15°C for further analysis and study. **GC-MS analysis:** The extracts were subjected for GC-MS analysis at IIT Bombay. Mobile phase: Ethanol electron impact (EI)-MS spectrum was scanned at 70 eV with instrument details as mentioned in **Table 1 and Table 2**: The compounds were identified by comparing their mass spectra with NIST MS 200 structural library.

Table 1: GC Specification

MODEL	Agilent 7890A GC System
Detector Specification	Mass Spectrometer Model: The AccuTOFGCv/JMS-T100GCv Make: JEOL
COLUMN SPECIFICATIONS I. Name of column II. Length III. Dimension IV. Column material	HP5 Column (30m length*0.25mm internal diameter*0.25microfilm thickness) Column Material is Polysiloxane
Carrier Gas Used	Helium
Carrier Gas Flow Rate	1ml/min
Oven Temperature	280°C
Injection Temperature	200°C
Injection Volume	1 microL
Sample flow rate	1ml/min
Split Ratio	1:10

Table 2: MS Specification

MODEL	Joel, AccuTOF GCV
IONIZATION SOURCE USED	EI Positive
Mass range	35-800amu
Split Ratio	1:10
Ion Source Temperature	220°C
Solvent Delay	4mins

RESULTS AND DISCUSSION

In the present research work we have identified the constituents in the ethanolic extract of MC, belonging to important phytochemical class such as sugar alcohol (1,2,3,4-Butanetetrol). sesquiterpenes (caryophyllene oxide); fatty acids (Hexadecanoic acid, ethyl ester; n-hexadecanoic acid; Octadecanoic acid ethyl ester; Docosanoic acid, ethyl ester etc.), glucosinolate (Desulphosinigrin), acyclic diterpenoid (phytol), stigmasterol (γ -sitosterol), acyclic sesquiterpene alcohol [(2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-(E,E)] etc. These constituents have a potential pharmacological role. The detailed information of various compounds elucidated by GC-MS analysis is shown in **Table 3** and their chemical nature is described in **Table 4**. The mass spectrum peaks of MC extracts are shown in **Figure 1**. The mass spectrum of different components found in MC extract are shown from **Figure 2 to Figure 22**.

Sugar alcohols – low digestible carbohydrates are characterized with lower blood glucose response and metabolized without insulin, and so are widely used in pharmaceutical industries [1, 21, 22]. Long

chain fatty acids are important in health and disease and play an essential role in metabolic disorders and in chronic diseases where inflammation is associated [2, 3, 24]. Sesquiterpenes are extensively studied for chemistry, biochemistry and biological origin and display a wide range of pharmacological properties – antibiotic, antitumor, antiviral, cytotoxic, immunosuppressive, antifungal etc. [5, 6-14, 25, 26] Sesquiterpene alcohol - Farnesol identified in this study has reported to show antimicrobial, anti-inflammatory, antitumor, antioxidant activity [27]. Glucosinolates are extensively studied for anti-carcinogenic effect [15]. Desulphosinigrin a glucosinolate identified in MC extract has been reported to show anticancer and antimicrobial effects [28, 29].

CONCLUSION

The study demonstrates the presence of numerous phytoconstituents in plant extracts belonging to sugar alcohol, long chain fatty acids, terpenoids, glucosinolate, sesquiterpene alcohol chemical classes. The glucosinolate desulphosinigrin was identified first time in this extract of study. The literature has shown various pharmacological activities especially

immunomodulatory and anticancer, associated with these phytochemical groups observed in this research. Therefore, the observed phytoconstituents can be of significant with respect to pharmacological aspects and could provide researchers to work with different animal models and activities. However, the isolation of these constituents from the plant and screening it

for biological role by various *in-vitro/in-vivo* studies would be more beneficial.

