

Phytochemical Analysis of leaves Extract of *Abutilon pannosum* for its Bioactive Components through Gas Chromatography-Mass Spectrometry (GC-MS)

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Abstract: *Abutilon pannosum* usually recognized as khapat is a significant therapeutic plant used in a traditional system. Its extract is also used in treating against bronchitis, gonorrhea, diarrhea in relieving thirst, and inflammation of the bladder and in reducing fever, cleaning wound and ulcer, treating a vaginal infection, diabetics, hemorrhoids and can also be used as an anemia. The present study has been carried out on the qualitative and quantitative analysis of the major bioactive components of therapeutically significant plant *A. pannosum* leaves (APL) by the use of GC-MS, whereas the mass fragment spectra of the compounds were compared with the National Institute of Standards and Technology (NIST) library. The Soxhlet extraction of a sample was done by use of continuous hot percolation method using n- Butanol as a solvent. After extraction, it was concentrated by using distillation method. Crude n- Butanol extracts were introduced in GC/MS instrument for isolation and identification of valuable phytochemicals. GC-MS analysis has revealed the existence of 25 compounds. The result exhibited that there are very significant phytochemicals found in n-Butanol leaves extract of *A. pannosum* like Fatty acid, Hydrocarbons, Carbohydrate, Diterpenoid, Diterpenes, Triterpene, Sesquiterpenoids, Phytosterol, Vitamin E and Steroid compounds.

Key words: *Abutilon pannosum*, GC/MS, Phytochemicals, n- Butanol extract, Soxhlet extraction

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I. INTRODUCTION

Information about the chemical components and its mechanism & application are very useful in divulging innovative sources of economic phytochemicals for the production of complex chemical substances and for discovering the real importance of folkloric therapies. And also need to authentication of the herbal drugs has established as a new branch of science, emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have a complementary and overlapping mechanism of action. Also essential to experiment the biological activities of locally grown medicinal plants will expose the herbs as potential sources for therapeutic agents, through isolation, identification, and characterization the new phytochemical constituents.^[1]

This work will help to identify the therapeutic value of butanol extract of *A. pannosum* (Forst.f) leaves by use of GC-MS. *Abutilon* belongs to the family Malvaceae. ^[2]The genus *Abutilon* is used for the dealing with several diseases in ethnic medicines.^[3 to 5]The leaves were used as an adjunct to medicines used for relieving dehydration, diarrhea, treating bronchitis, for pile grumbles, gonorrhea, in reducing fever, diabetics, hemorrhoids, and anemia, treating a vaginal infection and in impatience of the bladder and it is also used in cleaning wound and ulcer.^[6]Gas chromatography coupled with Mass Spectrum (GC/MS) has been widely useful unequivocally to recognize the structures of various Phytoconstituents from plant extracts with great achievement.^[7]The GC-MS analysis is a mutual validation experiment. It splits all the parts in an extract and provides a descriptive mass spectra. Through the injection port, the extract is inserted into of the GC device, convert to vaporized form. That could travel according to their mass by charge ratio and examines by mass spectra. Every constituent electronically produces on a paper chart. The time intervened between injection and elution is called the "retention time." It can help to distinguish among some composites.^[8]

II. MATERIALS AND METHODS

A. pannosum leaves were collected from the Punitvan, Bhuj- Kachchh. Leaves were washed with tap & distilled water and dried. Using electric grinder it has been converted into the fine powder and prepared to use for further study.

2.1 Preparation of Plant Extraction

15 gm of leaves powder was extracted with 2-3 liter of n-Butanol (117.6° C) for 12 hours using plant tissue homogenization method. After extraction, it was filtered and the exclusion of solvent was done under pressure by distillation process to afforded extract. Extracts were collected in an airtight glass tube. ^[9]

2.2 GCMS Data Analysis Study

Shimadzu made GC-MS QP2010 instrument was used for GC-MS analysis. The composition of the volatile constituents was established by GCMS analysis. It was performed on a Shimadzu GCMS-QP2010 system in EI mode prepared with a split/splitless injector (300.00°C), at a split ratio of 1/10 using SGE make BPX5WCOT (Wall coated open tubular) capillary column (30m,0.25mm i.d., 0.25µm film thickness). Helium was used as a carrier gas at a flow rate of 2.5ml/min and Hold Time was 2.00 min. The injection volume of each sample was 3µl Column Oven Temperature was maintained at 70.0°C to 300.0°C. The flow rate of Career gas was 1.47 ml/min. The chromatogram is shown in Figure 2 and identified by Comparison with NIST and Wiley compound library which is presented in Table 1.

2.3 Identification of Phytochemicals

The identification of bio-component in the n-butanol extract of (*A. pannosum*) was done by Mass Spectroscopy comparing retention indices and mass spectra fragmentation patterns with the computer library of NIST08s and Wiley Registry of Mass Spectral Data's, New York (Wiley 8) have been used to identify the compound in above extract.

III. RESULT AND DISCUSSION

In the present study, nine different types of bioactive chemical constituents were identified in the *A. pannosum* leaves with important chemical properties. It has been described below in table 1 & 2 and mass spectra of that bioactive compound have been shown in figure 1.

