Extraction of Essential Oils from Flower and Flower Stalk of *Samadera indica* by Steam Distillation

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Abstract **- Essential oils are made of higher hydrocarbons and their oxygenated derivatives. The main constituents of essential oils are terpenoids (mono and sesquiterpenes). Generally, the process of steam distillation is the most widely accepted method for the production of essential oils on a large scale. Essential oils were isolated by steam distillation of flower and flower stalks of *Samadera indica*. By Gass chromatography and mass spectroscopy GCMS analysis, forty-two components were identified from flower essential oil and seventeen components were identified from flower stalk oils of the plant. The major components of flower essential oil are phytol, 2-Naphthalene methanol,1,2,3,4,4a,5,6,7-octahydro-alpha. alpha.4a,8-tetramethyl-(2R-cis)- Hexadecanoic acid, methyl ester, Heptanoic acid, 3-hexenyl ester, (Z)-, n-Hexadecanoic acid, Isophytol, Alpha-Cadinol, Tricosane, 3-Hexen-1-ol, benzoate, (Z). *Samadera indica* flower stalk essential oil contains 1,2-Benzenedicarboxylic acid butyl methyl ester, 1,2- Benzenedicarboxylic acid, bis (2-methyl propyl) ester, 2-ethyl-1-Hexanol and phytol. These include esters, phenols as well as terpenoids, which are reported to be antioxidants, antimicrobials and cytotoxic in nature.**

Index Terms **- Essential oil, Steam distillation, GCMS analysis, phytocomponents, Bioactive.**

INTRODUCTION

Essential oils are volatile phytoproducts and have many bio-properties such as cytotoxicity, antioxidant property, anti-microbial property, and psychoactive property including stimulating and sedative effects of fragrances, behavioural changes etc[1]. Actually, it is the essential oils which give spices and herbs their specific scent and flavour and flowers and fruits their smell. Minute droplets of these oils are present in tiny glandular cells in the outer cells of fruits and flowers[2]. Essential oils are present relatively more in younger plants. However, older plants are rich in darker, resinous oil.

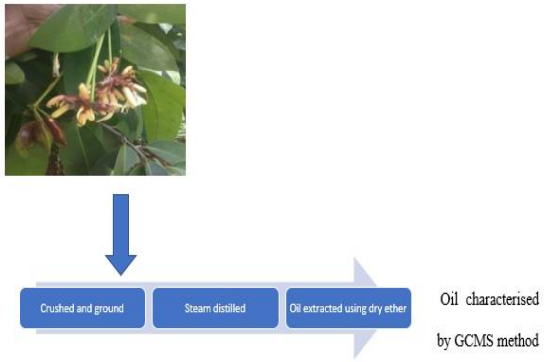
India is considered as the ancient home of fragrances due to the country having a wide variety of soils in which different kinds of plants can grow. More odour-giving plants are present in tropical countries, like India, due to the availability of more solar energy. Solar energy influences the quality and quantity of plant products. Much work has not been done on the essential oils of *S. indica*. This may be due to the plant having small flower and also due to small amount of oil secreted by the plant. Essential oils are made of higher hydrocarbons and their oxygenated derivatives. The main constituents of essential oils are terpenoids (mono and sesquiterpenes) and the oxygenated derivative[3].

Steam distillation, pressing, solvent extraction and extraction by using fat are the major methods used for isolation of essential oil from plant parts. Generally, the process of steam distillation is the most widely accepted method for the production of essential oils on a large scale. In steam distillation, the steam is produced in a boiler and is blown through a pipe into the bottom of the still, where the plant material rests on a perforated tray or in a basket for quick removal after exhaustive extraction. The condensed distillate, consisting of a mixture of water and oil, is separated in a flask. There the distillate separates into two layers from which the oil and the water can be separately withdrawn by extracting with pure diethyl ether[4].

The coupling of a gas chromatograph with a mass spectrometer is the most often used and a well-established technique for the analysis of essential oils. The very first application of a GC-MS coupling for the identification of essential oil constituents. The proliferation of GC-MS applications is also a result of commercially available easy-to-handle dedicated mass spectral libraries (e.g., NIST/EPA/NIH 2005; WILEY

Registry 2006; Mass Finder 2007; and diverse printed versions such as Jennings and Shibamoto, 1980; Joulain and König, 1998; and Adams, 1989, 1995, 2007 inclusive of retention indices) providing identification of the separated compounds[5].

Fig 1 Extraction of flower and flower stalk of *Samadera indica*



METHODS AND MATERIALS

214g of the flower and 262g of flower stalks are separately crushed and ground to reduce the particle size and to rupture some of the cell walls of oil-bearing glands using an electric mixer grinder. The ground masses were then subjected to steam distillation for 3 hours. About 2 litres of the distillate were collected and were extracted using pure organic solvent ie diethyl ether (3×100ml) and dried using anhydrous sodium sulphate. The dry ether extract on evaporation yielded 0.45g flower oil with a pale-yellow colour (0.21% of fresh weight of the sample) and 0.34g flower stalk oil with pale orange yellow colour (0.13% of fresh weight of the sample). 2 µL of the essential oils of flower and flower stalk of *Samadera indica* employed for GC/MS analysis.

GAS CHROMATOGRAPHY- MASS SPECTROMETRY

The GC-MS analyses (fig. 4) were carried out on Agilent 6890 GC system equipped with a 5973 inert mass selective detector (Agilent Technologies, USA). A CO Sil 8 CB (Varian, Middleburg, Netherlands) column of 30m length, 0.25mm i.d, and 0.25µm film thickness was used. The oven was programmed from an initial temperature 50 ^{0}C (hold for 2 min) to the final temperature 280^{0}C at the rate of 100C/min. The final temperature holds up time was 5min. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode. The inlet and interface temperatures were kept at 280^{0}C. The EI source was operated at 230^{0}C and the quadrupole temperature was 150^{0}C. The MS was scanned from 30 to 500 mass units. One micro litre of the sample was injected in split mode at a split ratio of 10:1. for compound identifications.

Interpretation on mass spectrum of GC-MS was done using the database of CSIR- Indian Institute of chemical technology (IICT) Hyderabad. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the IICT library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSIONS

On comparison of the mass spectra of the constituents with NIST library, 42 peaks for flower oil and 17 peaks for flower stalk were identified (Tables 1,3). The extracts show presence of many methyl ethyl esters, phenols, terpenoids in them. Phytol, a diterpene alcohol, is the major component in flower and 1,2-Benzenedicarboxylic acid, butyl methyl ester in flower stalk extracts. Both are reported as potential antioxidants and antimicrobials, cytotoxic (Tables 4) hinting the use of the flower and flower stalk of *Samadera indica* for pharmaceutical advantages.[6,7,8]

Figure 2: GCMS of flower essential oil of *Samadera indica*

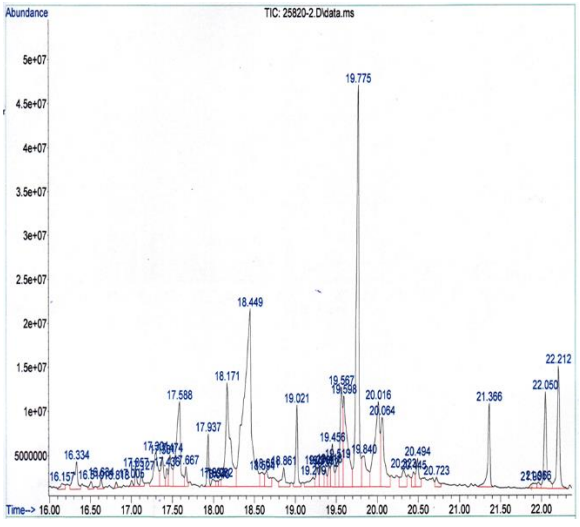
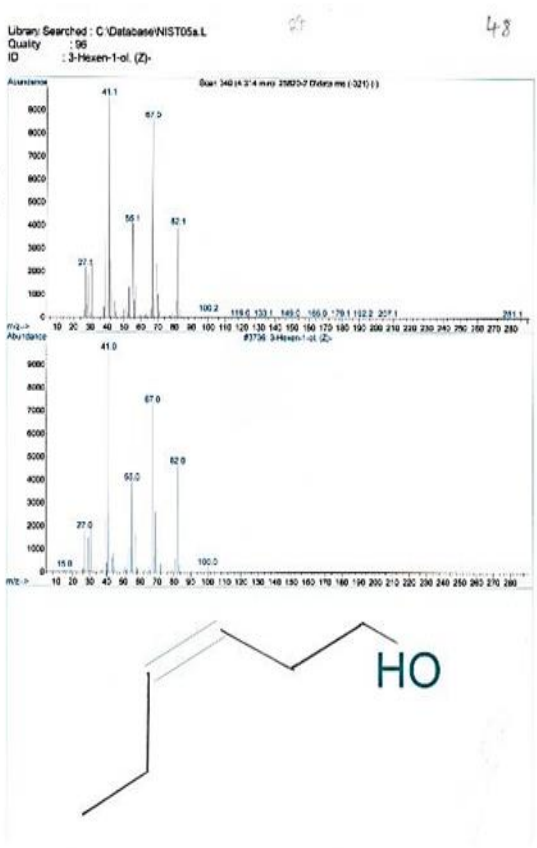


Table 1: Phytochemical present in flower essential oil of *Samadera indica*

Sl. No	RT	Name of the component	Percentage composition (%)
1	4.314	3-Hexen-1-Ol, (Z)	0.83
2	4.445	1- Hexanol	0.78
3	6.258	2- Butanoicacis, 2-methyl- (E)	0.64
4	6.876	Hexanoic acid	0.82
5	7.388	2- Hexenoic acid	0.90
6	7.697	2-Hexenal, (E)	0.84
7	7.795	1,6- Octadien-3 Ol, 3,7-dimethyl	0.86
8	7.822	Nonanal	0.72
9	8.032	Phenyl ethyl Alcohol	1.06
10	8.124	2-Cyclohexen-1-one,3,5,5-trimethyl	0.67
11	8.465	Bicyclo[2,2,1]heptan-2-one,1,7,7trimethyl-(1S)	0.69
12	9.188	3-Cyclohexen-1-methanol, alpha., alpha,4-trimethyl	0.89
13	9.641	Benzothiazole	1.25
14	9.694	Cyclopropane,1,1′-ethenylidenebis-	0.83
15	10.042	2,6-Octadien-1-Ol,3,7-dimethyl- (E)	0.73
16	10.449	Hydroquinone	1.62
17	10.869	2-Methoxy-4-vinylphenol-	3.96
18	11.789	Heptanoic acid, 3-hexenyl ester, (Z)-	2.98
19	11.828	3 Hexenoic acid (E)	1.90
20	11.888	1-Hexene,6-bromo	1.95
21	12.919	1-Decene	1.64
22	13.352	Alpha- Farnesene	1.07
23	13.523	Dodecanoicacid, methyl ester	0.78
24	13.589	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7, dimethyl-(1-methyl ethyl)-, (1S-Cis)-	1.4
25	13.753	(E)- 9,11-Dodecadien-1-ol	2.88
26	14.134	3-Hexen-1-ol, benzoate, (Z)-	2.25
27	14.285	Phenyl tert-butyl ketone	
28	14.377	Azulene,1,2,3,3a,4,5,6,7-Octahydro-1,4-dimethyl-7- (1-methyl ethenyl)-[1R-(1. alpha.,3a. beta.,4-alpha.,7. Beta)]	0.84
29	14.916	2-Naphthalene methanol,1,2,3,4,4a,5,6,7-octahydro-alpha., alpha.,4a,8-tetramethyl-(2R-cis)-	3.36
30	15.014	Tau-Muurolol	0.57
31	15.172	Alpha-Cadinol	2.88
32	15.310	Cyclotetradecane	0.48
33	15.717	2-Tridecenal, (E)	1.08
34	16.328	Benzyl Benzoate	1.77
35	17.937	Hexadecanoicacid,methyl ester	6.4
36	18.174	Isophytol	4.88
37	18.436	n-Hexadecanoic acid	4.96
38	19.566	9-Octadecyne	1.39
39	19.757	Phytol	22.8
40	20.006	9,12-Octadecadienoic acid (Z, Z)	2.8
41	21.360	Tricosane	2.05
42	22.212	Z-12-Pentacosene	1.34

