Determination of bioactive compounds from the hexane extract of ipomoea *pes-caprae* (l) r. Br by GC-MS analysis

A. RAMESH[1], P. SUNDARRAJ[2], Dr.J. BALAMANI[3]

[1,2,3] Department of Chemistry, Chikkanna Government Arts College, Tirupur

Abstract- **The bioactive compounds of the hexane extracts of Ipomoea pes-caprae (L.) R. Br stem and leaf were investigated using Perkin-Elmer Gas Chromatography – Mass Spectrometry (GC-MS), while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Twenty compounds were identified from the stem and leaf of I. pes-caprae respectively. GC-MS analysis of hexane extract of stem of I. pes-caprae revealed the presence of β-sitosterol (21.28%), (6E, 10E)-3,7,11,15-Tetramethyl – 1,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen – 3 –ol (7.73%), Lanosterol (5.93), Stigmasterol (5.54%) and Geranylgeraniol (4.35%), while the leaf of I. pes-caprae revealed the existence of γ-sitosterol (20.35%), 2, 6, 10, 15, 19, 23 – Hexamethyl-1, 6, 10,14,18,22-tetracosahexaen-3-ol (12.84%), Hexatriacontane (6.71%), Lanosterol (6.12%), α-Amyrin (5.07%), Stigmasterol (5.05%), Vitamin E (4.86%) and Campesterol (3.72%).**

Index Terms - Ipomoea Pes-caprae, GC-MS, Convolvulaceae, Medicinal plant.

INTRODUCTION

Medicinal plants are gift of nature to cure immeasurable diseases among human beings. It is believed that for many years utilization of the medicinal plants for the treatment of various diseases has been practiced by human beings. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. So, plant origin can be used as an alternative to synthetic drugs.

One such plant Ipomoea pes-caprae belongs to genus convovulaceae consists of more than 200 species widely distributed in tropical and subtropical countries [1]. The generic name Ipomoea is derived from the Greek word, which means worm like, about the twining habit. The hyphenated specific pes-caprae is from the Latin pes, foot and caprae, goat or literally "foot of a goat" [2]. It grows widely just above the high tide line along coastal beaches, forming large mats that supports in stabilizing sand. This is an evergreen permanent with a large, thick root that can be 10 ft. long and 2 inches in diameter [3]. I. pes-caprae has the potential in searching free radicals. In addition to this, due to the presence of compounds, such as betulinic acid, beta-amyrin acetate and iso-quercitin, it can be a vital source of antioxidant photochemical [4] and good antinociceptive properties [5]. The leaves are used in rheumatism, and stomachic and laxative properties and it has many biological activities like antioxidant, analgesic and anti-inflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic [6]. It is also used in inhibition of platelet aggregation, diarrhoea, vomiting and piles [7]. The leaf extract of I. pes-caprae also demonstrates ability to neutralize crude jellyfish venoms [8].

Considering of the medicinal importance of the plant, the hexane extract of I.pes-caprae was analyzed in the GC-MS to identify additional photochemical constituents. This work will help to identify the bioactive compounds of therapeutic value. GC-MS is one of the techniques to identify the phytochemical constituents of long chain, branched chain hydrocarbons, alcohols, acids, esters, etc.

EXPERIMENTAL

Materials and methods

The leaf and stem of I.pes-caprae were collected from the east coastal area, Tuticorin, Tamilnadu, India. The plants were shaded dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to scot Duran bottle (50.0 ml), treated with n-hexane until the powder was fully immersed. The bottle was placed in ultrasonication for 1 hour at 80oC at frequency of 20 MHz. Then the extract was filtered through Whatman (No.1) filter paper and evaporated to dryness by using a rota evaporator. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of these extracts was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a fused silica capillary column (30 mm X 0.25 mm ID X 0.25 µ, composed of 5% Dimethyl poly siloxane). An electron ionization system with ionizing energy of 70 ev was applied

for GC-MS detection. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min. An injection volume of 1 µl was also utilized (split ratio of 10: 1); and sustained at injector temperature 290oC; ion-source temperature 230oC. The oven temperature was programmed from 50oC (isothermal for 1min.), with an increase of 30oC/min, to 180oC, then 15oC/min to 260oC (isothermal for 3 min.), with an increase of 25 oC/min to 270oC (isothermal for 4 min.), ending with an increase of 10 oC/min to 300oC, 5-min isothermal at 300oC.Mass spectra were taken at 70 ev; a scan interval of 0.5 seconds and fragments from 45 to 550 Da. Total GC running time was 26 minutes. The relative % amount of each constituent was calculated by balancing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