Table 1 Bioactive compound detected from n-butanol extract of *A. pannosum*

Sr No.	Name	Synonyms	Formula	RT	CAS ID	M.W	% of P.A
1	Tetradecanoic acid	Myristic acid	C ₁₄ H ₂₈ O ₂	8.615	544-63-8	228	2.16
2	n-Hexadecanoic acid	Palmitic acid	C ₁₆ H ₃₂ O ₂	8.616	57-10-3	256	2.16
3	Dotriacontane	Bicetyl	C ₃₂ H ₆₆	8.855	544-85-4	451	2.75
4	Eicosane	n-Eicosane	C ₂₀ H ₄₂	8.857	112-95-8	282	2.77
5	Octacosane	n-Octacosane	C ₂₈ H ₅₈	8.859	630-02-4	394	2.79
6	Heptacosane	n-Heptacosane	C ₂₇ H ₅₆	8.861	593-49-7	380	2.80
7	Docasane	n-Docosane	C ₂₂ H ₄₆	8.863	629-97-0	310	2.81
8	Neophytadiene	2,6,10-trimethyl,14-ethylene-14-pentadecne	C ₂₀ H ₃₈	9.020	0-00-0	278	11.02
9	(E)- (7R,11R)-3,7,11,15-tetramethyl-2-hexadecene-1-ol	Phytol	C ₂₀ H ₄₀ O	9.022	150-86-7	296	11.04
10	9-Eicosyne	9-Eicosyne	C ₂₀ H ₃₈	9.200	71899-38-2	278	11.06
11	Citronellylvalerate	Citronellylvalerate	C ₁₅ H ₂₈ O ₂	9.202	0-00-0	240	11.08
12	Pentacosane	n-Pentacosane	C ₂₅ H ₅₂	9.275	629-99-2	352	3.32
13	Tricosane	n-Tricosane	C ₂₃ H ₄₈	9.277	638-67-5	324	3.34
14	Pentadecanoic acid methyl ester	Pentadecylic acid	C ₁₅ H ₃₀ O ₂	9.470	1002-84-2	242	16.81
15	Hexatriacontane	n-Hexatriacontane	C ₃₆ H ₇₄	9.680	630-06-8	507	3.16
16	Heneicosane	n-Heneicosane	C ₂₁ H ₄₄	9.682	629-94-7	296	3.16
17	Pentadecane	n-Pentadecane	C ₁₅ H ₃₂	10.065	629-62-9	212	2.82
18	Hexadecane	Cetane	C ₁₆ H ₃₄	10.067	544-76-3	226	2.84
19	dl-Citronellol	Dihydrogeraniol	C ₁₀ H ₂₀ O	10.090	26489-01-0	156	3.81
20	9,12,15-Octadecatrienoic acid	Methyl linolenate	C ₁₉ H ₃₂ O ₂	10.155	301-00-8	296	21.01
21	9,12,15-Octadecatrien-1-ol	Octadeca-9,12,15-trien-1-ol	C ₁₈ H ₃₂ O	10.157	2774-90-5	264	21.03
22	11,14,17-Eicosatrienoic acid	Methyl-11,14,17-eicosatrienoate	C ₂₁ H ₃₆ O ₂	10.159	55682-88-7	320	21.05
23	Squalene	Spinacene	C ₃₀ H ₅₀	12.740	111-02-4	410	2.75
24	Farnesol	Dihydrofarnesol	C ₁₅ H ₂₆ O	12.742	4602-84-0	222	2.77

25	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	Vitamin E, α -Tocopher	C ₂₉ H ₅₀ O ₂	14.545	59-02-9	430	4.48
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The chemical compounds in the n-butanol leaf extract of *A. pannosum* were found to be in the order of 11,14,17-Eicosatrienoic acid (21.05%), 9,12,15-Octadecatrien-1-ol (21.03%), 9,12,15-Octadecenoic acid (21.01%), Pentadecanoic acid (16.81%), Citronellylvalerate (11.08%), 9-Eicosyne (11.06%), Phytol (11.04%), Neophytadiene (11.02%), α -Tocopherol (4.48%), n-Hexadecanoic acid (2.16%), Squalene (2.75%), were obtained at high concentration. All this major component (described in table 2) have higher biological activity like anticancer, antimicrobial, antiarthritic, antidiabetic, antioxidant, anti-inflammatory, antihypertensive, anti-atherogenic and antitumor activities etc. [9] The leaves n-butanol extract also possess Pentadecane, Hexadecane, tricosane, pentacosane, heptacosane, octacosane, docasane, n-heneicosane, eicosane, triacontane, dotriacontane, hexatriacontane types of carbohydrates as well as hydrocarbons which are good exhibited antibacterial, antiviral and antioxidant activity. [10][11]