Figure 3 Phytochemical present in flower essential oil of *Samadera indica*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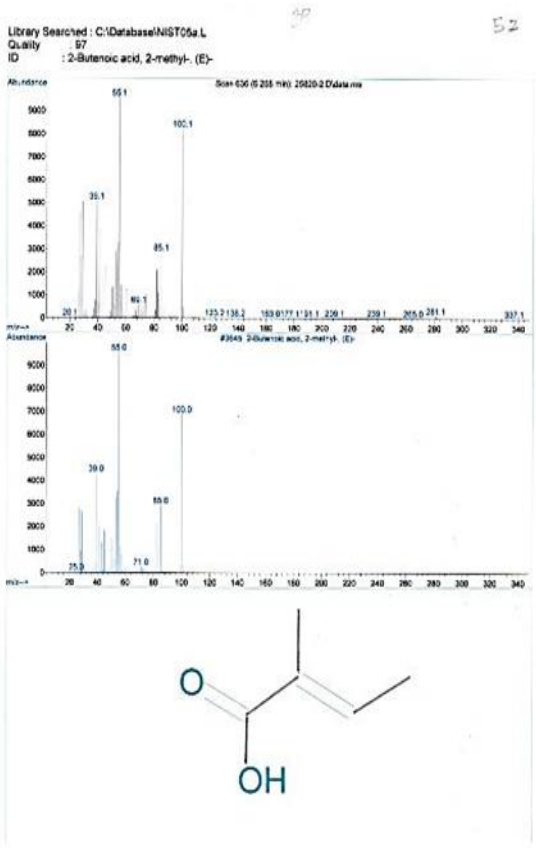
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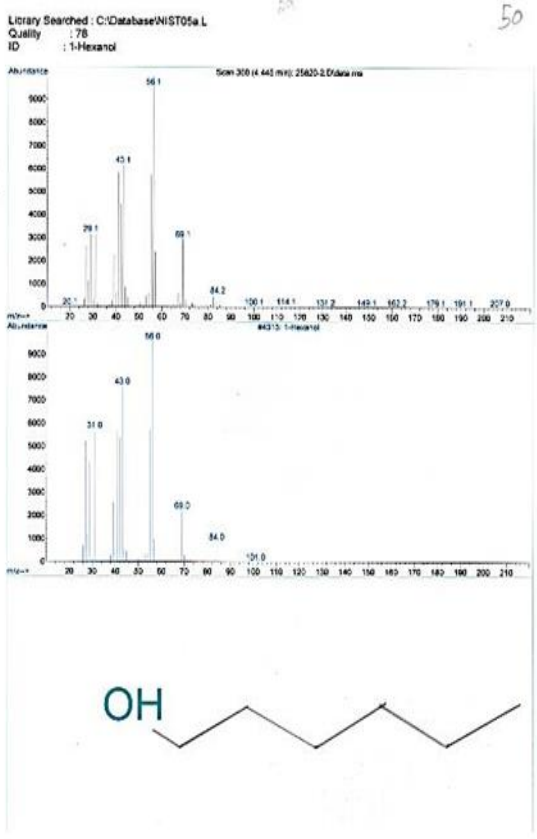
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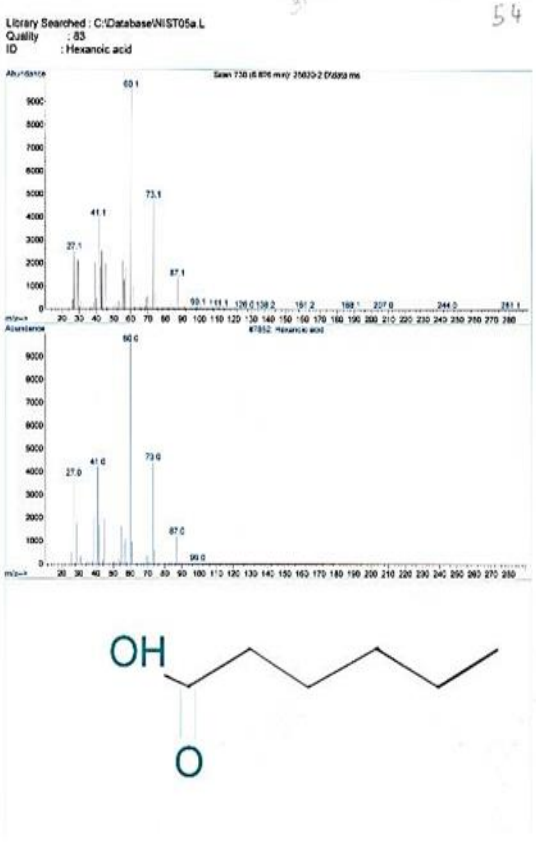
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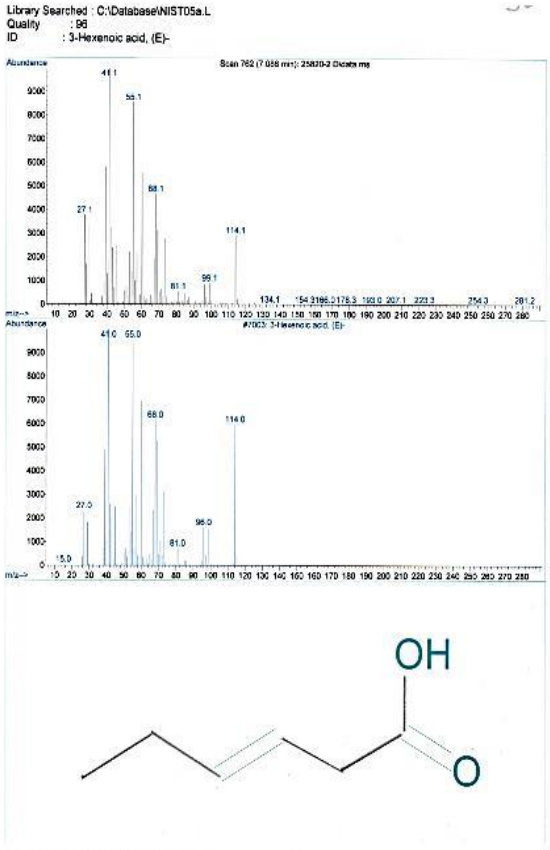
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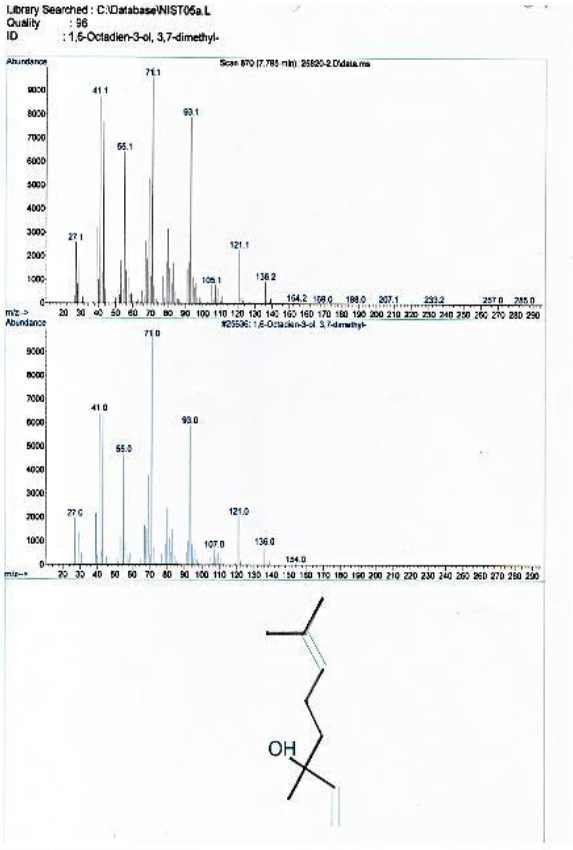
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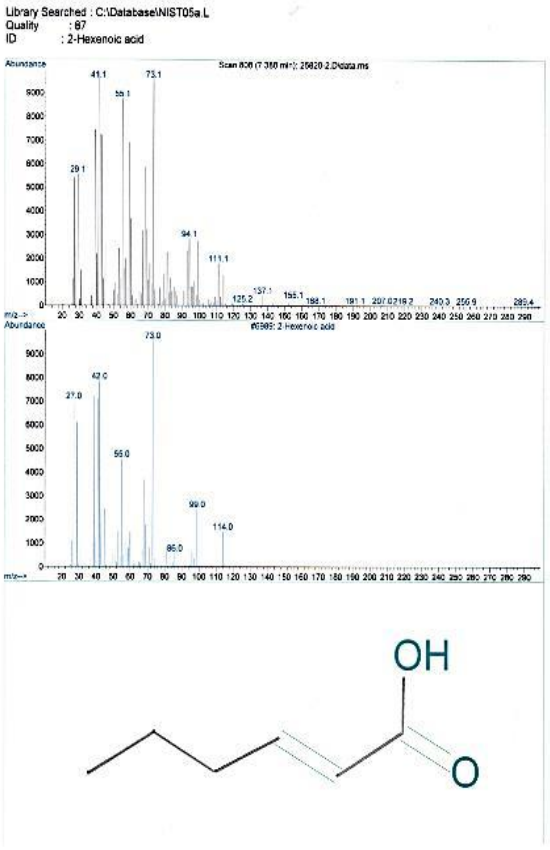
