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GC-MS DETERMINATION OF BIOACTIVE COMPOUNDS OF *DOLICHANDRONE ATROVIRENS* (SPRAGUE) BARK

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ABSTRACT

Plants have been an important source of medicine with qualities of years. The present study focuses on the analysis of the methanol extract of *Dolichandrone atrovirens* bark by Gas Chromatography and Mass Spectrometry (GC-MS). The phytocomponents of the methanol extract of *Dolichandrone atrovirens* were investigated by using Gas Chromatography-Mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The study revealed the presence of 10 phytocomponents.

Keywords: *Dolichandrone atrovirens*, GC-MS, Phytocomponents

INTRODUCTION

In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects [**1**]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate [**2**]. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. [**3**]. The medicinal actions of plants unique to particular plant species or groups are

consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinet [**4**]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity [**5-11]**. Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer [**12**].

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [**13**]. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [**14**]. Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemical have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants.

The lack of pharmacological and clinical data on the majority of herbal medicinal products is medicinal practice. For valid integration, pharmacological studies must be conducted on those plants lacking such data [**15** and **16]**. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties [**17**]. The list of many such undocumented, unexploited and uncharacterized plants of medical importance have been brought to light by many researchers [**18**]. With this background, the present study was aimed to identify the phytoconstituents present in one such unexplored plant is *Dolichandrone atrvirens* using GC-MS analysis. *Dolichandrone atrovirens* plant bark was collected from Boda hills, Rasipuram taluk, Namakkal district, Tamilnadu, India. This plant bark was used for cancer treatment by Malayali tribes in Boda hills.

MATERIALS AND METHODS

GC-MS Analysis

25 gm of the powdered *Dolichandrone atrovirens* stem bark were soaked in 95% methanol for 12 hrs. The extracts were then

filtered through Whatmann filter No. 41 along with 2gm of sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was made wet with 95% ethanol along with sodium sulphate. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents in the plant material. 2µl of this solution was employed for GC-MS analysis [**19**].

The plant powder was extracted with methanol and analyzed using GC-Ms (GC Clarus 500 Perkin Elmer) analyzer. The data were obtained on an Elite-1 (100% Dimethyl poly siloxane) column (30 × 0.25mm ID × 1µm df). Helium (99.999%) was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2 µl of ethanol solution of the sample was injected into the column with the injector temperature at 250°C. GC oven temperature started at 110° C and holding for 2 min and it was raised to 200 ° C at the rate of 10° C/min, without holding. Holding was allowed at 280° C for 9 min with program rate of 5° C/min. The injector and detector temperature were set at 250° C and 280° C respectively. Ion source temperature was maintained by electron ionization at 70 eV and the detector was operated in scan mode from 45- 450amu (atomic mass units). A scan interval of 0.5 seconds and fragments from 45 to 450 Da was maintained. The total running time was 36 minutes.

Identification of Components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database on National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were aascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomass 5.2.

RESULTS

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters etc. The GC-MS analysis of *Dolichandrone atrovirens* stem bark revealed the presence of ten compounds (phytochemical constituents) that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak

area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in **Table 1 and Figure 1**. The first compound identified with less retention time (3.67min) was 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, whereas á-Sitosterol was the last compound which took longest retention time (31.27min) to identify. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in **Table 2**. The biological activities listed are based on Dr. Duke's phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA. The major phytochemical constituent's present in methanolic extract of *Dolichandrone atrovirens* are presented as mass spectra and compound structures are in **Figure 2-9**.

DISCUSSION

The more precise information in qualitative analysis can be obtained by gas chromatography coupled with mass spectrometry (GC-MS) [**20**]. For quantitative determination, gas chromatography with flame ionization detector (GC-FID) and GC-MS are preferred [**21**, **22**, **23**].