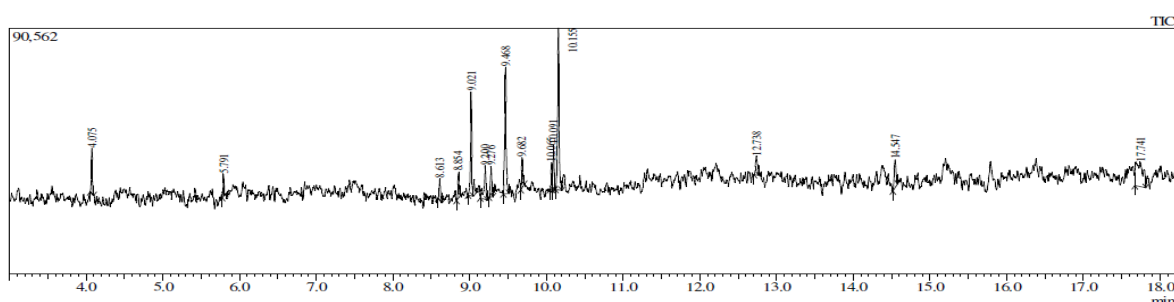
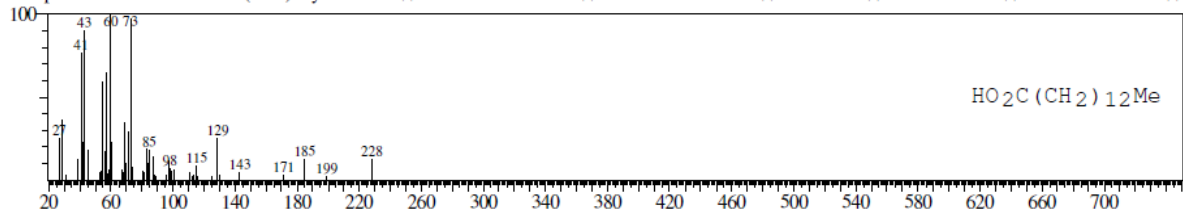
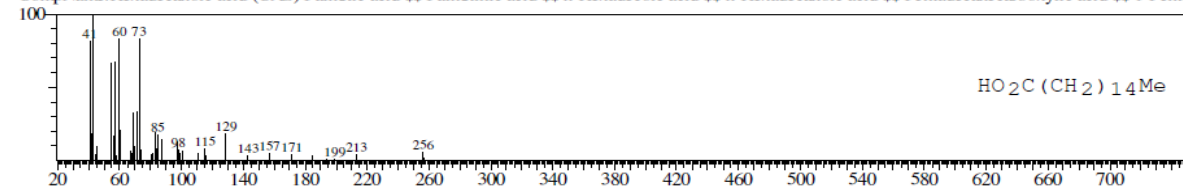


Figure 1 Chromatogram of the bioactive compound of n-butanol extract of *A. pannosum* leaves sample

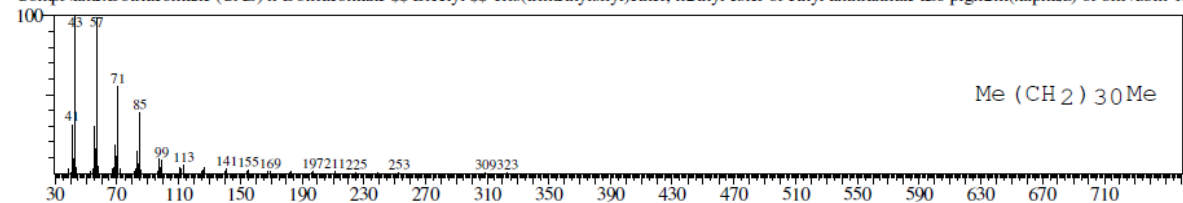
CompName: Tetradecanoic acid (CAS) Myristic acid $\bar{\bar{S}}$ MYRISTIC ACID $\bar{\bar{S}}$ n-Tetradecanoic acid $\bar{\bar{S}}$ neo-Fat 14 $\bar{\bar{S}}$ Univol U 316S $\bar{\bar{S}}$ n-Tetradecic acid $\bar{\bar{S}}$



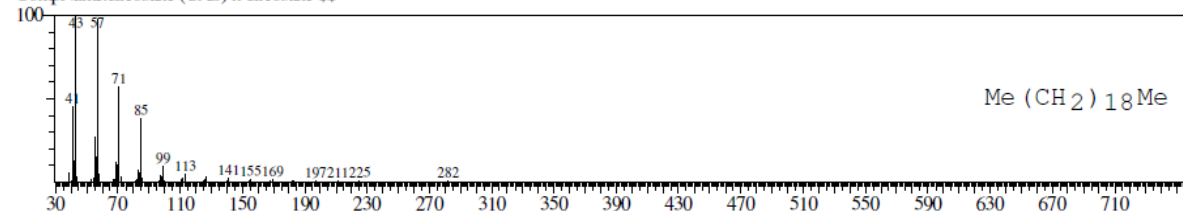
CompName: Hexadecanoic acid (CAS) Palmitic acid $\bar{\bar{S}}$ Palmitic acid $\bar{\bar{S}}$ n-Hexadecic acid $\bar{\bar{S}}$ n-Hexadecanoic acid $\bar{\bar{S}}$ Pentadecanecarboxylic acid $\bar{\bar{S}}$ 1-Penta