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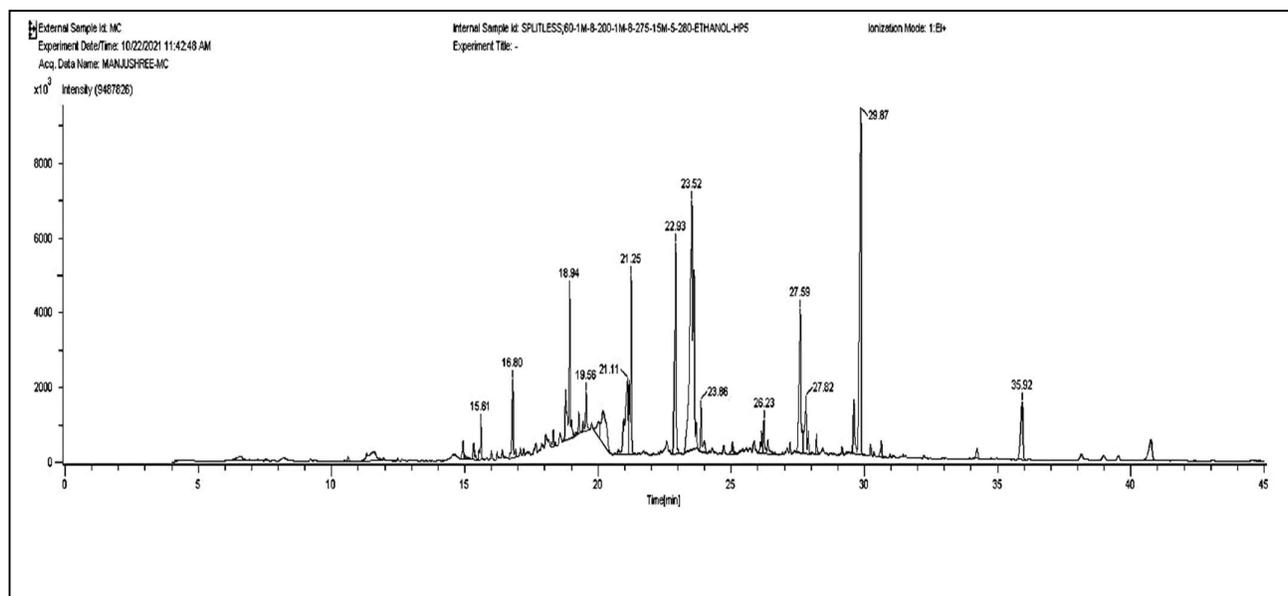
We would like to acknowledge IIT Bombay for GC-MS analysis. We would like to acknowledge the management of Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan for their constant support in providing the required facilities for conducting this work.

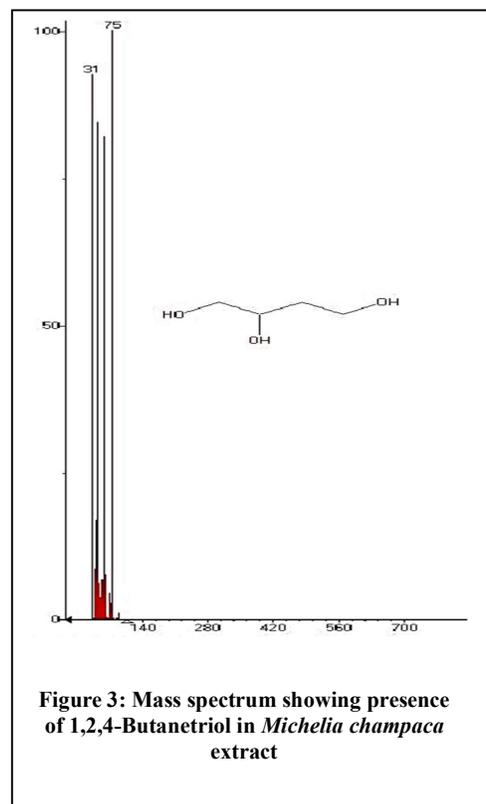
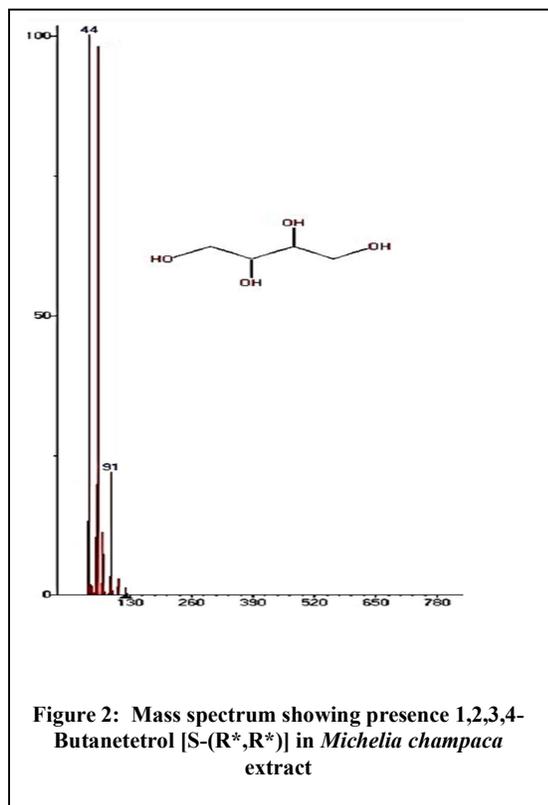
Table 3: The components found in ethanolic extract of *Michelia champaca* by GC-MS analysis