Using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns, clarification on mass spectrum was conducted using GC-MS. In the NIST library the spectrum of the unknown component was compared with the spectrum of the known components stored. The name, molecular weight and structure of the components of the test materials were determined.

RESULTS AND DISCUSSION

Twenty compounds were identified in I.pes-caprae stem and leaf by GC-MS analysis. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW), and the concentration (%) were shown in Table 1and Fig. 1 pictures the GC-MS chromatogram of hexane extract of stem. The prevailing compounds were β-Sitosterol (21.24%), n-Heptacosane (10.9%), 2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol (7.73%), Lanosterol (5.93%), Stigmasterol (5.54%), Geranylgeraniol (4.35%), Campesterol (3.46%), 1-Docosanol (3.39%), Urs-12-ene (3.32%), Dimethyl(bis{[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]oxy})silane(2.76%), 9,19-Cyclo-9β-lanost-24-en-3β-ol acetate (2.38%), 2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-Tetracosahexaene,[synonyms: Squalene](2.08%), vitamin E (2.01%), 2-cis-9-Octadecenyloxyethanol (1.18%), α-Amyrin (1.01%), Methyl ester Octadecanoic acid [synonyms: Methyl stearate] (0.96%), 1-Heptatriacotanol (0.85%), n-Eicosanol(0.82%), 3,7,11-trimethyl-2.6,10-dodecatrien-1-ol [synonyms: Farnesol] (0.80%). Figs. 3, 4, 5, 6, 7, 8, 9 and 10 shows structures of important constituents of n-hexane extracts of I.pes-caprae. Similarly, twenty compounds were identified in I.pes-caprae leaves by GC-MS analysis. The active principles with their (RT), molecular weight (MW) and concentration (%) were presented in Table 2 and Fig. 2 shows the GC-MS chromatogram of hexane extract of leaf. The prevailing compounds were γ-Sitosterol (20.35%). Additionally, eight different compounds were identified in leaves from stem, Hexatriacontane (6.71%), γ-Tocopherol (2.55%), Lupeol (2.59%), Methyl 11-(3-pentyl-2-oxiranyl) undecanoate (2.62%), tetradecyl-Oxirane (0.82%), 2,4-diisopropenyl-1-methyl-1-vinyl-Cyclohexane (0.68%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.65%). Fig. 11, 12, 13, 14, 15, and 16 show structures of important constituents of hexane extract of I.pes-caprae leaf. Table 3 listed the various photochemical constituents which contribute to the medicinal activity of hexane extract of stem and leaf of I.pes-caprae.

Among the identified phytochemicals, Vitamin E is noticed in I.pes-caprae whole plant which was found to be effective antioxidant and belongs to the class of compounds identified to enhance sperm quality and prevent sperm agglutination, thus making more motile with forward progression and hence promote male fertility. Vitamin E was thought to be important chain breaking antioxidant, which plays an important role in various stages of carcinogenesis through its contribution and immunocompetence, membrane and DNA repair and decreasing oxidative DNA damage [9]. Phytol (3,7,11,15-tetramethylhexadec-2-en-ol) is a diterpene, a member of the group of branched chain unsaturated alcohols [10] was also identified which was the product of chlorophyll metabolism in plants.it was also known to inhibit the growth of staphylococcus aureus [11]. The compound stigmasterol was identified in both stem and leaf of I.pes-caprae was found to possess anticervical cancer property [3]. Squalene has antioxidant, antibacterial, antitumor, immunostimulant and lipoxygenase inhibitor activity. Recently, it has been established that, squalene possesses chemo preventive activity against the colon carcinogenesis [12, 13]. Sitosterol limits the amount of cholesterol entering the body by inhibiting cholesterol absorption in the intestines, therefore decreasing the intensity of cholesterol in the body. It was helpful with benign prostatic hyperplasia (BPH), due to its anti-inflammatory effects and its ability to improve urinary symptoms and flow. Betulunic acid and α-Amyrin compounds has antinociceptive activity [14]. Also, studies have proven the in vivo anti-tumour activity I.pes-caprae against mice melanoma (B16F10) cancer cells has been explored [15].