The GC-MS analysis of *Dolichandrone atrovirens* stem bark revealed the presence of ten compounds. The identified compounds possess many biological properties. For instance, 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl (RT-3.67) possesses antimicrobial and anti inflammatory properties. Hexadecanoic acid, methyl ester (RT-12.19) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, anti androgenic, flavor, hemolytic, 5-alpha reductase inhibitor. Hexadecanoic acid, methyl ester (RT- 15.51) is an antioxidant, flavor, hypocholesterolemic, pesticide, 5-alpha reductase inhibitor (Sermakkani and Thangapandian, 2012). Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid (Z,Z)-, 9-Octadecenoic acid (Z)-, methyl ester were presented in methanol extract of *Cinnamon zeylanicum* stem bark [**24**]. Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)- were presented in methanol extract of *Justicia wynaadensis* leaves and stem [**25**]. Similar types of compounds were identified among the ten compounds of this present study [**26**]. The main components 9,12-Octadecadienoic acid (Z,Z)-, 9-Octadecenoic acid (Z)-, methyl ester were presented in *Croton tiglium* seed. These compounds were found to have potential antioxidant and anticancer activities. Hexadenoic acid has earlier been reported as a

component in alcohol extract of the leaves of *Kigelia pinnata* [**27**] and *Melissa officinalis* [**28**]. **[29]** Identified seventeen compounds with n-Hexadecanoic acid and Octadecanoic acid as the major comounds in the leaves of *Cleistanthus collinus*. GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid **[30]**. n-Hexadecanoic acid, hexadecanoid acid, 9,12-octadecadienoic acid and squalene were identified in the ethanol leaf extract of *Aloe vera* **[31]** and *Vitex negundo* **[32]**. Squalene is used in cosmetics as a natural moisturizer. **[33]** *Euphorbia longan* leaves mainly contained h- hexadecanoic acid and 9, 12-octadecadienoic acid. These reports are in accordance with the result of this study.

The constituent compounds in the essential oil are long chain aliphatic carboxylic acid, (saturated and unsaturated) and their derivatives including alcohols, aldehyde as well as benzene carboxylic acid ester and a steroidal compound **[34]**. The present study, Squalene (RT- 23.99) possesses antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide. Terpenoids are an important part of volatiles from plants. It suggested as potential anticarcinogenic agent(**35**), exhibit cytotoxic activity against several solid tumor cell lines **[36]**. Many medicinal plants are rich in varieties of secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids **[37, 38, 39**]. The activities of some phytocomponents with compound nature of flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecaonoic acid), unsaturated fatty acid and linolenic (decosatetraenoic acid and octadecatrienoic acid) as antimicrobial, anti inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antienzemic and anticoronary.

CONCLUSION

The source of many plants can often be identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of many alcoholic beverages. The technique of fingerprint could really identify the false herbal products. The construction of chromatographic fingerprints aims at evaluating the quality of herbal medicines [**40**]. The fundamental reason of quality control of herbal medicines is based on the concept of phytoequivalence of herbs, and then to use this conception to identify the real herbal medicine and the false one, and further

to do quality control. The importance of the study is due to the biological activity of some of these compounds [**41**]. The present study, which reveals the presence of components in *Dolichandrone atrovirens* suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

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Table 1: GC-MS Analysis Revealed the Presence of Phytochemical Components in Methanol Stem Bark Extract of *Dolichandrone atrovirens*

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	3.67	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	18.08
2	8.42	D-Glucose, 4-O-à-D-glucopyranosyl-	$C_{12}H_{22}O_{11}$	342	40.96
3	12.19	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.31
4	12.74	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.69
5	14.21	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.82
6	14.29	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	3.58
7	19.66	Nonadecane	$C_{19}H_{40}$	268	1.54
8	23.99	Squalene	$C_{30}H_{50}$	410	1.81
9	30.15	Stigmasterol	$C_{29}H_{48}O$	412	8.07
10	31.27	á-Sitosterol	$C_{29}H_{50}O$	414	12.14