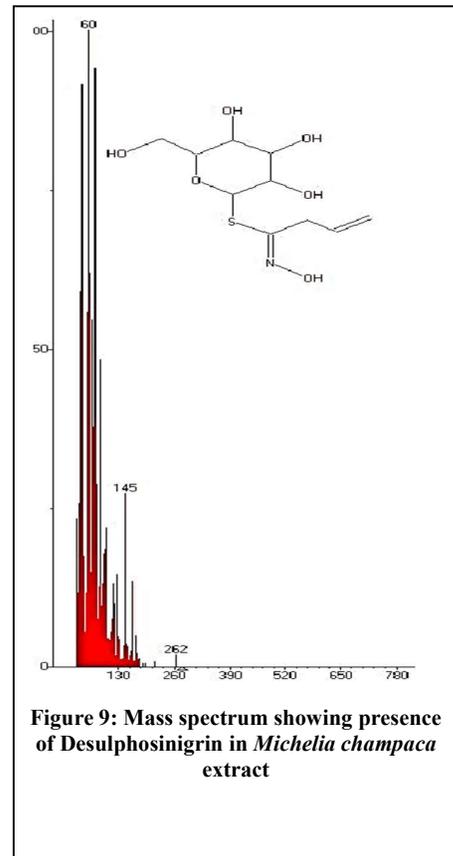
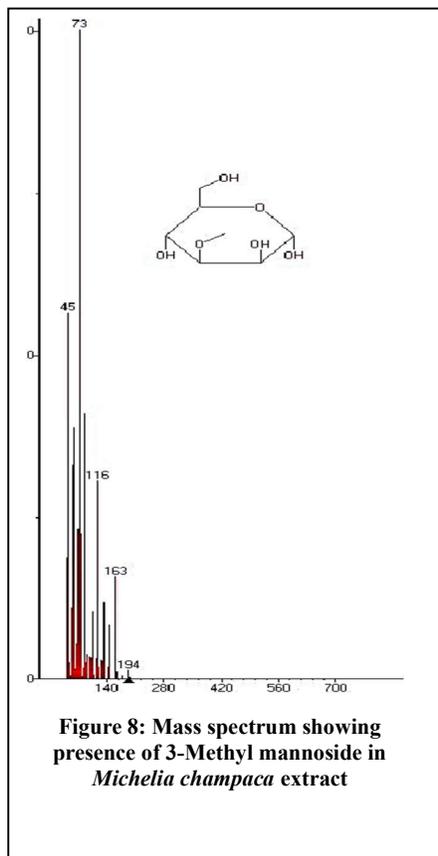
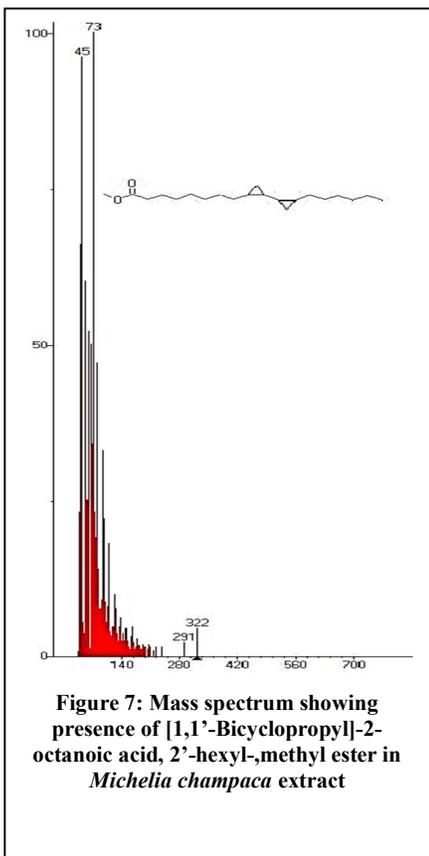
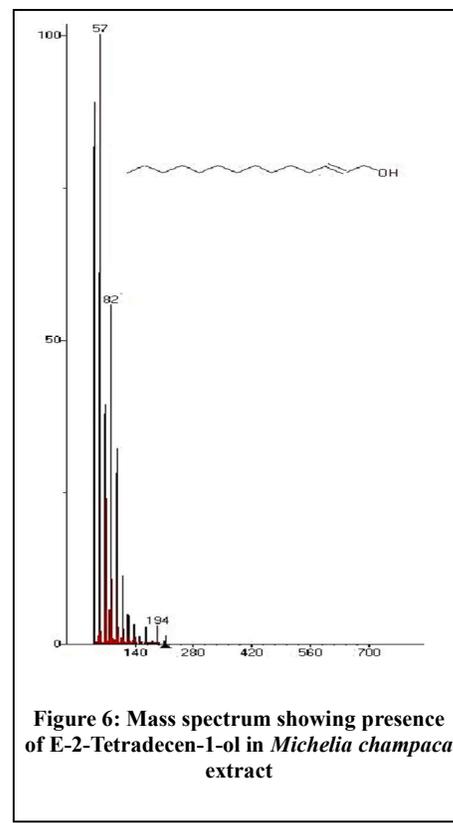
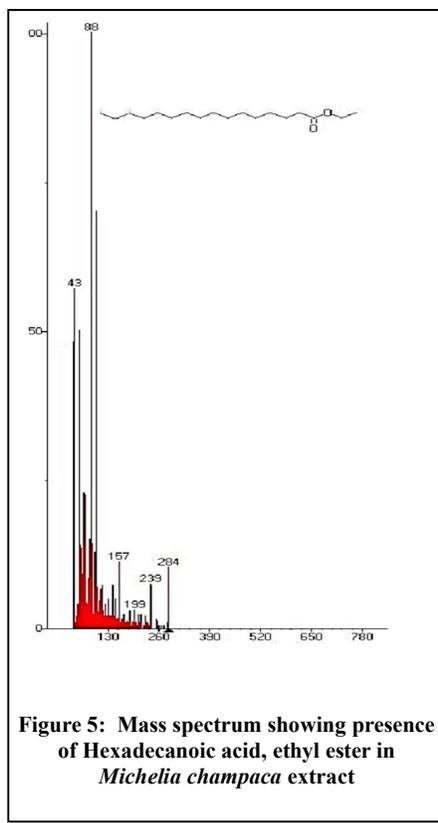
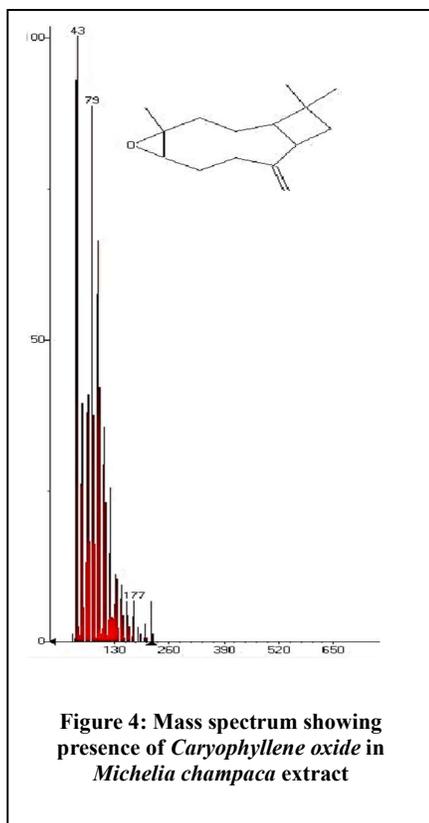
Sr. No.	Components	Retention time	Molecular formula	Molecular weight	Figure Number
1	1,2,3,4-Butanetetrol [S-(R*,R*)]	6.56	C ₄ H ₁₀ O ₄	122	2
2	1,2,4-Butanetriol	11.58	C ₄ H ₁₀ O ₃	106	3
3	Caryophyllene oxide	15.61	C ₁₅ H ₂₄ O	220	4
4	Hexadecanoic acid, ethyl ester	18.31	C ₁₈ H ₃₆ O ₂	284	5
5	E-2-Tetradecen-1-ol	18.93	C ₁₄ H ₂₈ O	212	6
6	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-,methyl ester	19.27	C ₂₁ H ₃₈ O ₂	322	7
7	3-Methyl mannoside	20.01	C ₇ H ₁₄ O ₆	194	8
8	Desulphosinigrin	20.17	C ₁₀ H ₁₇ NO ₆ S	279	9
9	n-hexadecanoic acid	21.10	C ₁₆ H ₃₂ O ₂	256	10