CONCLUSION

Ipomoea pes-caprae has broad spectrum of pharmacological activity. As relatively a little work has been done on it. The present study has been found useful in the identification of several constituents present in the hexane extract of the leaves and stems of Ipomoea pes-caprae by GC-MS analysis. The leaves of Ipomoea pes-caprae are used in rheumatism. It also has stomachic and laxative properties and it has many biological activities like antioxidant, analgesic and anti-inflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic Further investigation on these phytochemicals will route a way for the synthesis of cost effective drug with less side effects. Through research work was needed to be done on this potential plant which may yield many bio-active compounds.

REFERENCES

[1] Daniel E.P. Marie, B. Dejan and J.Q. Leclerc, "GC-MS analysis of the leaf Essential Oil of Ipomea pes-caprae, a traditional herbal medicine in Mauritius" NPC, vol.2(12), pp. 1225-1228, June 2007.

[2] D.Sahoo, J. Jena, and A. Dinda, "A review on Ipomoea pes-caprae (Beach Morning Glory): Value of medicinal plants in coastal area of India" IMT pharmacy College, Odisha.

[3] Arun Kumar, S. Paul, P. Kumari, S.T. Somasundaram, "Antibacterial and phytochemical assessment on various extracts of Ipomoea Pes-caprae (L.) R. Br through FT-IR and GC-MS spectroscopic analysis," AJPCR, Vol. 7, pp. 134-138, May 2014.

[4] Devall MS, J. "The biological flora of coastal dunes and wetlands" Roth Research, vol. 8(2), pp.442-456, June 1992.

[5] Devika R, Justin K, "Screening and evaluation of bioactive components of tageteserectalL. By GC-MS analysis" Pharma and clinical res, vol. 7(2), pp. 58-60, May 2014.

[6] Dunkic V, Bezic N, Vuko E, "Antiphytoviral activity of saturejamontanal. Essential oil and phenol compounds" on CMV and TMV Molecules, vol. 15, pp. 6713-6721, Jan 2010.

[7] Fu-An G, D. Huang V, J.P. Shea, "Evaluation of Antioxidant polyphenols from selected mangrove plants of Indian Asian," Vol. 20, pp. 1311-1322, July 2008.

[8] Pongprayoon U, Bohlin L, Wasuwat S., "Neutralization of toxic effects of different crude jelly fish venoms by an extract of Ipomoea pes-caprae (L.) R.Br." Journal of Ethnopharmacology, vol. 35, pp. 65-69, June 1991.

[9] Meena Rk, Patni V, Arora DK, J. "Study on phenolics and their oxidative enzyme in capsicum annumL, infected with Gemini virus," Asian, vol. 22, pp. 307-310, May 2008.

[10] Reichling J, Schnitzler P, Suschke U, Saller R, "Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties an overview" Forschende Komplementarmedizin, vol. 16(2), pp. 79-90, June 2009.

[11] Tohma HS, Glycyrrhizaglabra L, J. Gulcin I. "Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice Int properties," vol. 13, pp. 657-671, May 2010.

[12] C.V. Rao, H.L. Newmark, B.S. Reddy, "Chemopreventive effect of Squalene on Colon Cancer," Carcinogenesis, vol. 19, pp. 287-297, March 1998.

[13] N. Kulandai Therese, P.S. Tresina and V.R. Mohan, "GC-MS analysis of bioactive constituents of Hypericum mysorense(Hypericaceae)," Int. J. Appl. Biol. Pharmaceut. Technol., vol. 3, pp. 159-164, Jan 2013.