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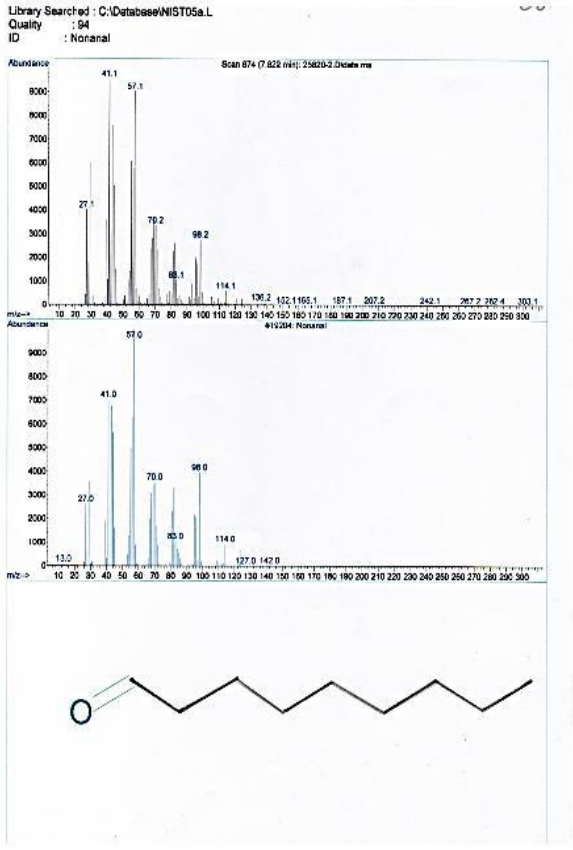
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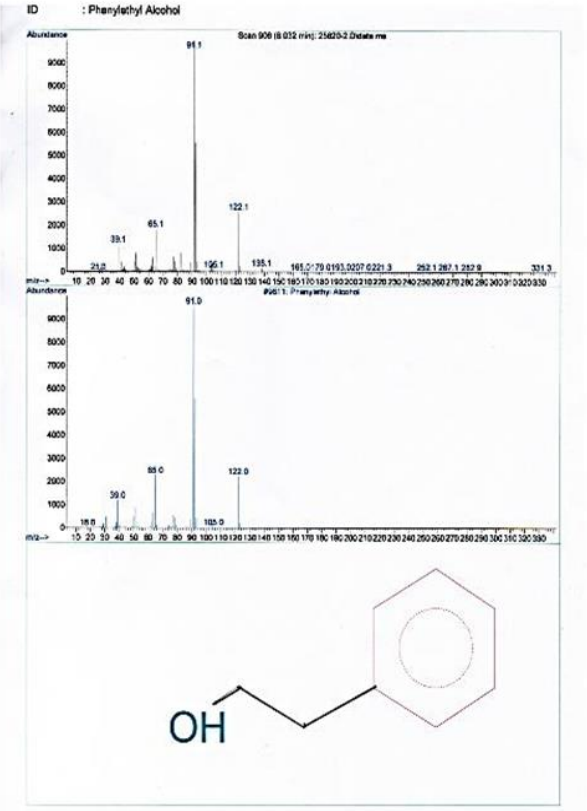
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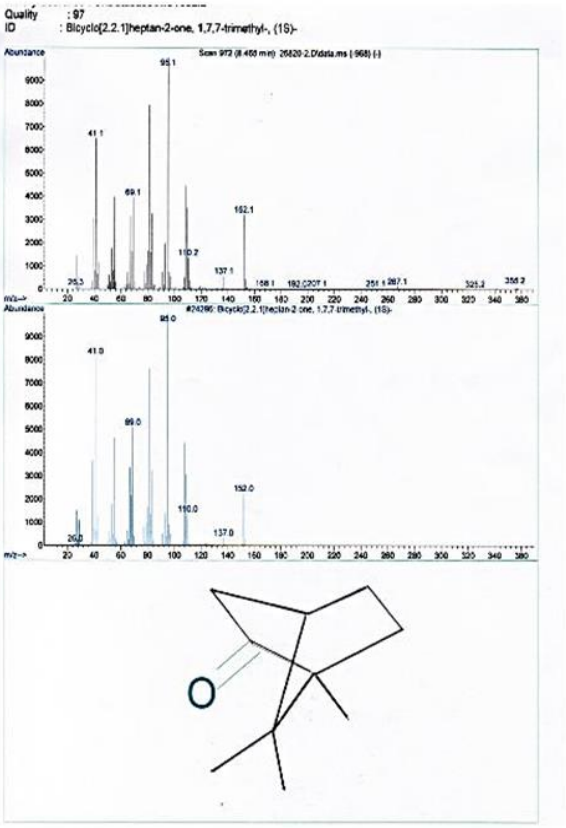
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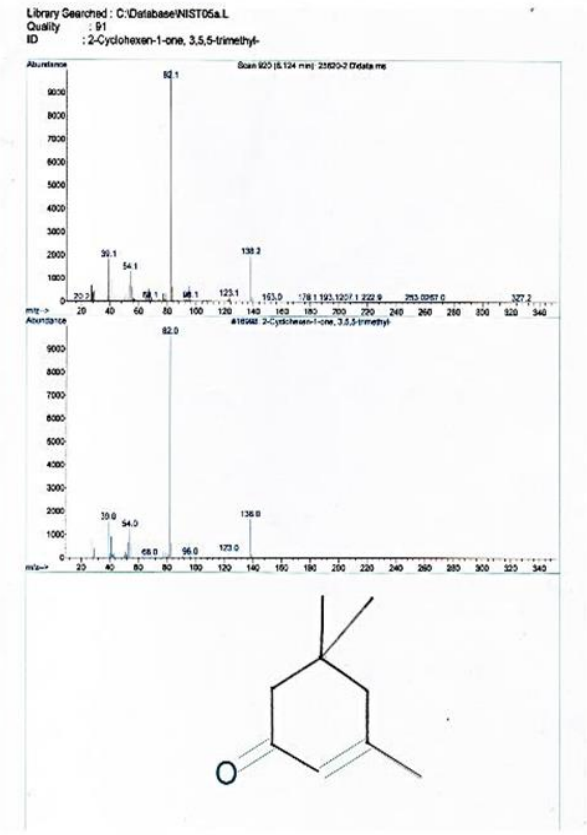
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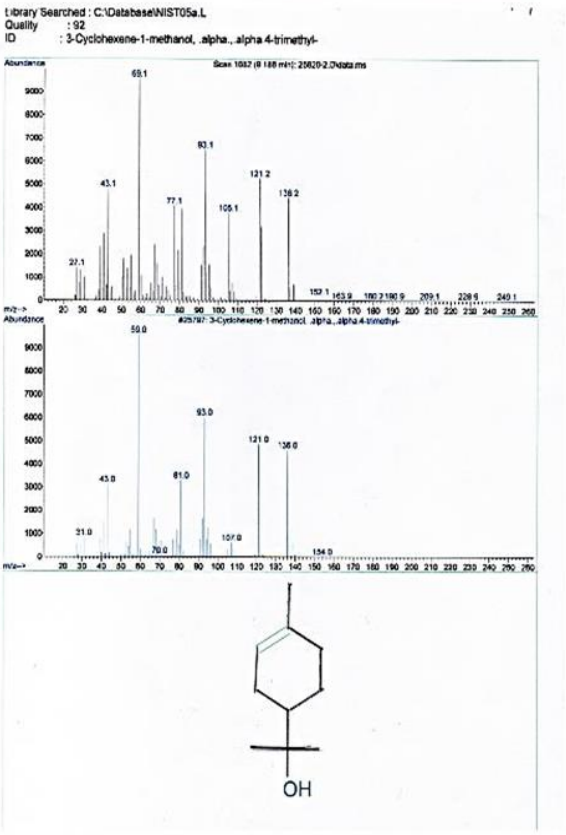
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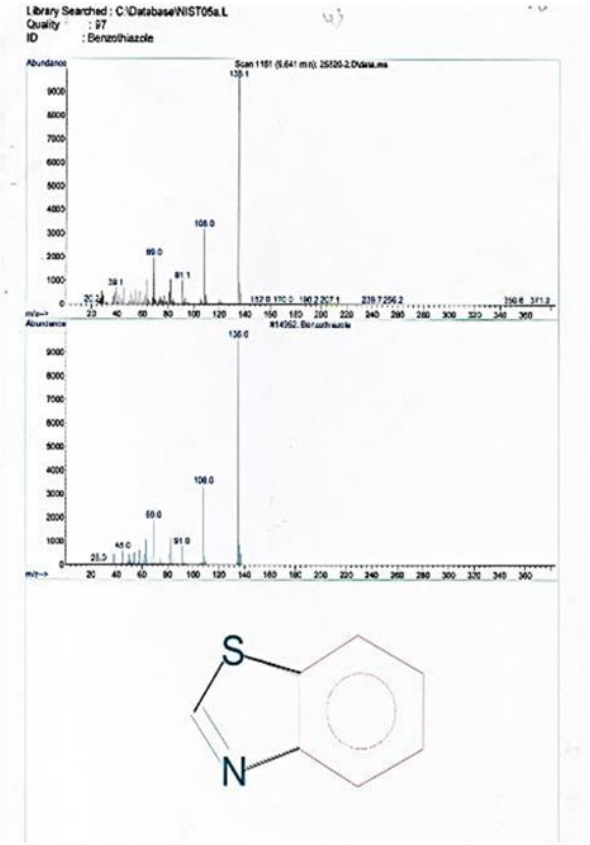
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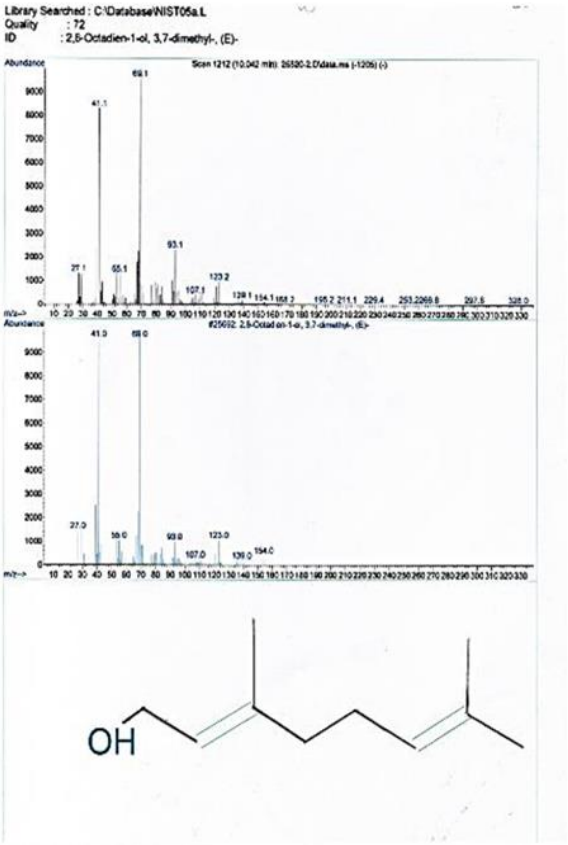