10	Phytol	22.91	C ₂₀ H ₄₀ O	296	11
11	9,12-Octadecadienoic acid, ethyl ester	23.50	C ₂₀ H ₃₆ O ₂	308	12
12	Octadecanoic acid, ethyl ester	23.85	C ₂₀ H ₄₀ O ₂	312	13
13	Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl]ethyl ester	25.03	C ₁₉ H ₃₈ O ₄	330	14
14	8,11-14-Eicosatrienoic acid (z,z,z)	26.22	C ₂₀ H ₃₄ O ₂	306	15
15	9,12,15-Octadecatrienoic acid,2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	26.38	C ₂₅ H ₄₀ O ₆	436	16
16	Docosanoic acid, ethyl ester	28.19	C ₂₄ H ₄₈ O ₂	368	17
17	Octadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester	29.60	C ₂₁ H ₄₂ O ₄	358	18
18	1,3-Benzenedicarboxylic acid-bis(2-ethyl hexyl) ester	29.85	C ₂₄ H ₃₈ O ₄	390	19
19	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-(E,E)	30.63	C ₁₅ H ₂₆ O	222	20
20	Vitamin E	35.91	C ₂₉ H ₅₀ O ₂	430	21
21	γ-Sitosterol	40.76	C ₂₉ H ₅₀ O	414	22