[14] Otuki MF, Ferreira J, Lima FV, Meyre-Silva C, Calixto JB, "Antinociceptive properties of mixture of alpha-amyrin and beta-amyrin triterpenes: evidence for participation of protein kinase C and protein kinase A pathways," NCBI, vol. 1, pp. 308-313, Apr 2005.

[15] Manigauha, M.D. Kharya, N. Ganesh, "In vivo antitumor potential of Ipomoea pes-caprae on melanoma cancer," Pharmacognosy magazine, vol. 11(42), pp. 426-433, June 2015.

Table 1: Components identified in the Hexane extract of Stem of ***Ipomoea pes-caprae***

S.NO	RT	Name of the compound	Molecular formula	MW	Peak area
1	8.63	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol (S: FARNESOL)	$C_{15}H_{26}O$	222	0.80
2	10.32	methyl ester Octadecanoic acid (S: Methyl stearate)	$C_{19}H_{38}O_2$	298	0.96
3	11.32	1-Docosanol	$C_{22}H_{46}O$	326	3.39
4	12.688	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	312	1.18
5	12.898	n-Eicosanol	$C_{20}H_{42}O$	298	0.82
6	14.897	9,19-Cyclo-9β-lanost-24-en-3β-ol, acetate	$C_{32}H_{52}O_2$	468	2.38
7	15.265	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-Tetracosahexaene (S: SQUALENE)	$C_{30}H_{50}$	410	2.08
8	18.158	(6E,10E)-3,7,11,15-Tetramethyl-1,6,10,14-hexadecatetraen-3-ol	$C_{20}H_{34}O$	290	8.49
9	19.053	Geranylgeraniol	$C_{20}H_{34}O$	290	4.35
10	19.789	Dimethyl(bis{[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl] oxy}) silane	$C_{32}H_{56}O_2Si$	500	2.76
11	20.631	n-Heptacosane	$C_{27}H_{56}$	380	10.9
12	20.788	Vitamin E	$C_{29}H_{50}O_2$	430	2.01
13	21.63	(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol	$C_{30}H_{50}O$	426	7.73
14	21.893	Campesterol	$C_{28}H_{48}O$	400	3.46
15	22.209	Stigmasterol	$C_{29}H_{48}O$	412	5.54
16	22.998	β-Sitosterol	$C_{29}H_{50}O$	414	21.2
17	23.576	α-Amyrin	$C_{30}H_{50}O$	426	1.01
18	24.313	Urs-12-ene	$C_{30}H_{50}$	410	3.32
19	24.966	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.85
20	25.522	Lanosterol	$C_{30}H_{50}O$	426	5.93

Table 2: Components identified in the Hexane extract of Leaf of ***Ipomoea pes-caprae***

S.NO	RT	Name of the compound	Molecular formula	MW	Peak area
1	6.218	2,4-diisopropenyl-1-methyl-1-vinyl-Cyclohexane	$C_{15}H_{24}$	204	0.68
2	10.321	Methyl 11-(3-pentyl-2-oxiranyl) undecanoate	$C_{19}H_{36}O_3$	312	2.62
3	11.32	1-Eicosanol	$C_{20}H_{42}O$	298	3.11
4	12.688	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	312	1.66
5	13.266	tetradecyl-Oxirane	$C_{16}H_{32}O$	240	0.82
6	14.897	(3β)-acetate-9,19-Cyclolanost-24-en-3-ol	$C_{32}H_{52}O_2$	468	2.7
7	16.317	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene (s: squalene)	$C_{30}H_{50}$	410	1.03
8	17.632	Heptacosane	$C_{27}H_{56}$	380	1.11
9	18.158	2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol	$C_{30}H_{50}O$	426	12.84
10	19.053	Geranylgeraniol	$C_{20}H_{34}O$	290	2.08
11	19.842	γ-Tocopherol	$C_{28}H_{48}O_2$	416	2.55
12	20.525	Hexatriacontane	$C_{36}H_{74}$	506	6.71
13	20.788	Vitamin E	$C_{29}H_{50}O_2$	430	4.86
14	21.63	1-Heptatriacotanol	$C_{37}H_{76}O$	536	3.2
15	21.893	Campesterol	$C_{28}H_{48}O$	400	3.72
16	22.209	Stigmasterol	$C_{29}H_{48}O$	412	5.01
17	22.998	γ-Sitosterol	$C_{29}H_{50}O$	414	20.35
18	23.576	Lupeol	$C_{30}H_{50}O$	426	2.59
19	24.26	α-Amyrin	$C_{30}H_{50}O$	426	5.07
20	25.522	Lanosterol	$C_{30}H_{50}O$	426	6.12