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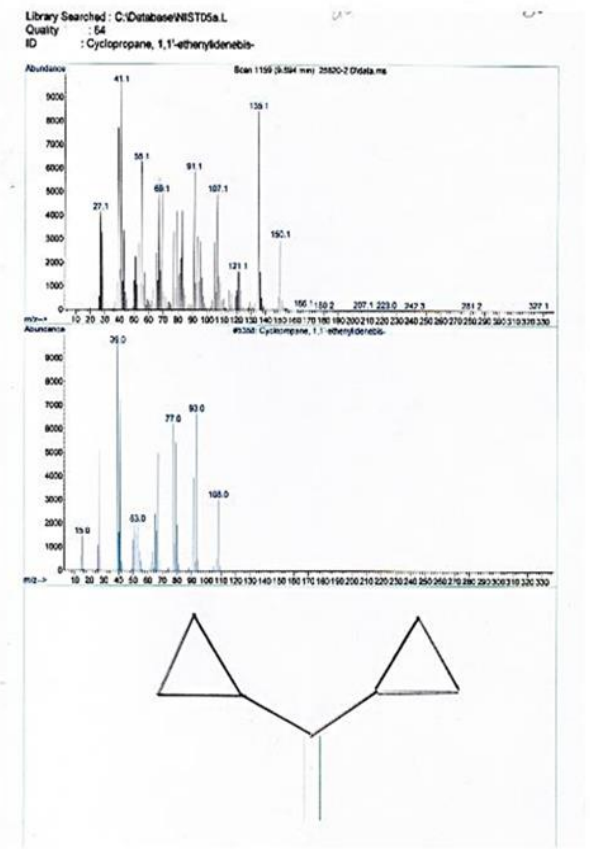
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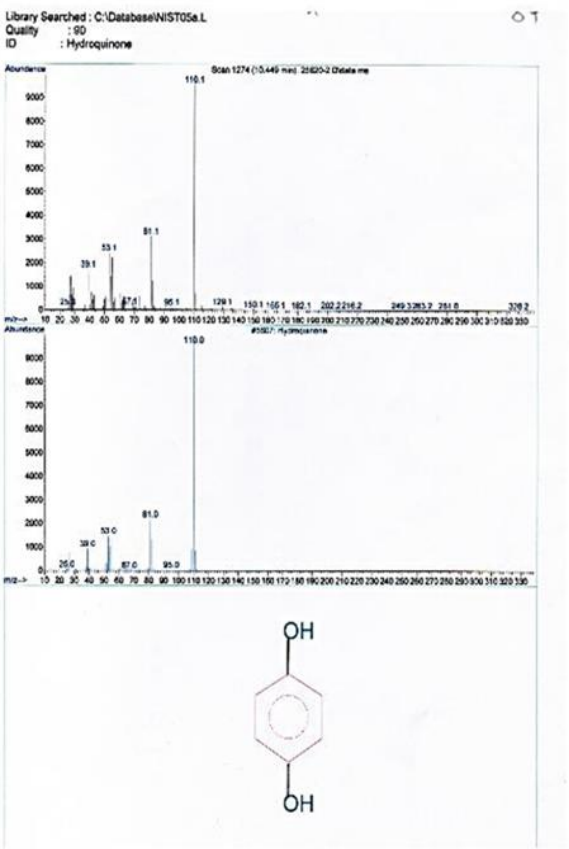
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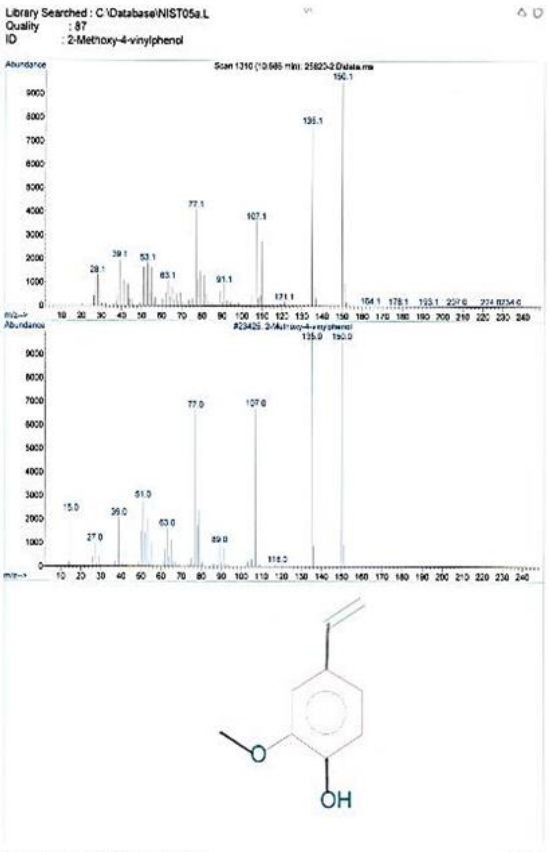
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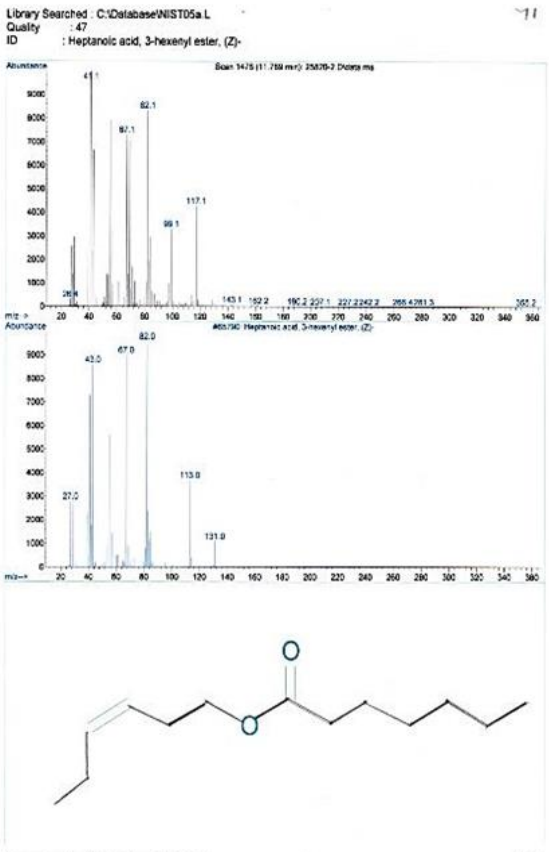
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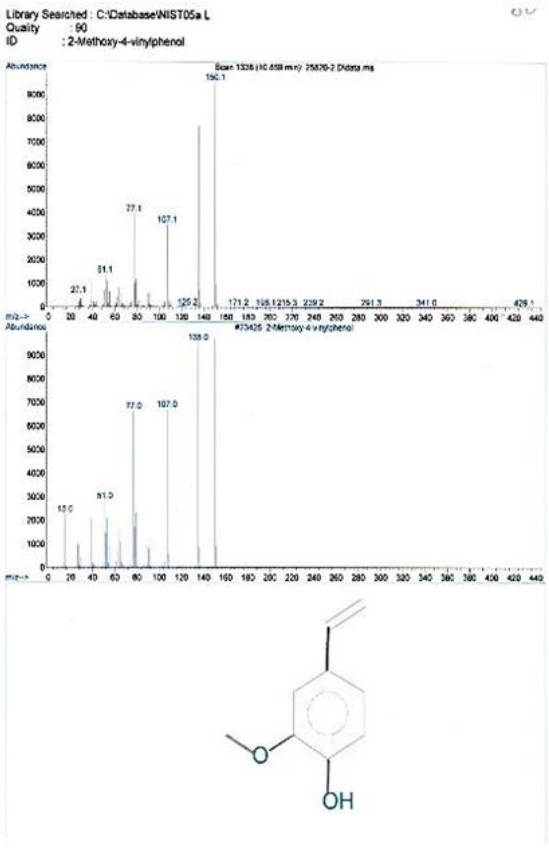
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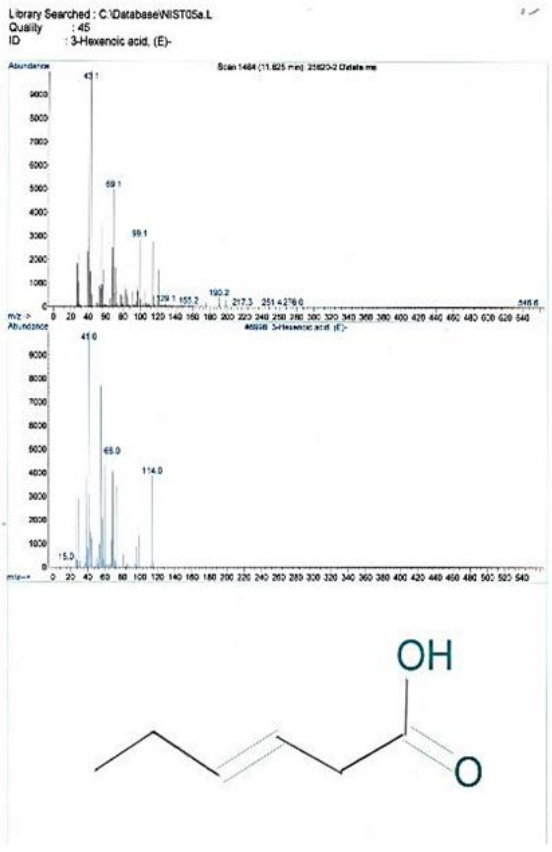
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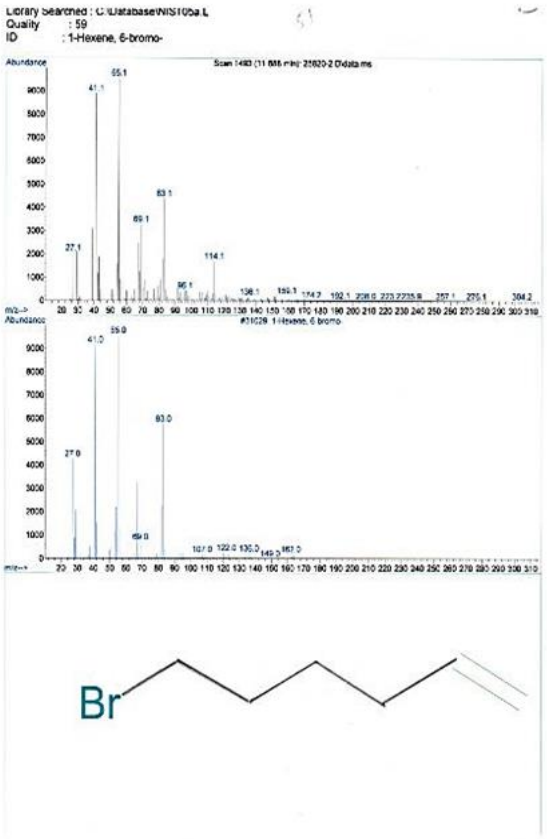