Table 2: GC-MS Analysis Showed Phytochemical Compounds, their Nature and Their Biological Activities of Methanol Stem Bark Extract of *Dolichandrone atrovirens*

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity

1	**3.67**	**4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-**	**$C_6H_8O_4$**	**144**	**18.08**	**Flavonoid Compound**	**Antimicrobial Anti-inflammatory**
2	**8.42**	**D-Glucose, 4-O-à-D-glucopyranosyl-**	**$C_{12}H_{22}O_{11}$**	**342**	**40.96**	**Sugar moiety**	**No activity reported**
3	**12.19**	**Hexadecanoic acid, methyl ester**	**$C_{17}H_{34}O_2$**	**270**	**2.31**	**Palmitic acid ester**	**Antioxidant Hypocholesterolemic Nematicide Pesticide Anti androgenic Flavor Hemolytic 5-Alpha reductase inhibitor**
4	**12.74**	**n-Hexadecanoic acid**	**$C_{16}H_{32}O_2$**	**256**	**10.69**	**Palmitic acid**	**Antioxidant Hypocholesterolemic Nematicide Pesticide Anti androgenic Flavor Hemolytic 5-Alpha reductase inhibitor**
5	**14.21**	**9,12-Octadecadienoic acid (Z,Z)-**	**$C_{18}H_{32}O_2$**	**280**	**0.82**	**Linoleic acid**	**Hypocholesterolemic Nematicide Antiarthritic Hepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase inhibitor Antihistaminic Anticoronary Insectifuge Antieczemic Antiacne**
6	**14.29**	**9-Octadecenoic acid (Z)-, methyl ester**	**$C_{19}H_{36}O_2$**	**296**	**3.58**	**Oleic acid ester**	**Anti-inflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor**
7	**19.66**	**Nonadecane**	**$C_{19}H_{40}$**	**268**	**1.54**	**Alkane compound**	**No activity reported**
8	**23.99**	**Squalene**	**$C_{30}H_{50}$**	**410**	**1.81**	**Triterpene**	**Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide**
9	**30.15**	**Stigmasterol**	**$C_{29}H_{48}O$**	**412**	**8.07**	**Steroid**	**Antimicrobial Anticancer Antiarthritic Antiasthma Diuretic Anti-inflammatory**
10	**31.27**	**á-Sitosterol**	**$C_{29}H_{50}O$**	**414**	**12.14**	**Steroid**	**Antimicrobial Anticancer**

							Antiarthritic Antiasthma Diuretic Anti-inflammatory

NOTE: **Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

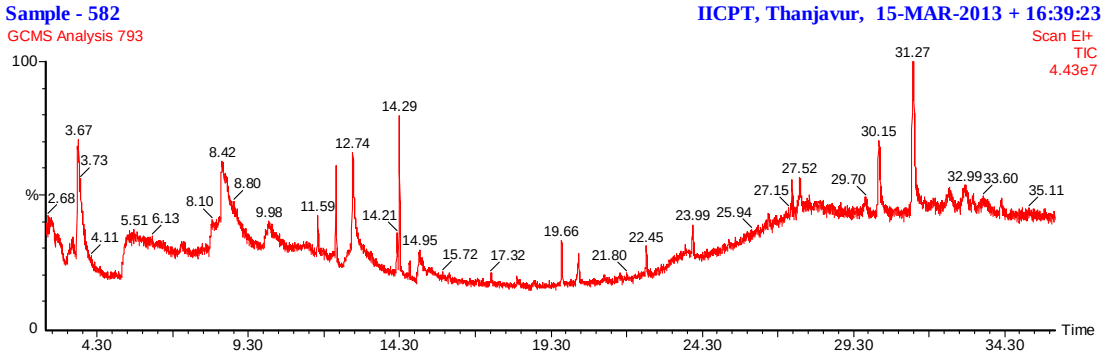

Figure 1: GC-MS Chromatogram of Methanol Extract of Stem Bark of *Dolichandrone atrovirens*