Table 3: Activity of Components in the Hexane extract of Stem and leaf of ***Ipomoea pes-caprae***

S.NO	Name of the compound	Molecular Formula	Nature of compound	Activity
1	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol (S: FARNESOL)	$C_{15}H_{26}O$	Sesquiterpene	Anti-tumour, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
2	methyl ester Octadecanoic acid (S: Methyl stearate)	$C_{19}H_{38}O_2$	Fragrance Agents	Antifoaming agent and fermentation nutrient, Food additives, Flavouring Agents
3	2-Hexadecanol	$C_{16}H_{34}O$	Aliphatic alcohol	Anti-acne agents, antidepressants
4	1-Docosanol	$C_{22}H_{46}O$	Aliphatic alcohol	Antiviral activity
5	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	Polyethylene Glycol	Antioxidant
6	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-Tetracosahexaene, (SQUALENE)	$C_{30}H_{50}$		Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase inhibitor, pesticide
7	Heptacosane	$C_{27}H_{56}$		
8	(6E,10E)-3,7,11,15-Tetramethyl-1,6,10,14-hexadecatetraen-3-ol	$C_{20}H_{34}O$		
9	Geranylgeraniol	$C_{20}H_{34}O$	Diterpene alcohol	potent inhibitor of *Mycobacterium tuberculosis*
10	n-Heptacosane	$C_{27}H_{56}$		
11	Vitamin E	$C_{29}H_{50}O_2$	Vitamin	Antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, anticancer, hepatoprotective, hypocholesterolemia, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary

12	Campesterol	$C_{28}H_{48}O$	Phytosterol	Anti-hypercholesterolemia prevention of certain cancer including ovarian, prostate, breast, and colon cancers, potent antioxidant, hypoglycemic and thyroid inhibiting properties.
13	Stigmasterol	$C_{29}H_{48}O$	Phytosterol	Antimicrobial, anticancer, antiarthritic,
14	β-Sitosterol	$C_{29}H_{50}O$	Steroid	antiasthma diuretic Cytotoxicity, Antitrypanosomal
15	α-Amyrin	$C_{30}H_{50}O$	Triterpene tetracyclic	activity Prevention of
16	Lanosterol	$C_{30}H_{50}O$	Triterpenoid	cataract