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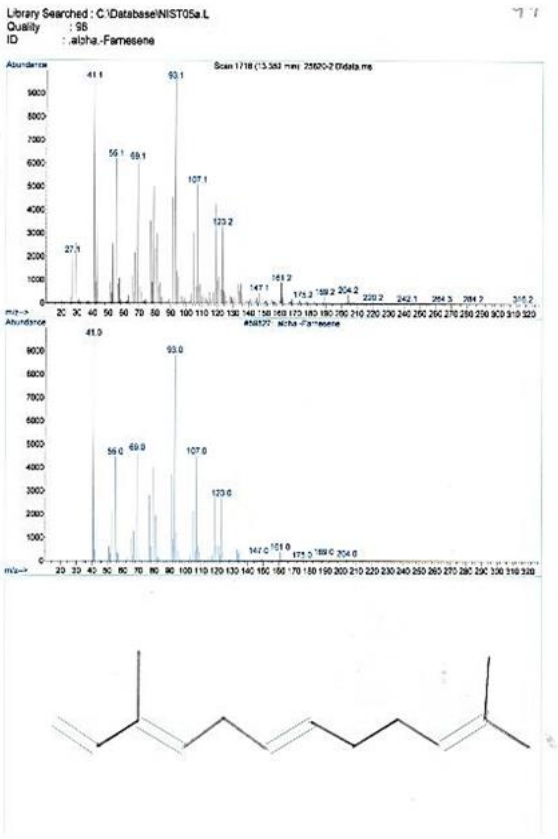
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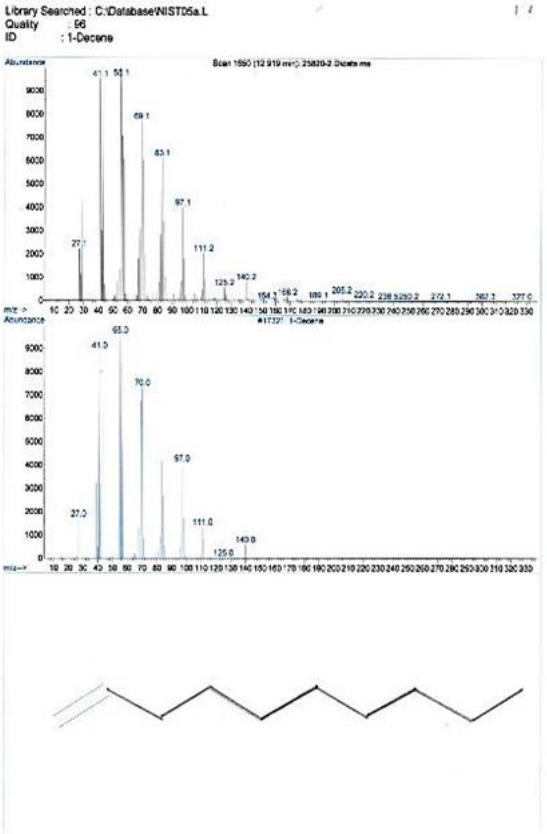
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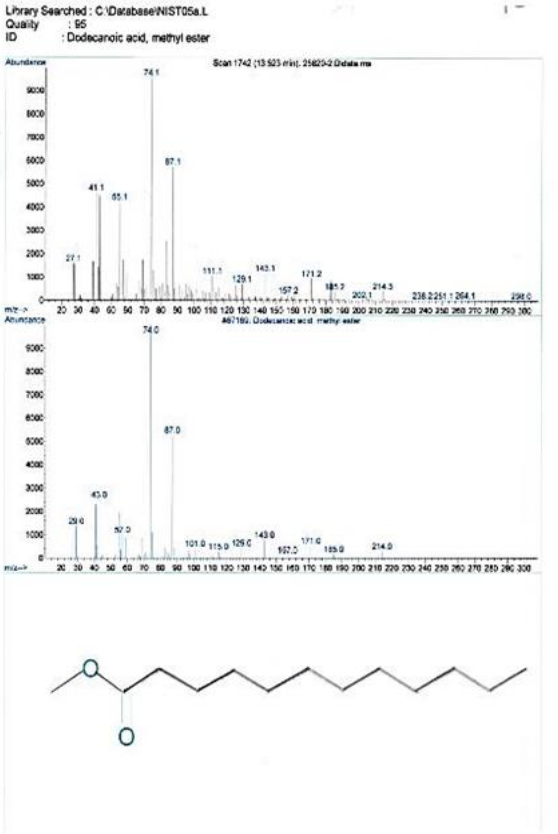
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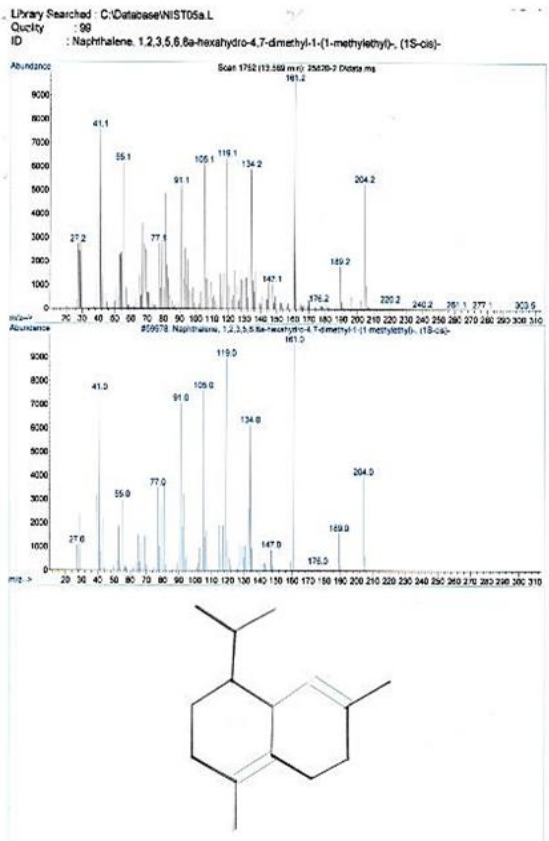
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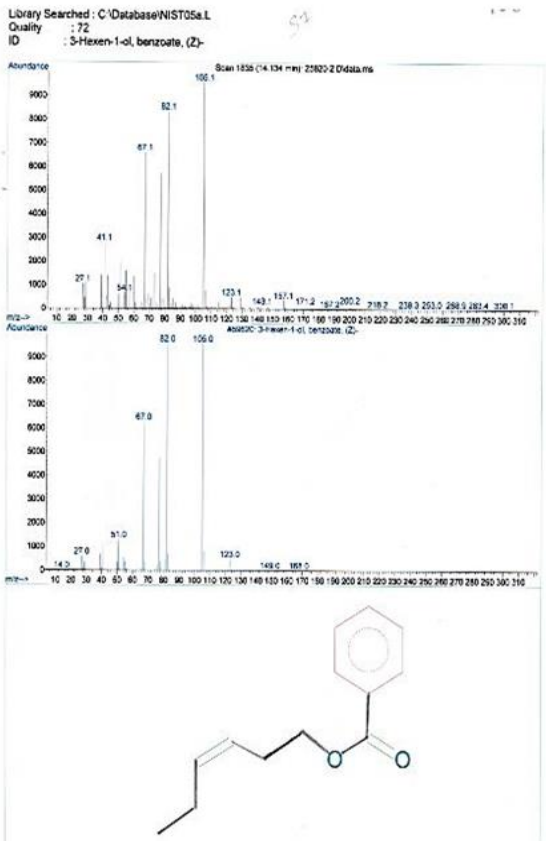
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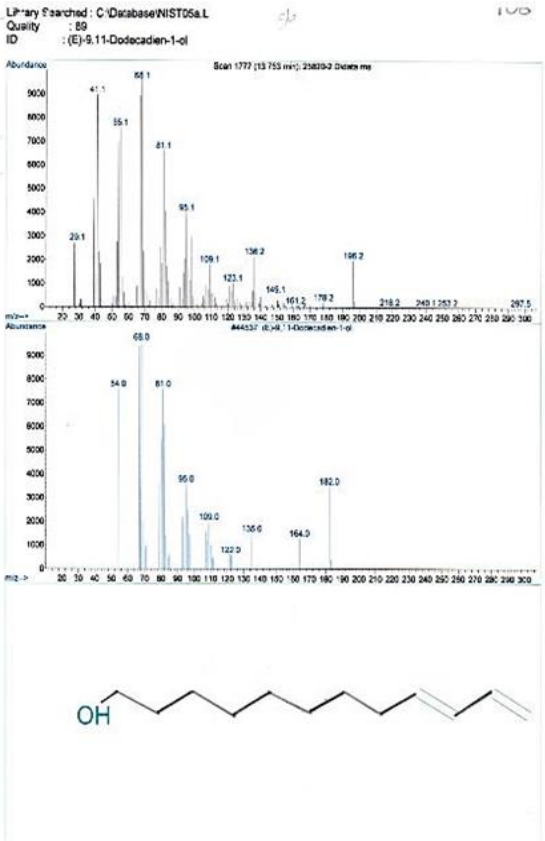
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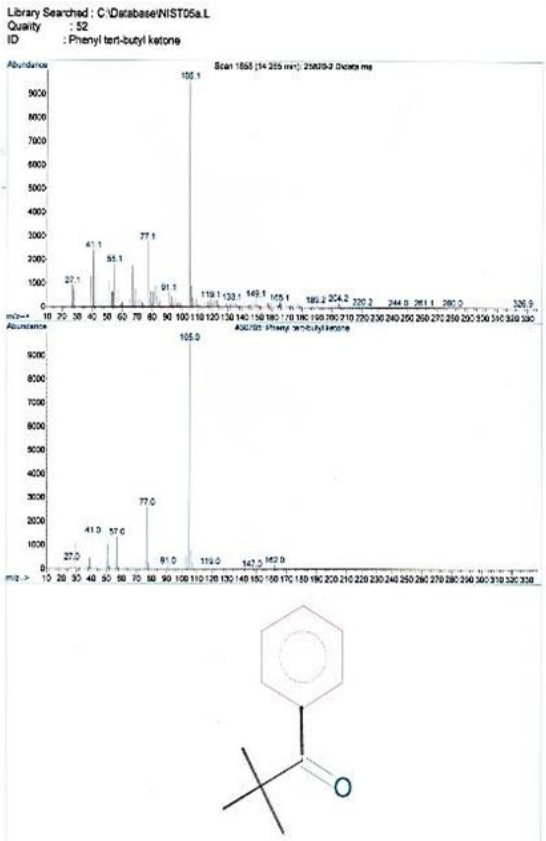