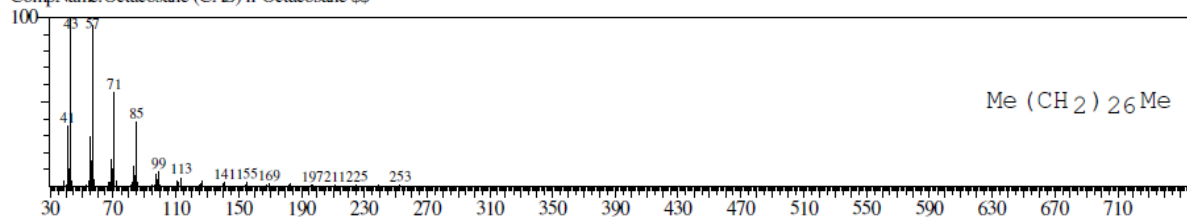
CompName: Dotriacontane (CAS) n-Dotriacontane $\bar{\bar{S}}$ Bicetyl $\bar{\bar{S}}$ Tris(trimethylsilyl)ether, methyl ester of ethyl anthranilate azo pigment(alpha.z) of bilivubin-1



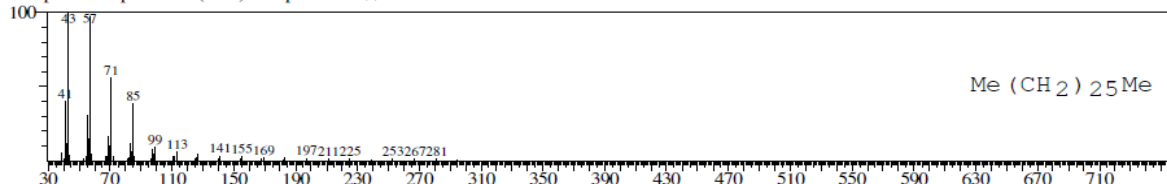
CompName: Eicosane (CAS) n-Eicosane $\bar{\bar{S}}$



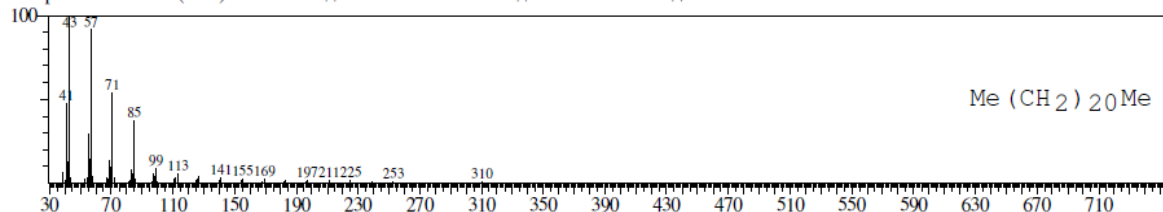
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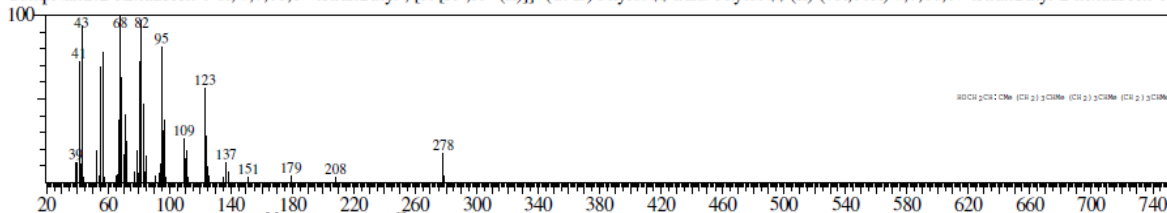
CompName: Heptacosane (CAS) n-Heptacosane SS



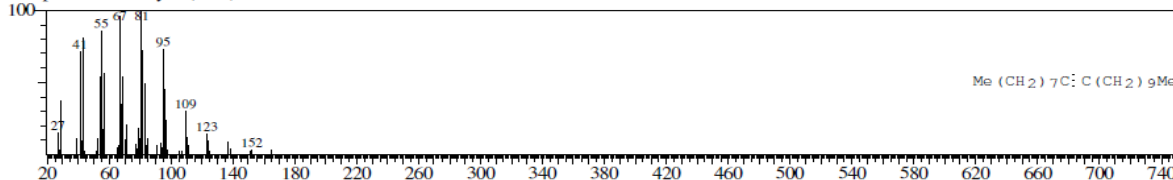
CompName: Docosane (CAS) n-Docosane SS C22H46 STANDARD SS Normal-docosane SS



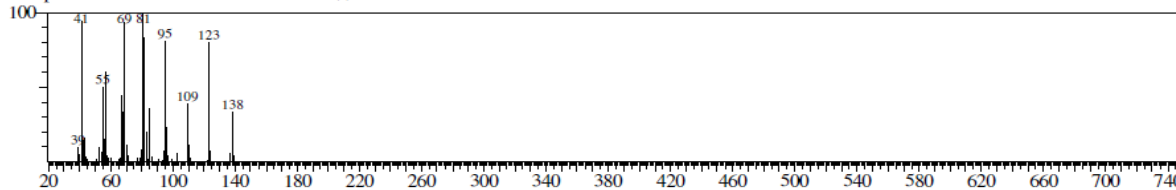
CompName: 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol SS trans-Phytol SS (E)-(7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol



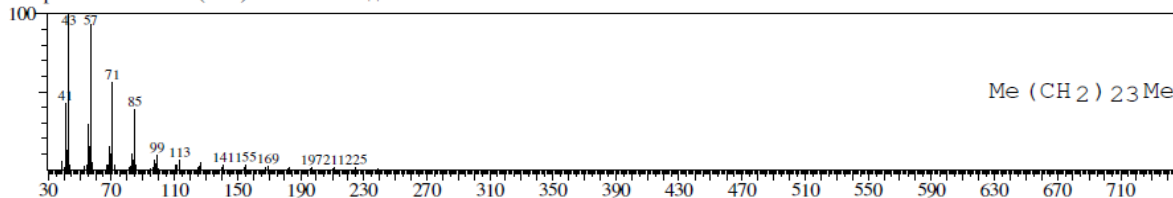
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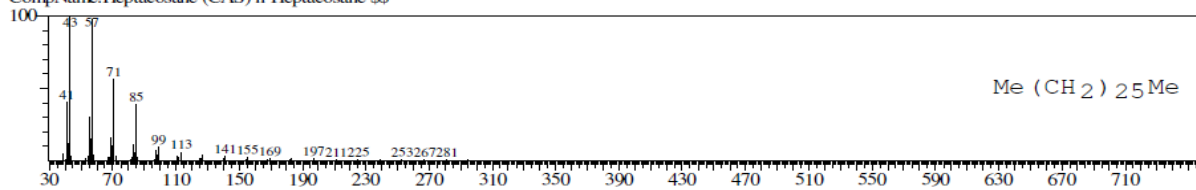
CompName: CITRONELLYL VALERATE SS



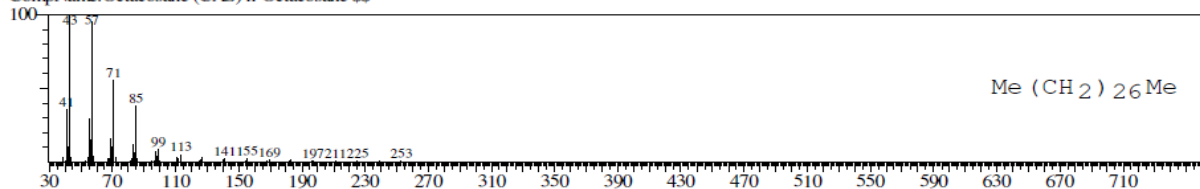
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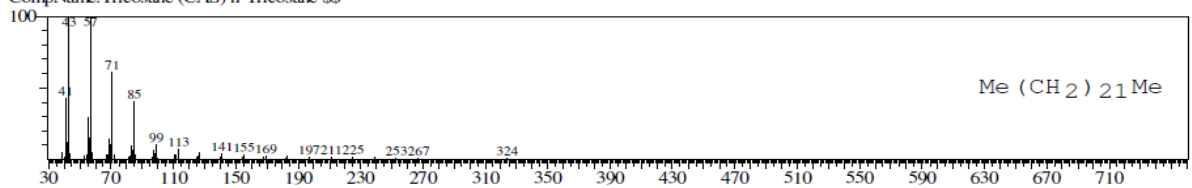
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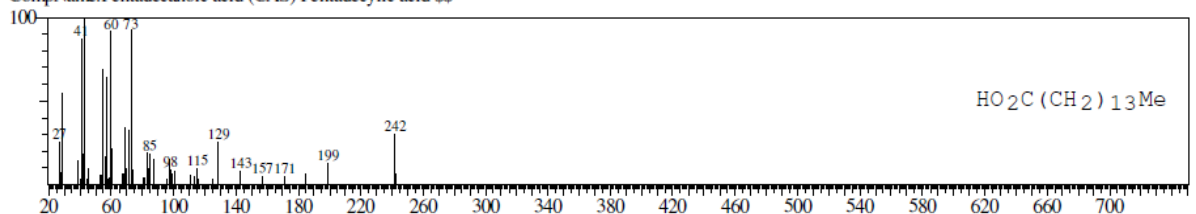
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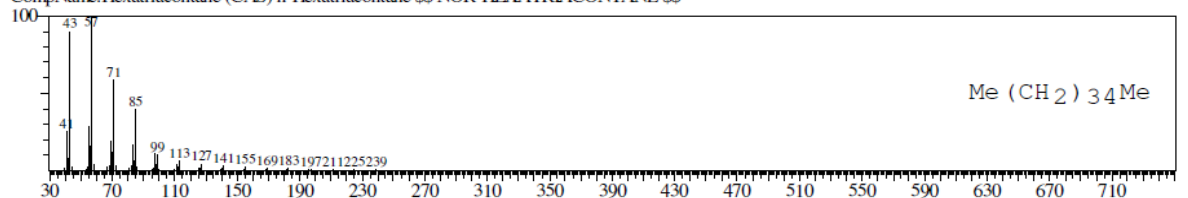
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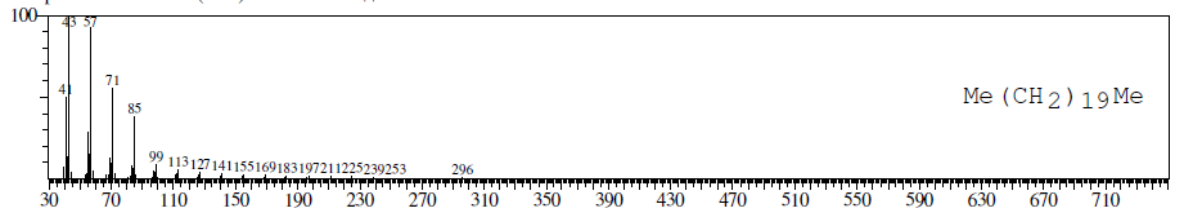
CompName:Pentadecanoic acid (CAS) Pentadecylic acid \$\$