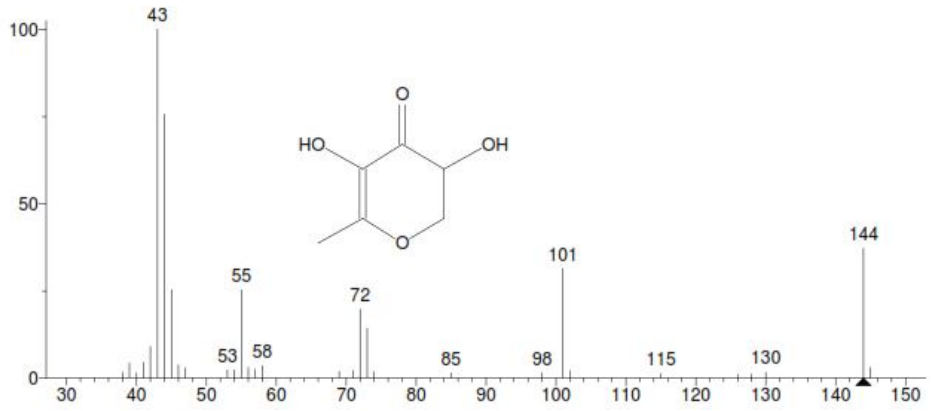

Figure 2: The Mass Spectrum Analysis and Structure of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl

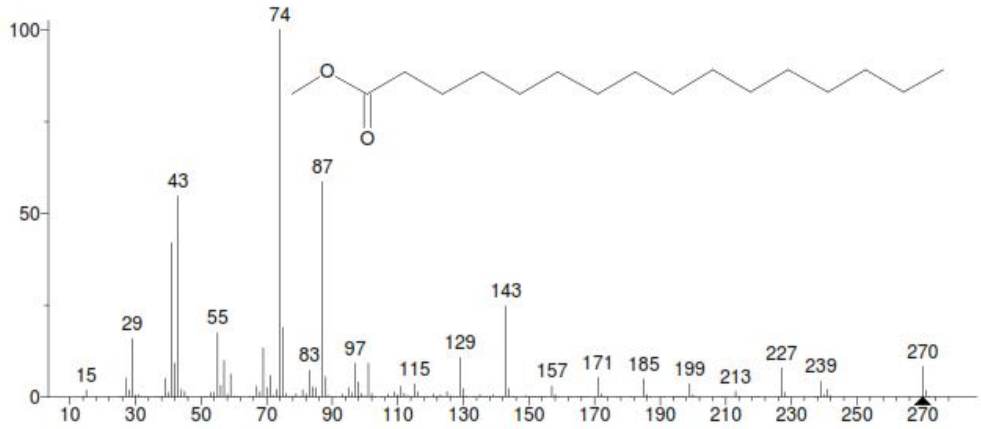

Figure 3: The Mass Spectrum Analysis and Structure of Hexadecanoic Acid, Methyl Ester