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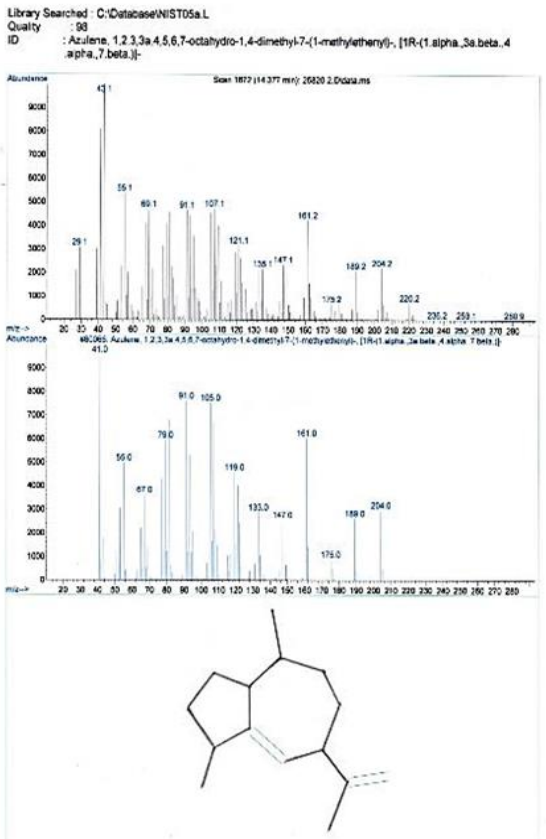
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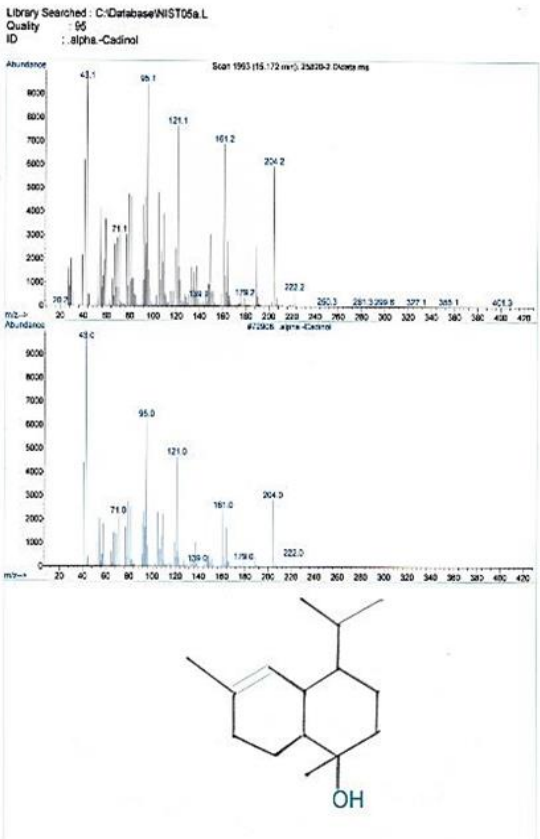
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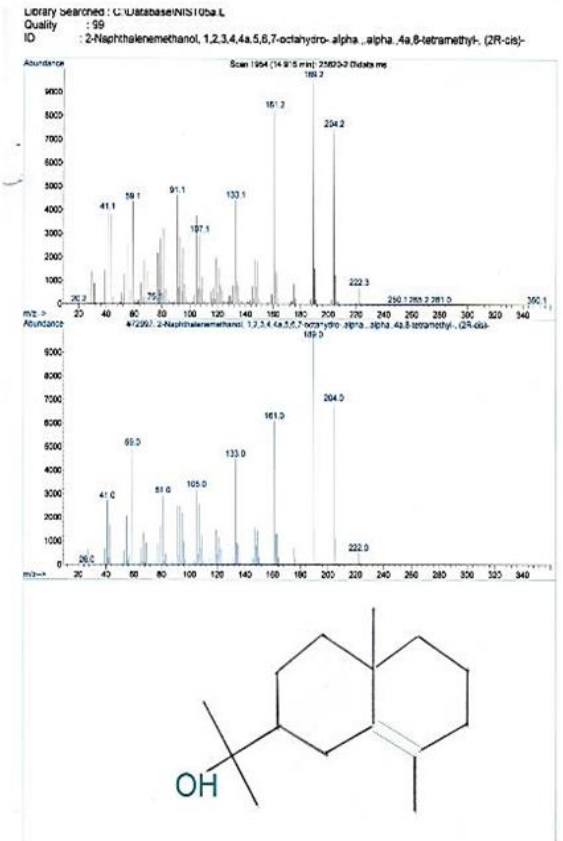
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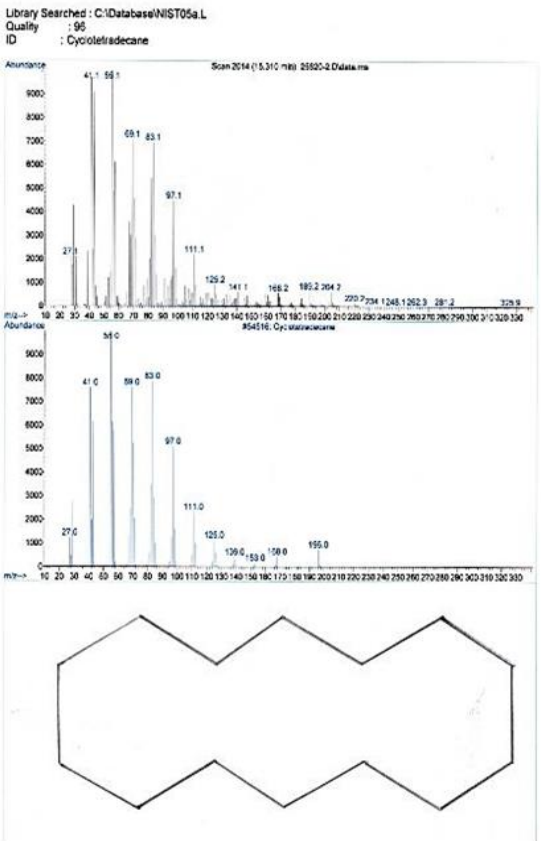
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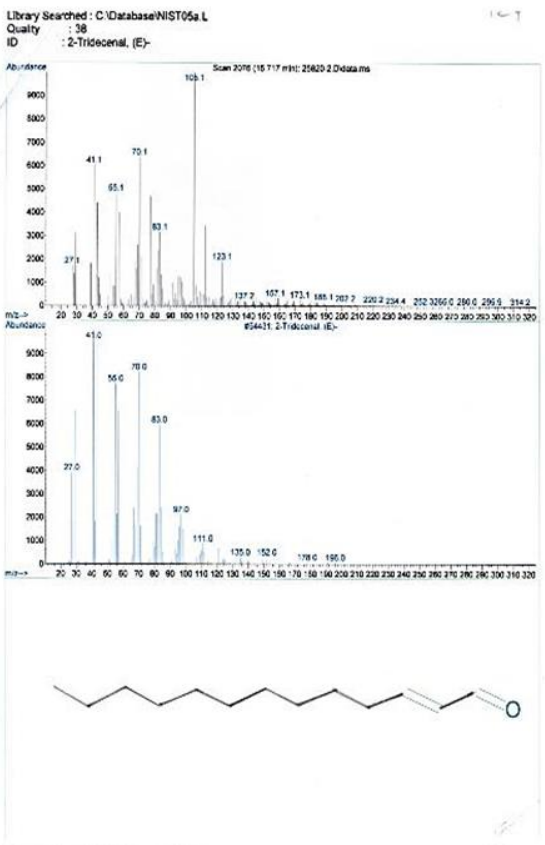
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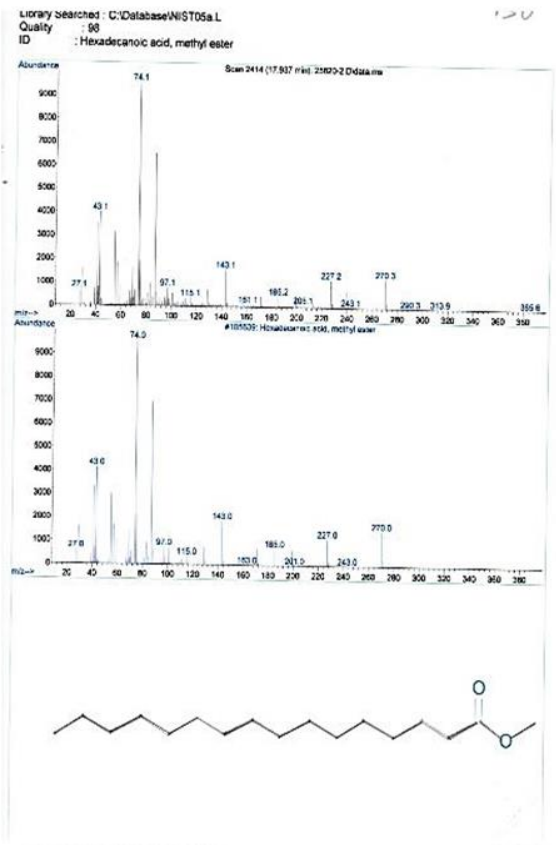
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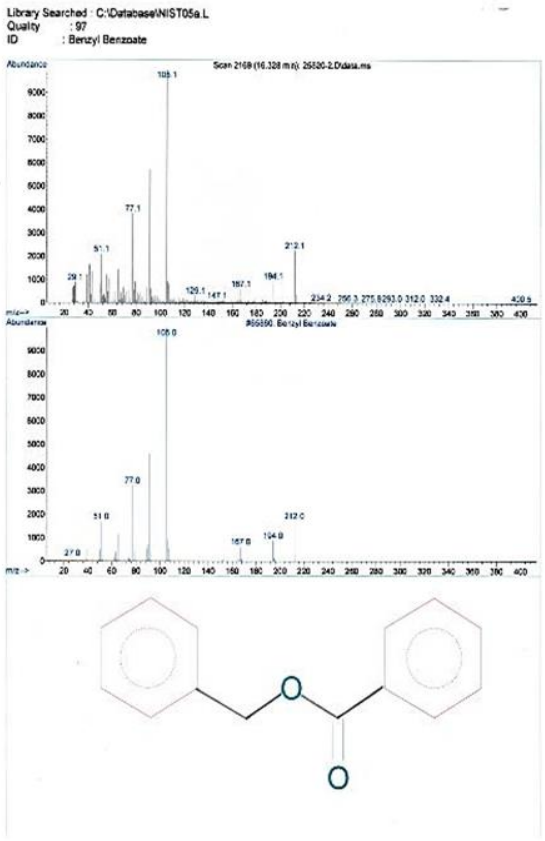
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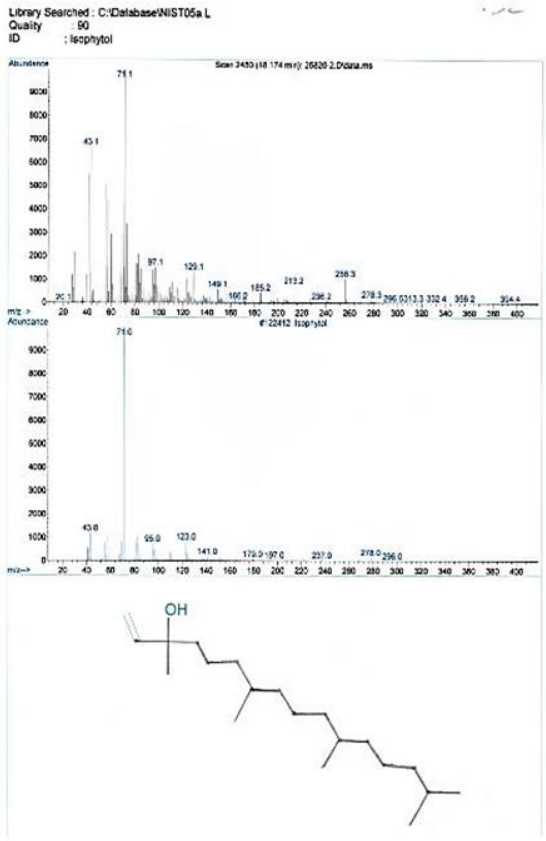