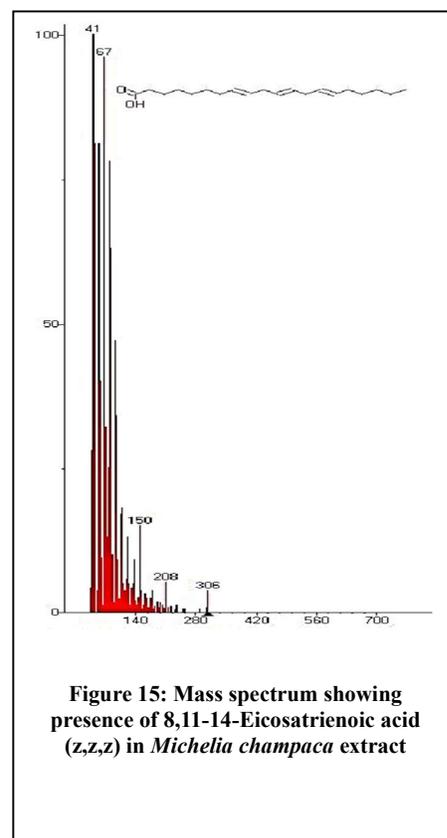
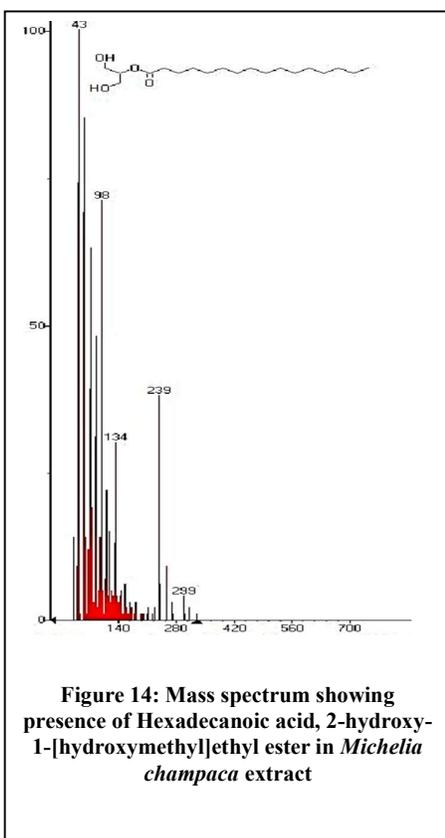
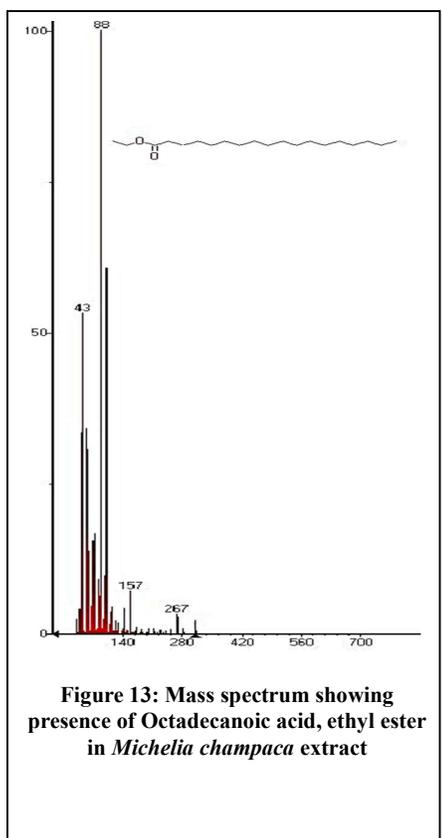
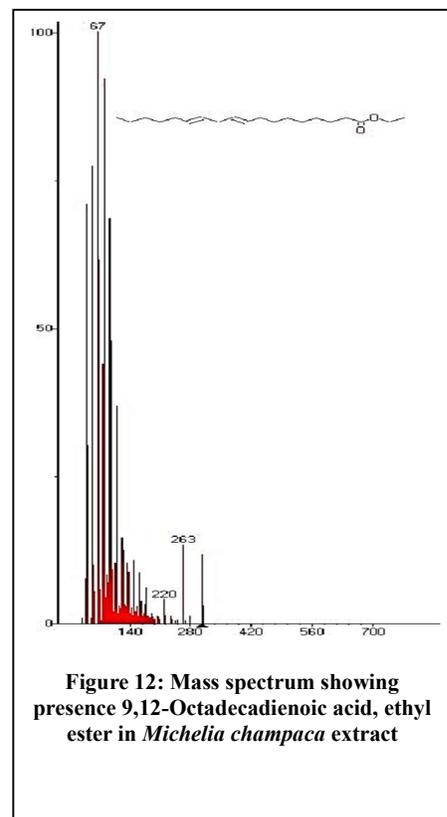