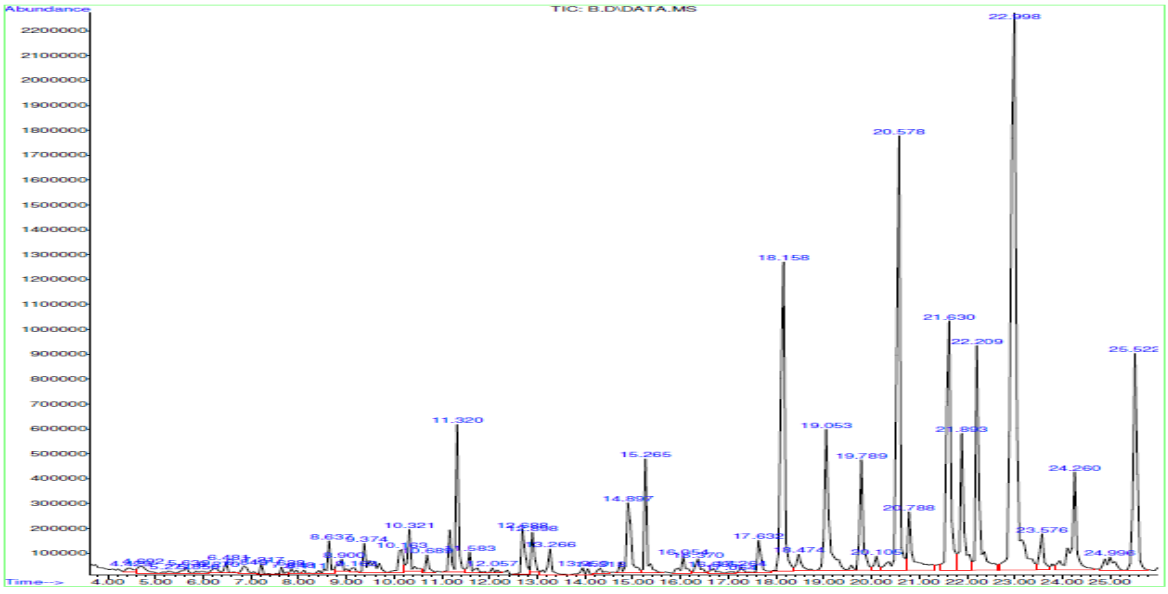

Fig. 1: GC-MS Chromatogram of Hexane Extract of Stem of ***Ipomoea pes-caprae***.