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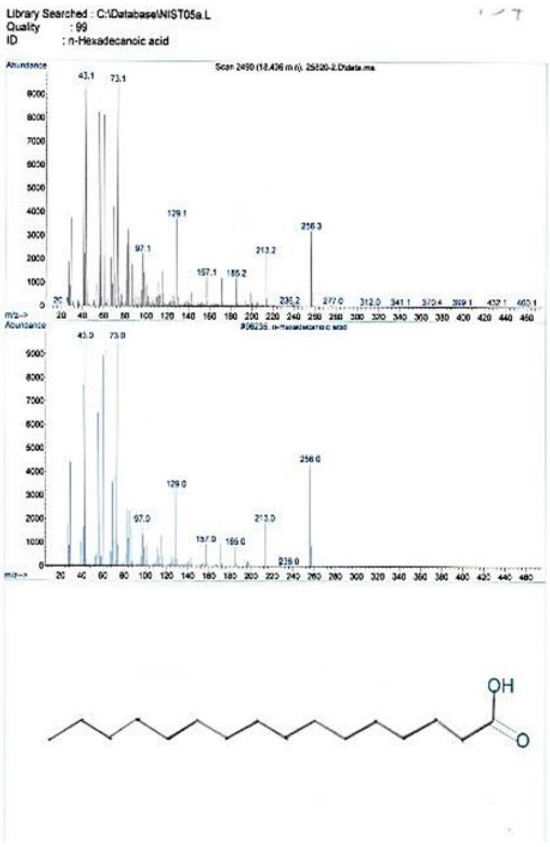
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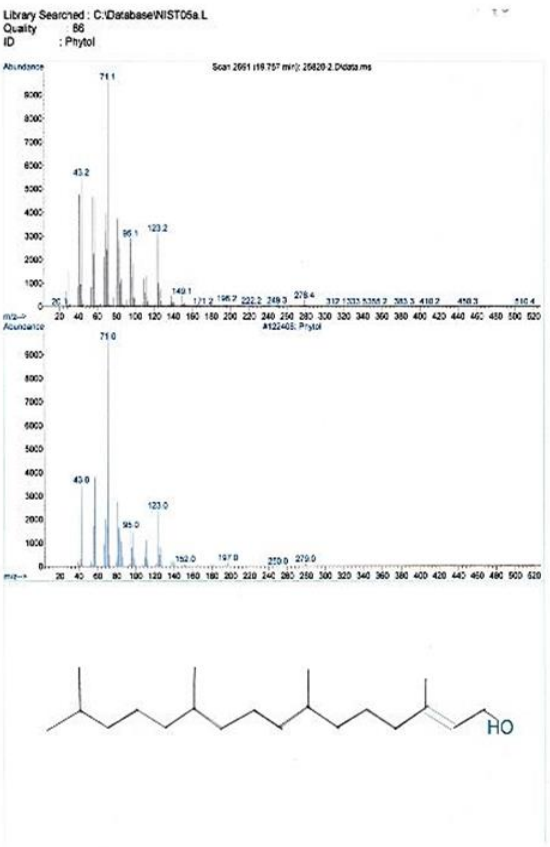
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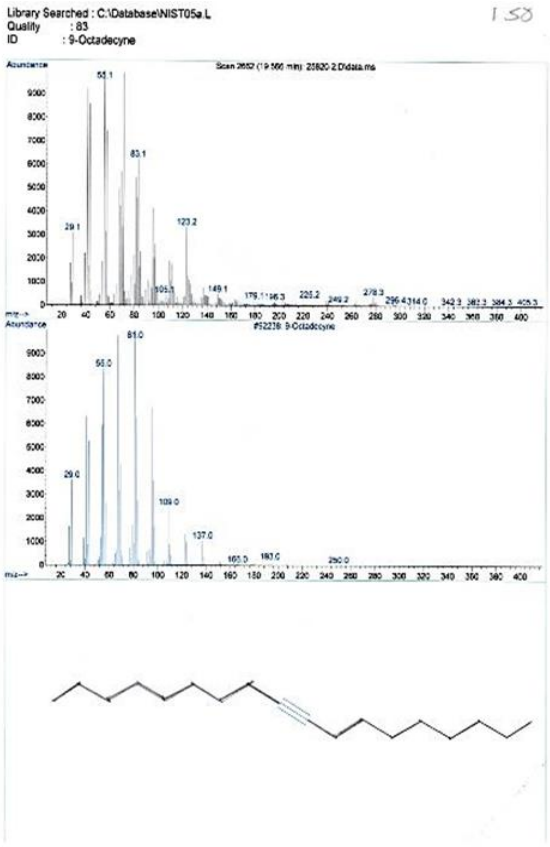
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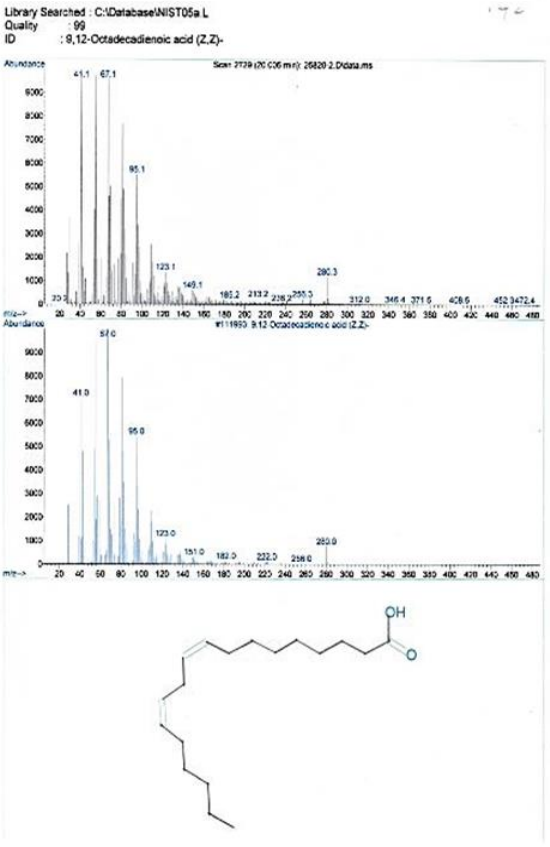
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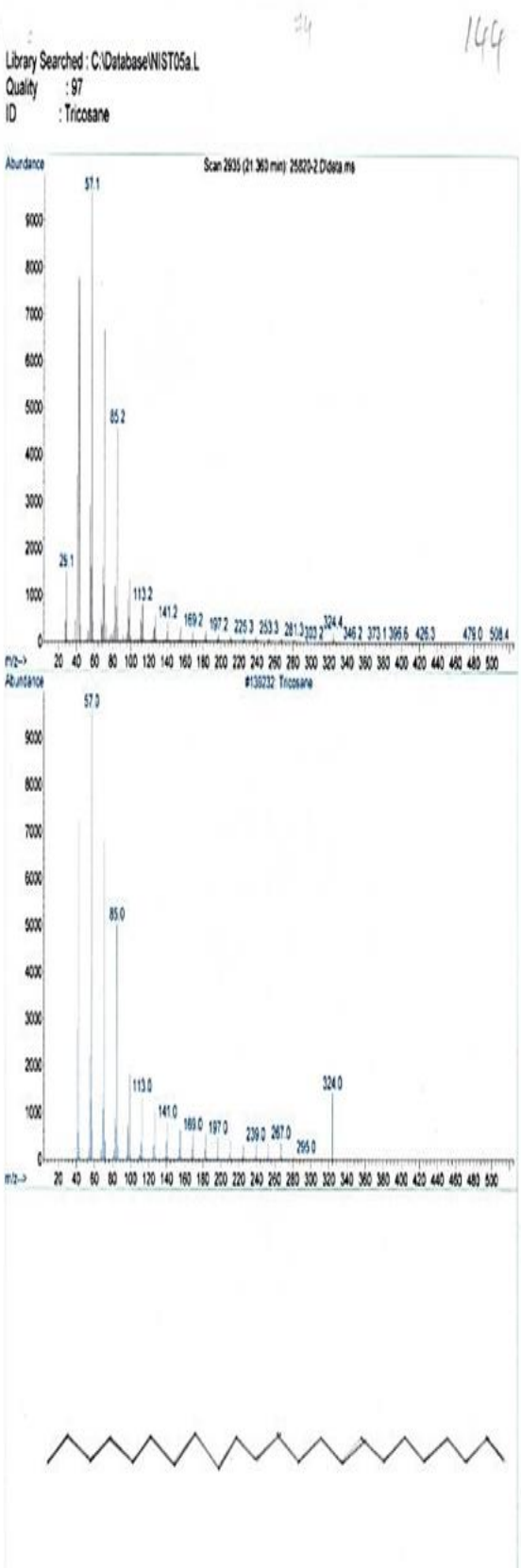
OH
O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Quality : 95
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#152786 Z-12-Pentacosene