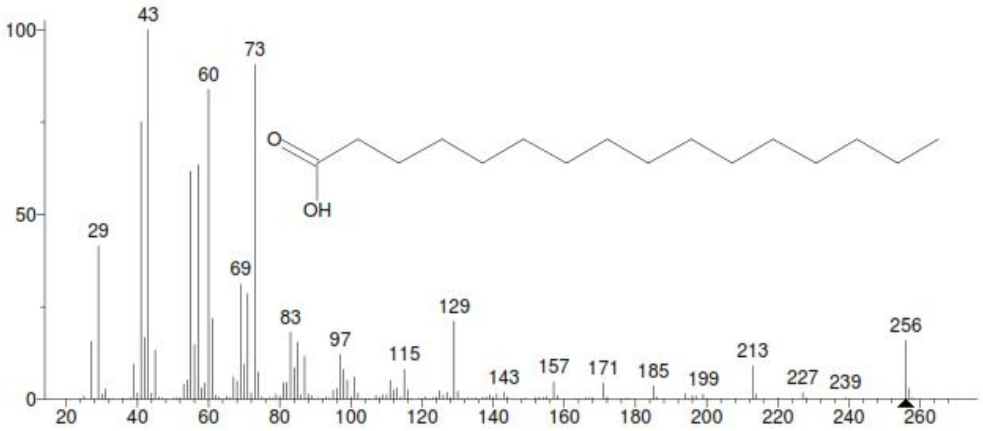

Figure 4: The Mass Spectrum Analysis and Structure of n-Hexadecanoic Acid

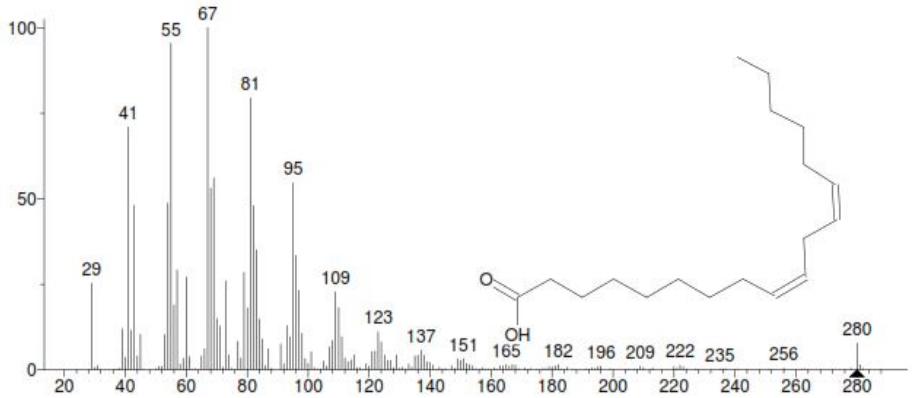

Figure 5: The Mass Spectrum Analysis and Structure of 9,12-Octadecadienoic Acid (Z,Z)-

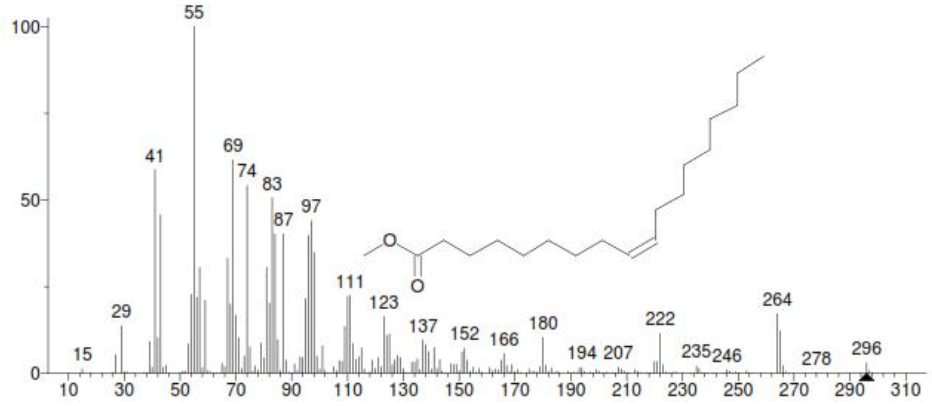

Figure 6: The Mass Spectrum Analysis and Structure of 9-Octadecenoic Acid (Z)-, Methyl Ester