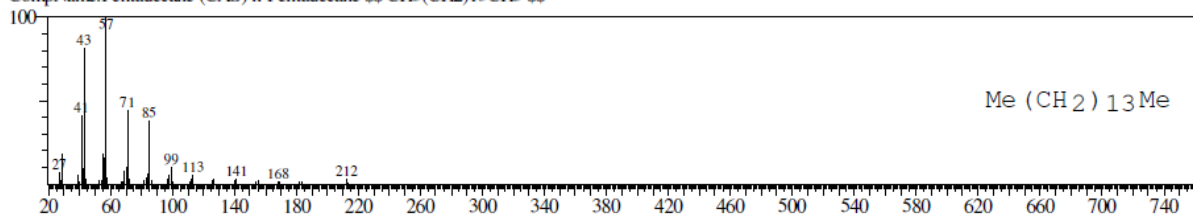
CompName:Hexatriacontane (CAS) n-Hexatriacontane \$\$ NOR-HEXATRIACONTANE \$\$



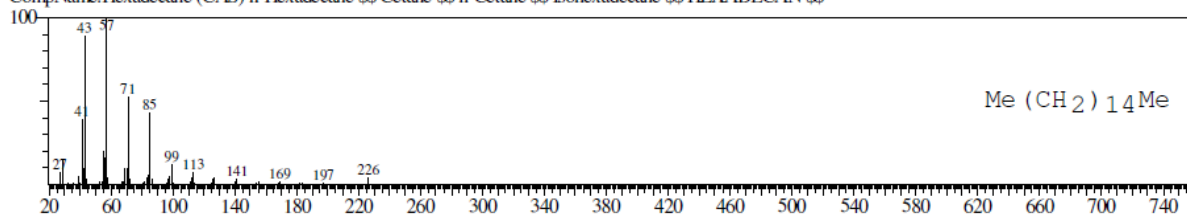
CompName:Heneicosane (CAS) n-Heneicosane \$\$



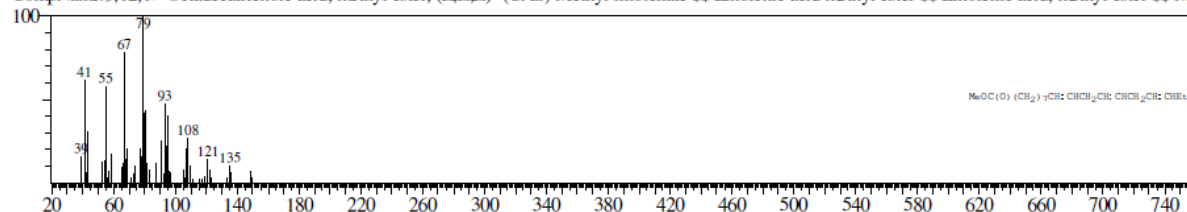
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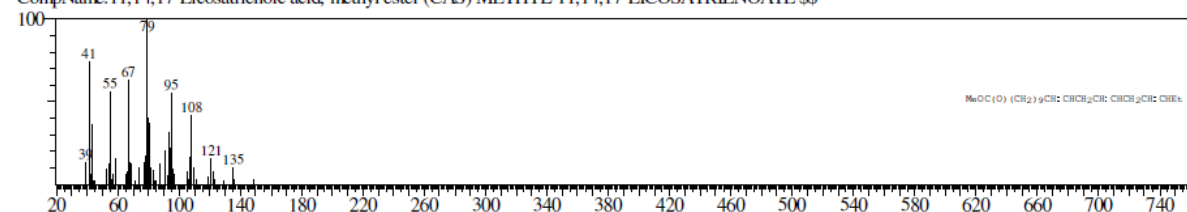
CompName: Hexadecane (CAS) n-Hexadecane \$\$ Cetane \$\$ n-Cetane \$\$ Isohexadecane \$\$ HEXADECAN \$\$



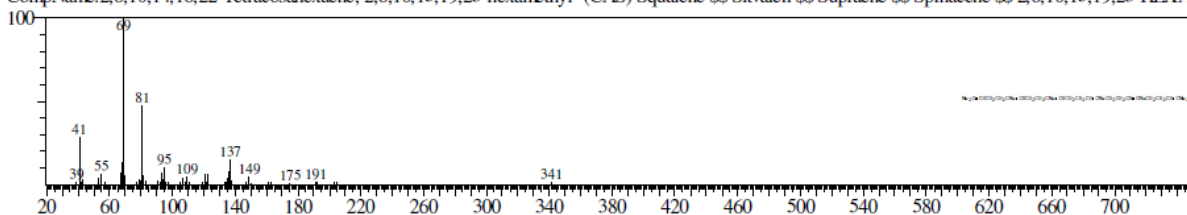
CompName: 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate \$\$ Linolenic acid methyl ester \$\$ Linolenic acid, methyl ester \$\$ Me