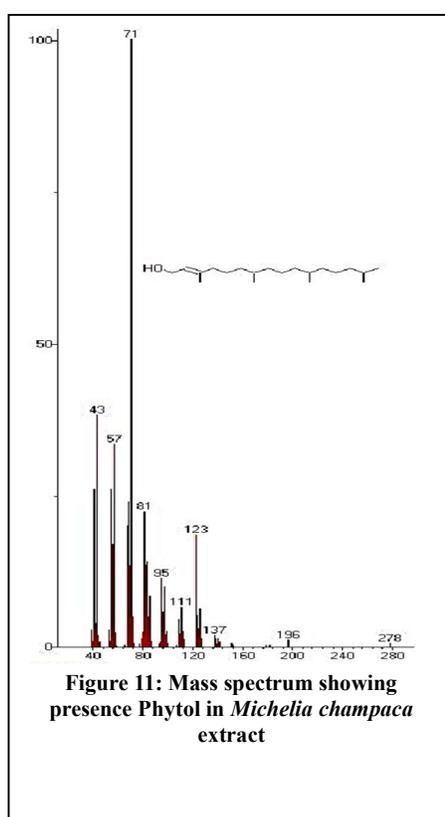
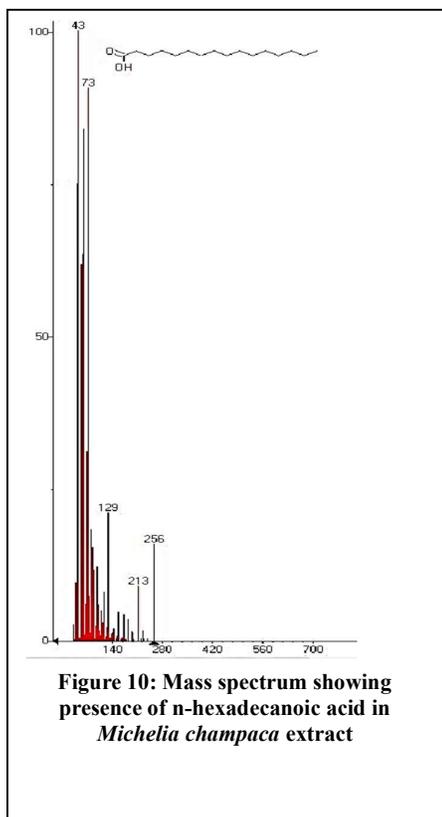
Table 4: Chemical nature of components found in ethanolic extract of *Michelia champaca*