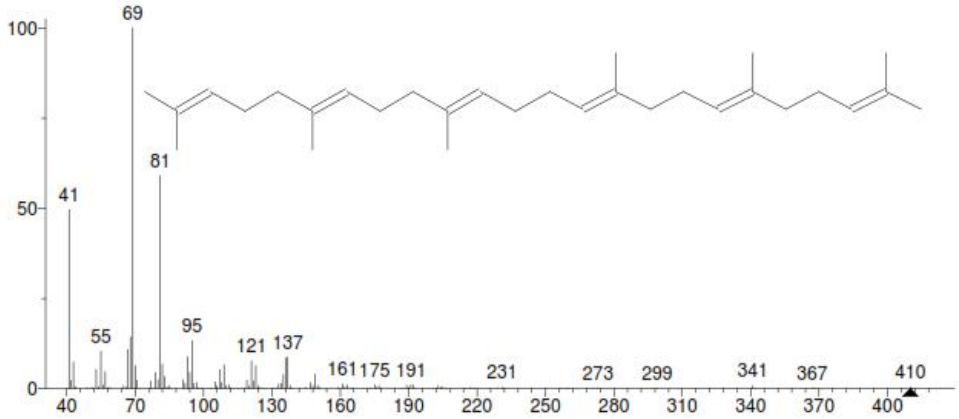

Figure 7: The Mass Spectrum Analysis and Structure of Squalene

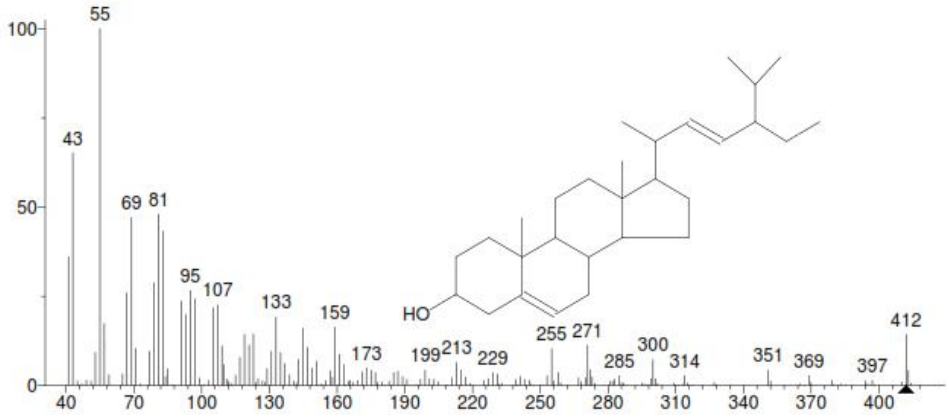

Figure 8: The Mass Spectrum Analysis and Structure of Stigmasterol

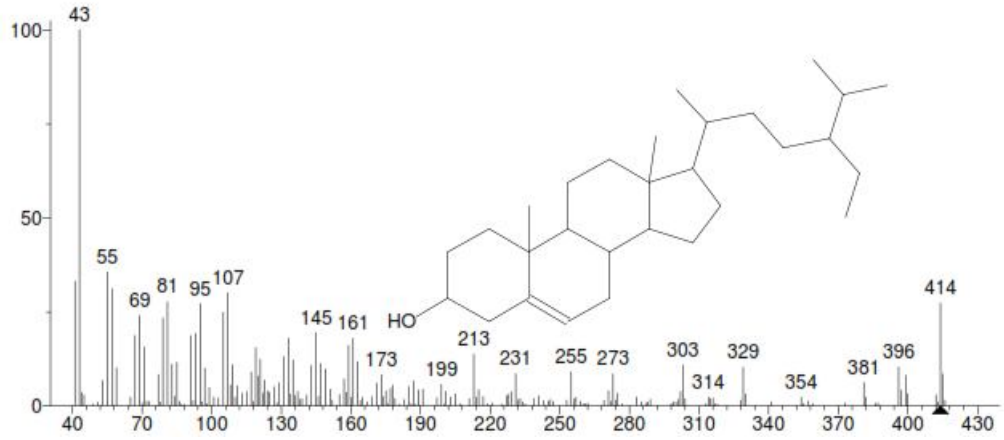

Figure 9: The Mass Spectrum Analysis and Structure of beta.-Sitosterol