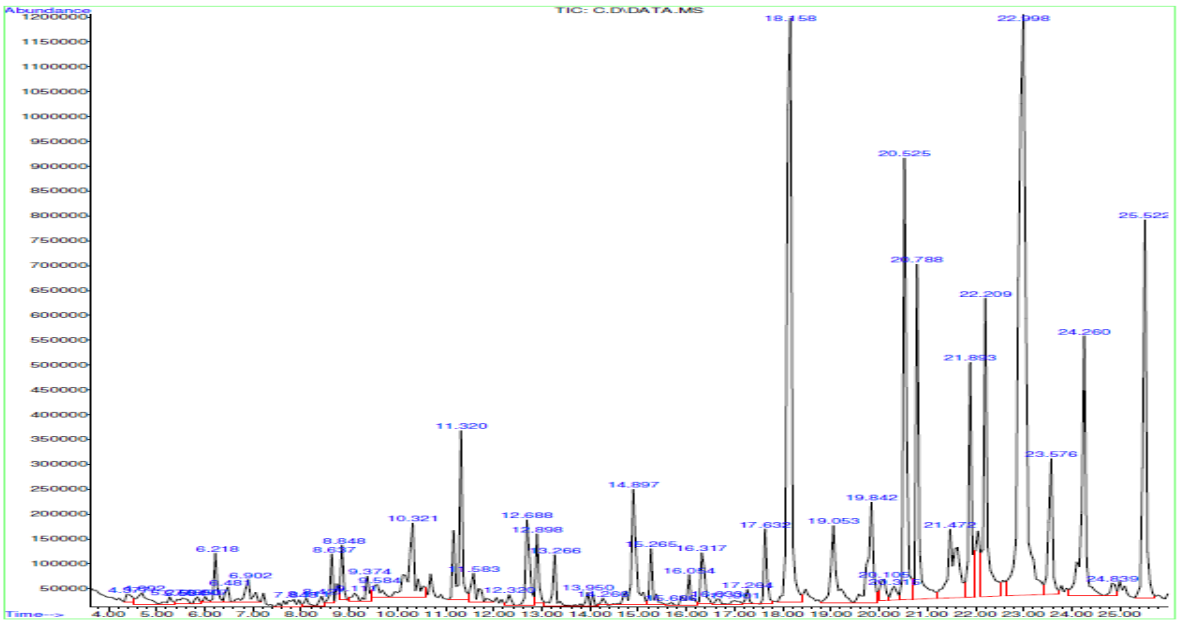

Fig. 2: GC-MS Chromatogram of Hexane Extract of Leaf of ***Ipomoea pes-caprae***

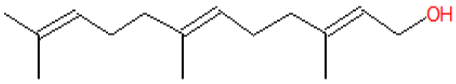

Fig. 3: 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl

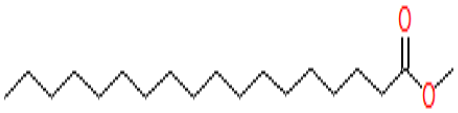

Fig. 4: Octadecanoic acid, methyl ester

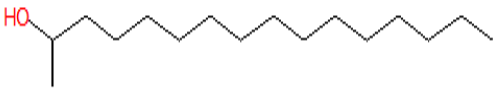

Fig. 5: 2-Hexadecanol

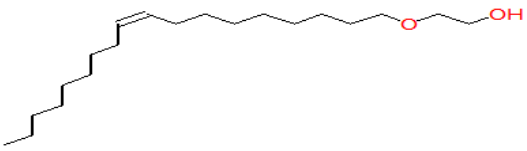

Fig.6: Ethanol, 2-(9-octadecenyloxy)-, (Z)-

Fig. 7: 1-Eicosanol

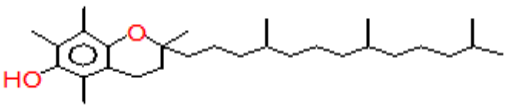

Fig. 8: dl-α-Tocopherol

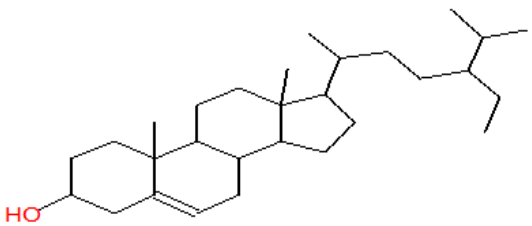

Fig. 9: β-Sitosterol

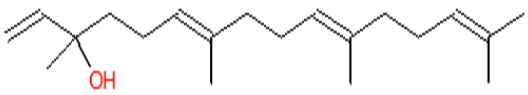

Fig. 10: 1,6,10,14-Hexadecatetraen-3-ol,

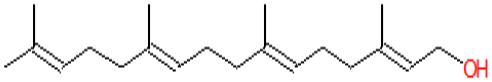

Fig. 11: Geranylgeraniol3,7,11,15-tetramethyl-, (E, E)-

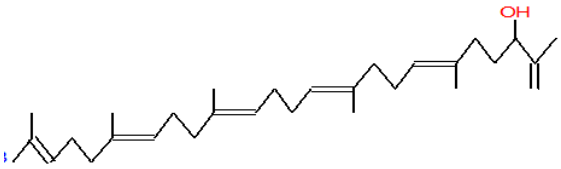

Fig. 12: 2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22

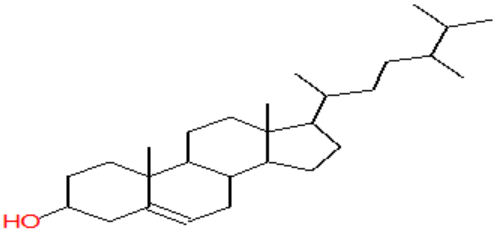

Fig. 13: campesterol -tetracosahexaen-3-ol

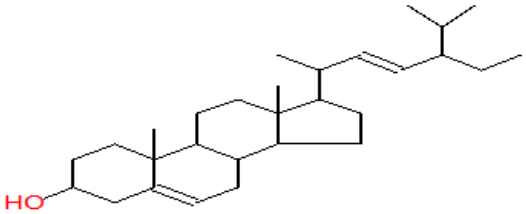

Fig. 14: Stigmasterol

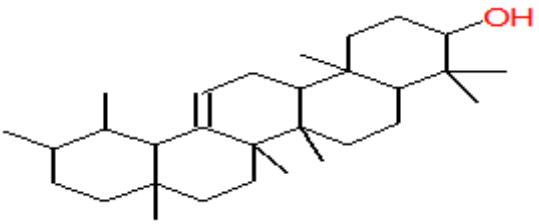

Fig. 15: α-Amyrin

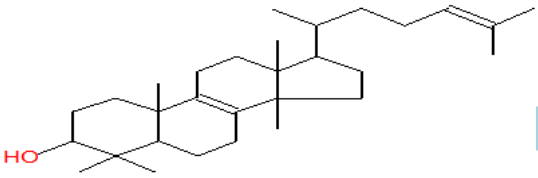

Fig. 16: Lanosterol