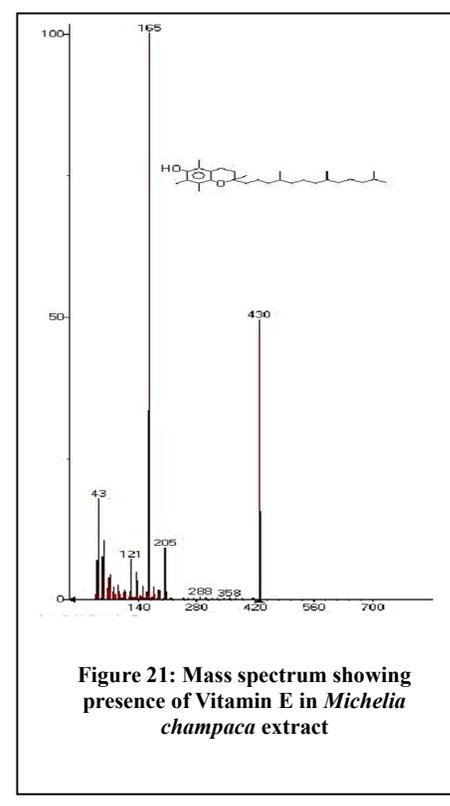
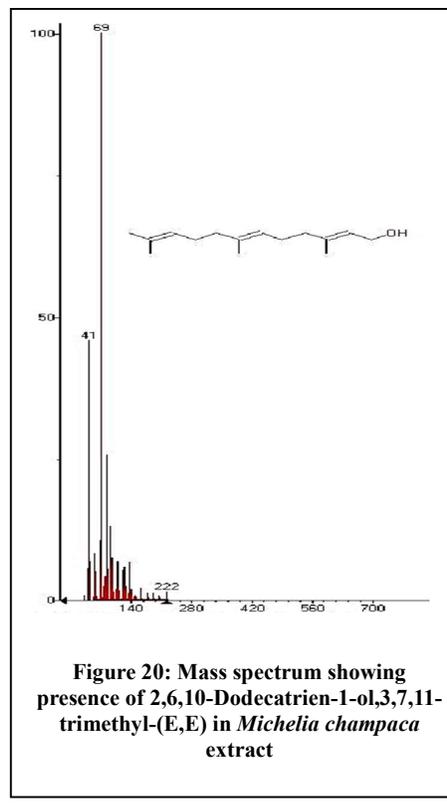
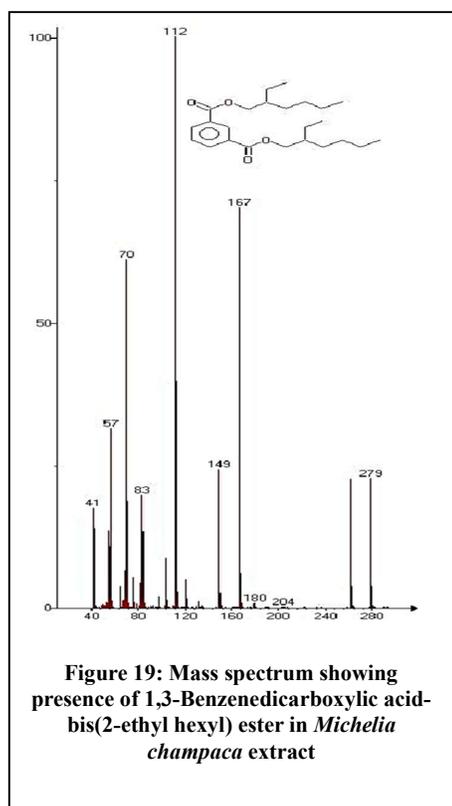
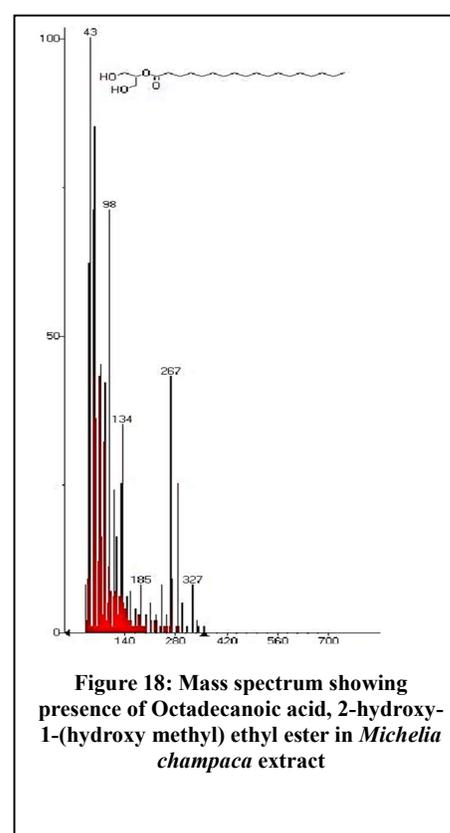
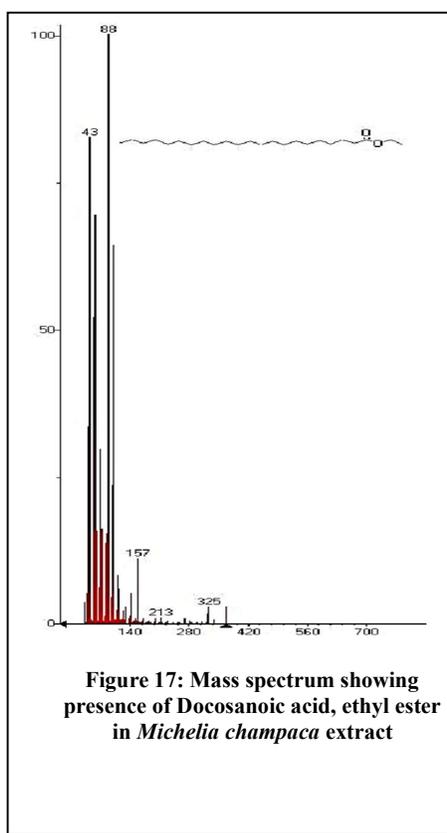
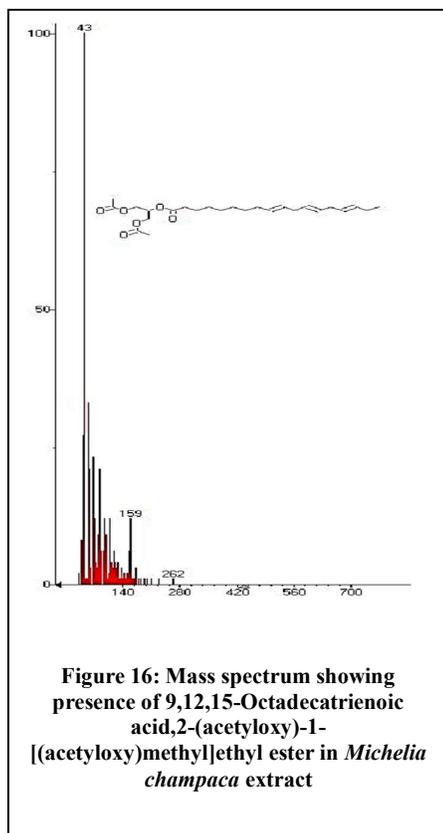
Sr. No.	Components	Chemical nature
1	1,2,3,4-Butanetetrol [S-(R*,R*)] (Erythritol)	Sugar alcohol
2	1,2,4-Butanetriol	Alcohol (four carbon polyol with three hydrophilic hydroxyl groups)
3	Caryophyllene oxide	Sesquiterpenoid
4	Hexadecanoic acid, ethyl ester	Saturated Long chain fatty acid ethyl ester (Palmitic acid ester)
5	E-2-Tetradecen-1-ol	Long chain fatty alcohol
6	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-,methyl ester	Saturated medium chain fatty acid
7	3-Methyl mannoside	O-glucosyl compounds
8	Desulphosinigrin	Glucosinolate
9	n-hexadecanoic acid	Saturated Long chain fatty acid (Palmitic acid)
10	Phytol	Acyclic diterpenoids
11	9,12-Octadecadienoic acid, ethyl ester	Polyunsaturated Long chain fatty acid,