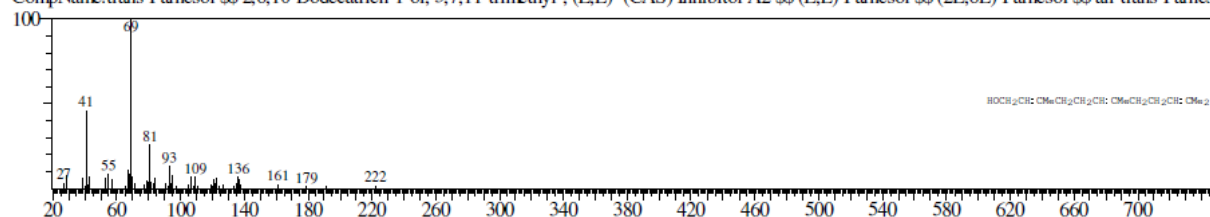
CompName: 11,14,17-Eicosatrienoic acid, methyl ester (CAS) METHYL-11,14,17-EICOSATRIENOATE \$\$



CompName: 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS) Squalene \$\$ Skvalen \$\$ Supraene \$\$ Spinacene \$\$ 2,6,10,15,19,23-HEXAL



CompName: trans-Farnesol \$\$ 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)- (CAS) Inhibitor A2 \$\$ (E,E)-Farnesol \$\$ (2E,6E)-Farnesol \$\$ all-trans-Farnesol



CompName: Vitamin E \$\$ 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]- (CAS) .alpha.-Tocopherol

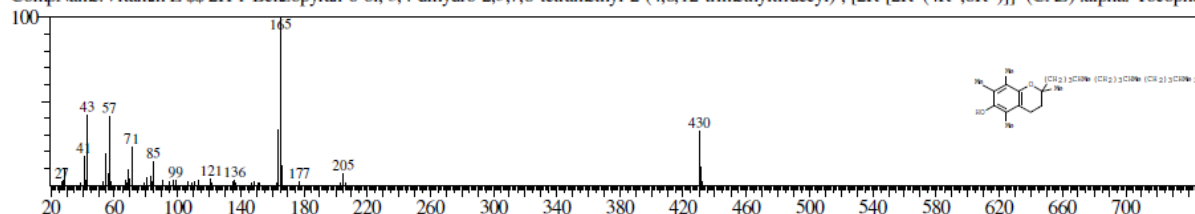

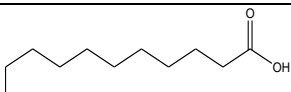
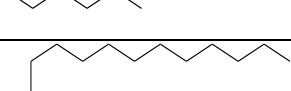
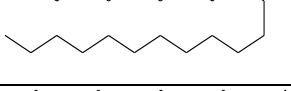
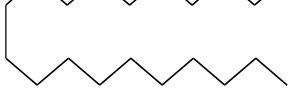
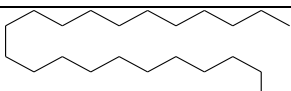
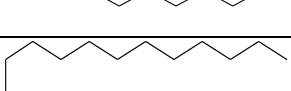
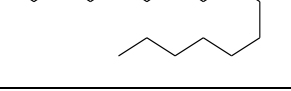
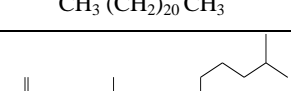
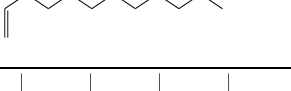

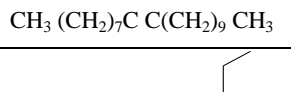
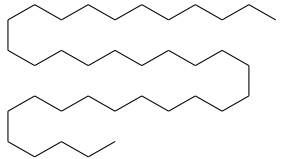
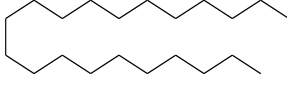


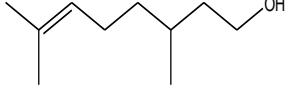
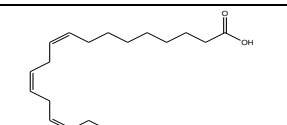

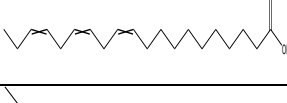
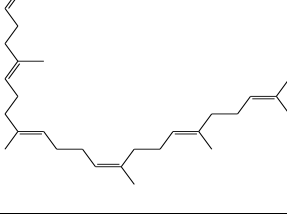

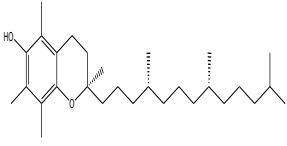


Figure 2 Mass spectra of the bioactive compound of n-butanol extract of *A. pannosum* leaves sample

Table 2: Components Identified and it's Activity/Uses in n-butanol Extracts of *A. pannosum* by GC-MS

Sr No	Bioactive Component	Structure	Nature	Importance	Ref.
.					