12	Octadecanoic acid, ethyl ester	Polyunsaturated Long chain fatty acid,
13	Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl]ethyl ester	Saturated Long chain fatty acid
14	8,11-14-Eicosatrienoic acid (z,z,z)	20-carbon-chain omega-6 fatty acid (polyunsaturated)
15	9,12,15-Octadecatrienoic acid,2-(acetyloxy)-1-(acetyloxy)methyl]ethyl ester	Polyunsaturated Long chain fatty acid
16	Docosanoic acid, ethyl ester	Fatty acid ester
17	Octadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester	Saturated Long chain fatty acid
18	1,3-Benzenedicarboxylic acid-bis(2-ethyl hexyl) ester	Phthalate ester
19	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-(E,E)	Acyclic sesquiterpene alcohol
20	Vitamin E	Tocopherols, antioxidant
21	γ -Sitosterol	Stigmastanes and derivatives

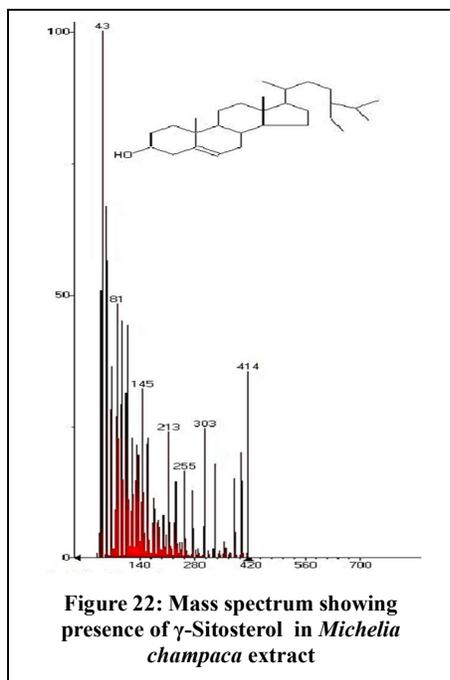
Figure 1: Mass spectrum of ethanolic extract of *Michelia champaca*











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