1	Tetradecanoic acid		Fatty acid	Antioxidant, anticancer, hypercholesterolemia	9, 12, 13
2	n-Hexadecanoic acid		Fatty acid	Anti-inflammatory, antioxidant, antipsychotic, antiallergic	14, 15, 16
3	Dotriacontane		Hydrocarbon	Antimicrobial, antioxidant, antispasmodic, antibacterial and antiviral	17, 18
4	Eicosane		Hydrocarbon	Thermal-regulating functional phase change material for clothing application	19
5	Octacosane		Carbohydrate s/Hydrocarbon	Act as good phase change materials	20, 21
6	Heptacosane		Hydrocarbon	Antibacterial	22
7	Docasane	$\text{CH}_3 (\text{CH}_2)_{20} \text{CH}_3$	Carbohydrate	Antifungal and antibacterial	23
8	Neophytadiene		Diterpenoid	Strong bactericidal, anti-inflammatory, antifungal compounds	24
9	(E)- (7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol		Diterpene	Diuretic, antimicrobial, anti-inflammatory anticancer, anti-inflammatory fragrance compound	25, 26
10	9-Eicosyne	$\text{CH}_3 (\text{CH}_2)_7 \text{C} (\text{CH}_2)_9 \text{CH}_3$	Sat. aliphatic hydrocarbon	Antimicrobial	22
11	Citronellylvalerate		Fatty alcohol esters	Flavour and fragrance substance, Insecticide	27
12	Pentacosane		Aliphatic hydrocarbon	Antibacterial	12
13	Tricosane		Carbohydrate/Hydrocarbon	Antibacterial	12, 13,
14	Pentadecanoic acid		Fatty acid	Antimicrobial, antifungal, antiallergic	28

15	Hexatriacontane		Terpene alcohol	Antioxidant activity	24
16	Heneicosane		Carbohydrate	Antibacterial, Inhibit larva growth	29
17	Pentadecane		Hydrocarbon	Antibacterial activity	30
18	Hexadecane		Hydrocarbon	Potent antifungal activity	30
19	dl-Citronellol		Essential oil	Antimicrobial, antifungal, antispasmodic and anticonvulsant activities	31
20	9,12,15-Octadecatrienoic acid		Fatty acid	Anti-inflammatory and anti-atherogenic properties	32
21	9,12,15-Octadecatrien-1-ol		Fatty acid	Antioxidant and antibacterial	33
22	11,14,17-Eicosatrienoic acid		Polyunsaturated fatty acid	Antiarthritic, anticoronary, antiinflammatory activity	34
23	Squalene		Triterpene	Antibacterial, Chemo preventive, immunostimulant, anti-tumor, antioxidant, anticancer, lipoxygenase-inhibitor, perfumery, pesticide, sunscreen	35, 36
24	Farnesol		Sesquiterpenoids	Antibacterial, antioxidant, antifungal anti-cancer agent, and chemoprotective effects	37
25	α -Tocopher		Vitamin E	Anticancer, antitumor, antioxidant, antiinfertility, anti-stroke	38

Tetradecanoic acid, n-Hexadecanoic acid, 11,14,17-Eicosatrienoic acid, 9,12,15-Octadecenoic acid and Pentadecanoic acid all are fatty acids. The human body needs essential fatty acids to construct and repair cell membranes enabling the cells to obtain optimum nutrition and expel harmful waste products. ^[39] A primary function of essential fatty acids, which support the cardiovascular, reproductive, immune and nervous systems, is the production of prostaglandins. These regulate body functions such as heart rate, blood pressure, blood clotting, fertility and play a role in immune system by regulating inflammation. ^[40] In the study of ^[41], the phytochemical investigation of *A. pannosum* resulted in the separation and identification of a new flavonoid, at

25.6358 RT that is, kaempferol 4'-O-(6''-O-E-p-coumaroyl)- β -D-glucopyranoside 1 from butanol fraction, along with the identification of the volatile constituents of petroleum ether and methylene chloride by GC/MS analysis. kaempferol 4'-O-(6''-O-E-p-coumaroyl)- β -D-glucopyranoside 1 is a representative acylated flavonol 3-O-glycoside, possess antioxidant activity. The identification of good amount of chemical components by gas spectrometry method in n-butanol crude extracts of *A. pannosum* leaves sample might have some ecological significance.

IV. CONCLUSION

The present study has been carried out on the qualitative and quantitative analysis of the major bioactive components of therapeutically significant plant *A. pannosum* leaves by use of GC-MS. Total nine different types of bio-compounds were identified from the *A. pannosum* leaves extracts by using n-butanol solvent. The biological activities of each of the identified phytochemicals range from anticancer, antimicrobial, antiarthritic, antioxidant, anti-inflammatory, antihypertensive, anti-atherogenic and antitumoral activities. These findings have provided the scientific basis for the therapeutic use of the plant. Though, isolation of these separate phytochemical components subject to biological activity and toxicity profile will give fruitful results.

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