Table.2. Activity of Phyto-Components identified in the flower essential oil of *Samadera indica* by GC-MS Dr.Duke's Phytochemical and Ethnobotanical Databases (Online Database)

SL. No	Name of compound	Activity
1.	2-Naphthalene methanol,1,2,3,4,4a, 5,6,7-octahydro-alpha., alpha.,4a,8-tetramethyl-(2R-cis)-	Anti-inflammatory, Cytotoxic,Antimicrobial
2.	Iso-phytol	Terpenes, antioxidants and antimicrobial
3.	Hexadecanoicacid, methyl ester	Antioxidant, hypocholesterolemicnematicide, pesticide, anti-androgenic flavor, haemolytic and 5-alpha reductase inhibitor
4.	Phytol	antidepressant, antiseptic, antispasmodic, expectorant

Figure 4: Phytol

Phytol

Figure 5: GCMS for Phytol

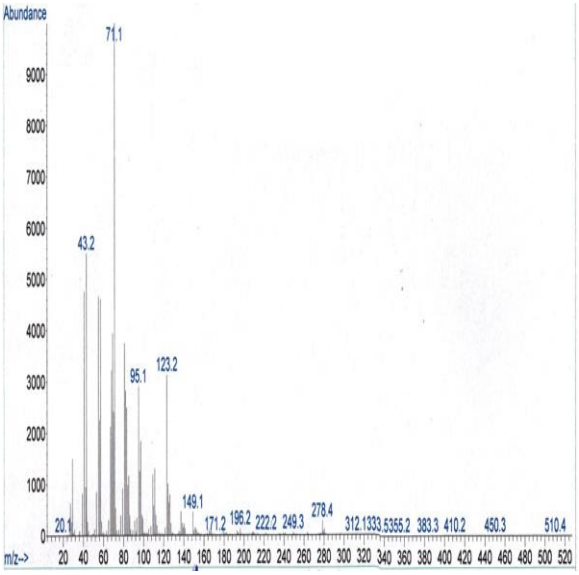


Figure 6: GCMS forflower stalk essential oil of *Samadera indica*

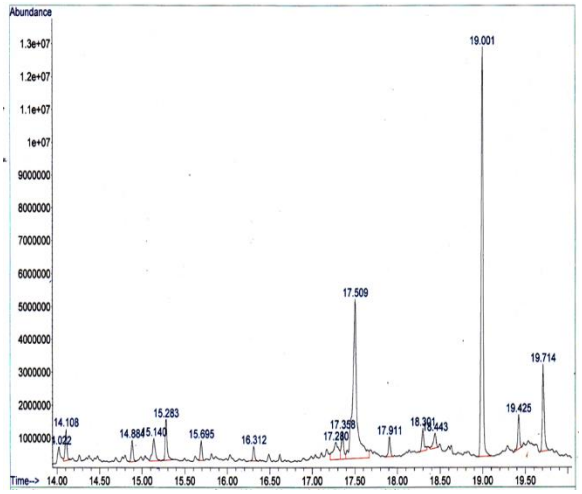
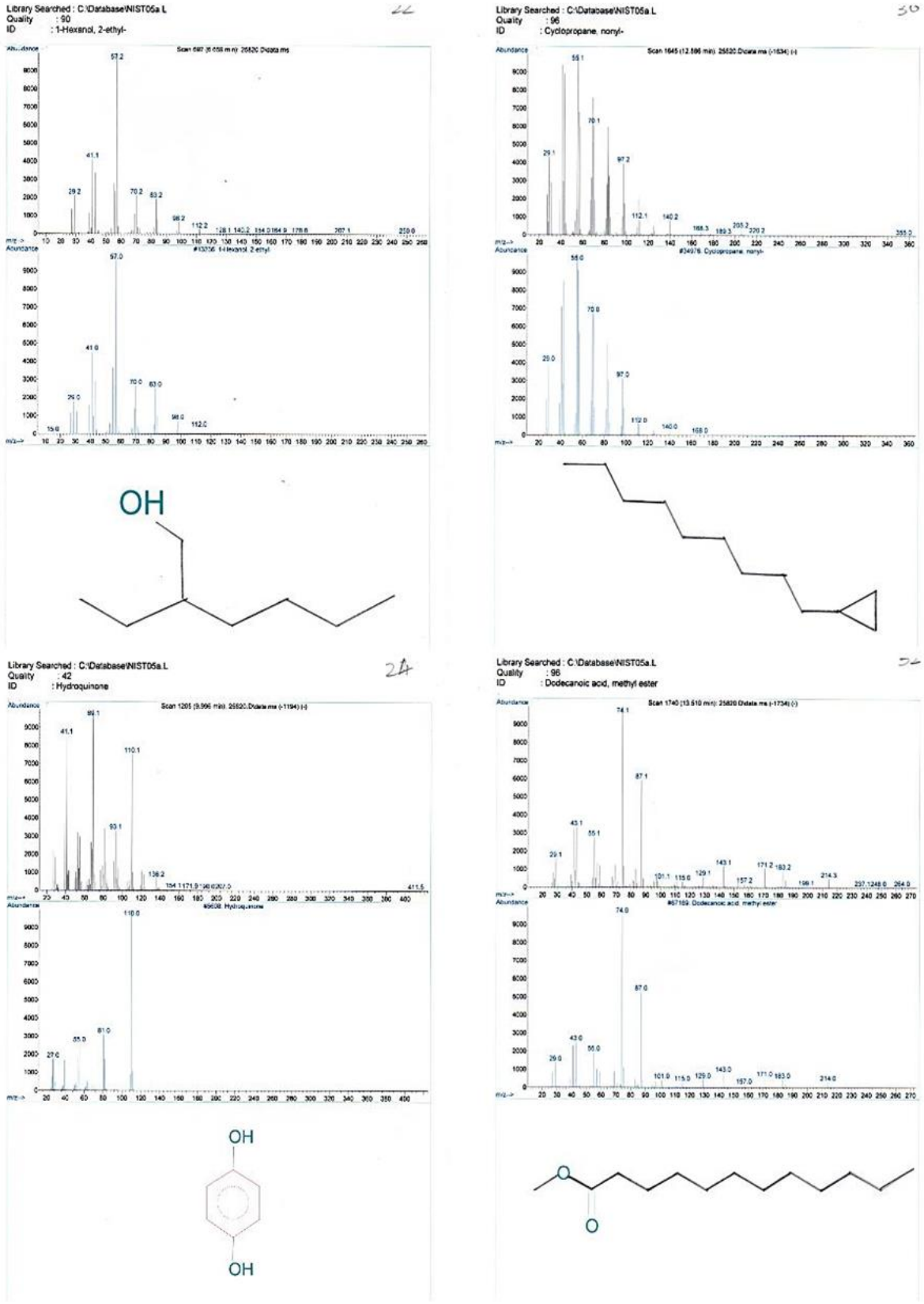
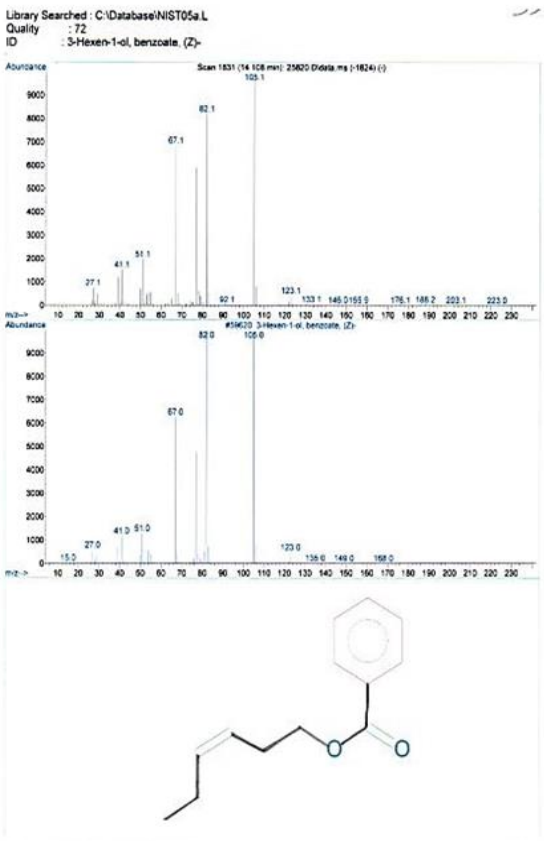


Table 3: Phytochemical present in the flower stalk essential oil of *Samadera indica*

Sl.No	RT	Name of the component	Percentage composition
1	6.659	2-ethyl-1-Hexanol	4.9
2	10.292	Hydroquinone	1.12
3	12.886	nonyl- Cyclopropane	1.5
4	13.510	Dodecanoic acid,methyl ester	0.24
5	14.108	3-Hexen-1-Ol, benzoate, (Z)	0.54
6	14.883	2- Naphthalene methanol,1,2,3,4,4a,5,6,7-Octahydro-alpha., alpha.,4a,8-tetramethyl-(2R- Cis)	0.5
7	15.139	Cyclohexene, 6-ethenyl-6-methyl-1- (1-methylethyl)-3-(1-methyl ethyllidene)-,(S)	0.45
8	15.284	Cycloteradecane	1.7
9	16.315	Benzoic acid,2-ethylhexyl ester	0.43
10	17.353	Benzyl Benzoate	0.68
11	17.504	1,2- Benzenedicarboxylic acid, bis (2-methyl propyl) ester	16.53
12	17.911	Propanil	0.46
13	18.301	Hexadecanoicacid,methyl ester	0.57
14	18.443	Dibutyl phthalate	0.54
15	18.995	1,2-Benzenedicarboxylic acid, butyl methyl ester	66.7
16	19.422	1-Octadecene	0.23
17	19.714	Phytol	2.5

Figure 7: Phytochemical present in the flower stalk essential oil of *Samadera indica*

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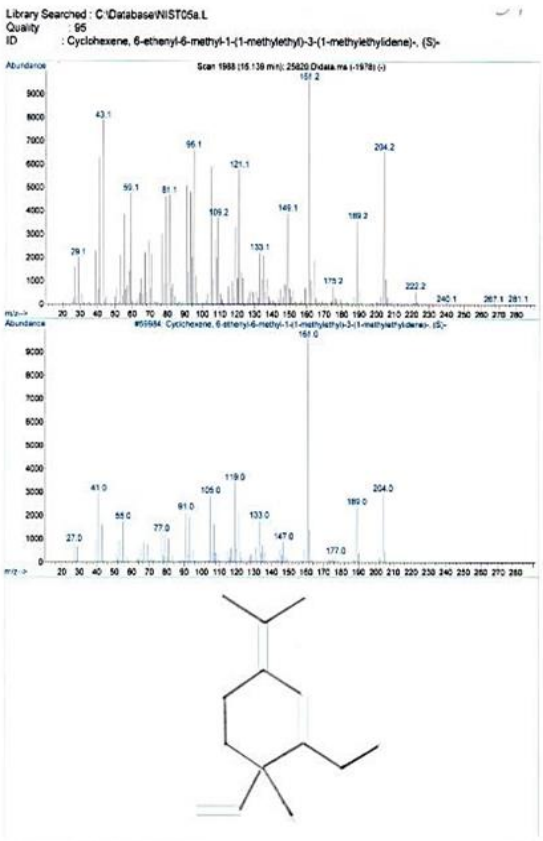
Abundance
m/z-->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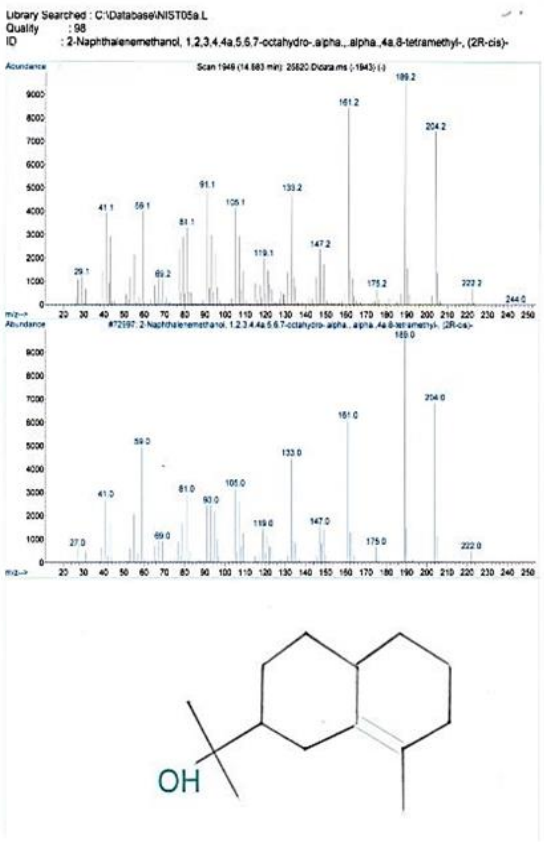
Abundance
m/z-->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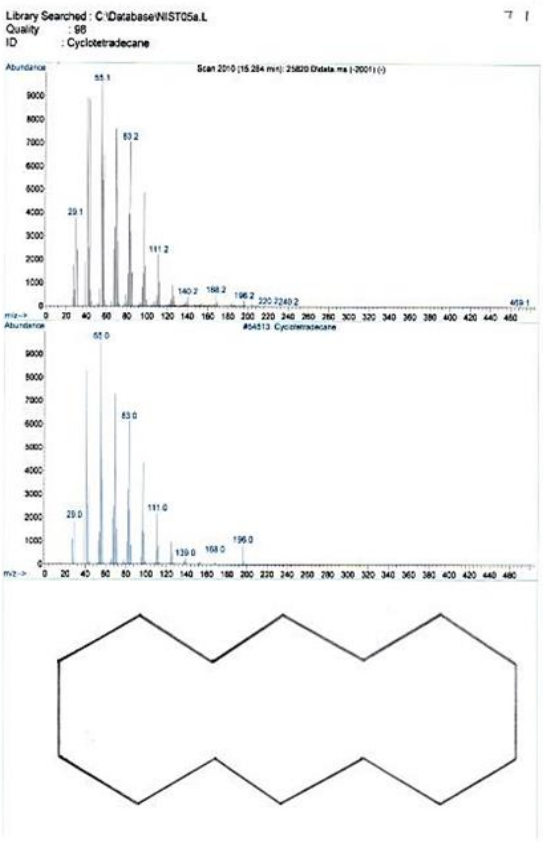
Abundance
m/z-->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Abundance
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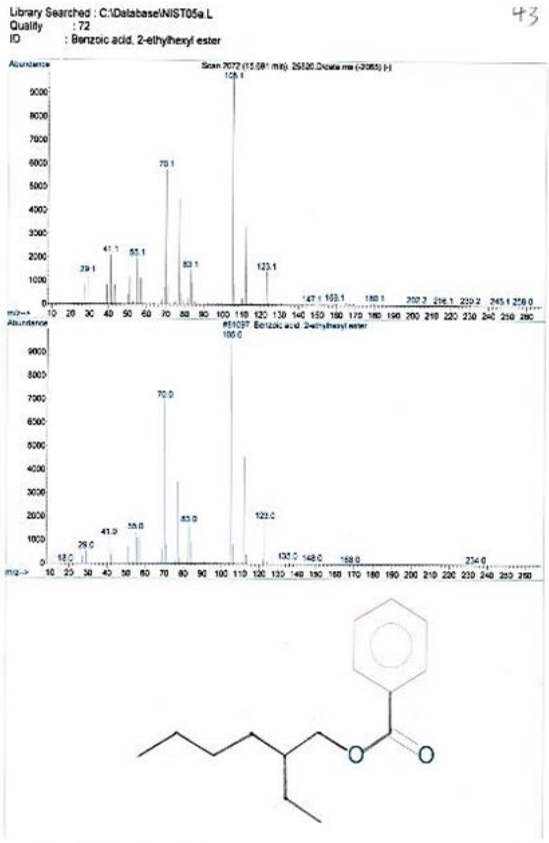
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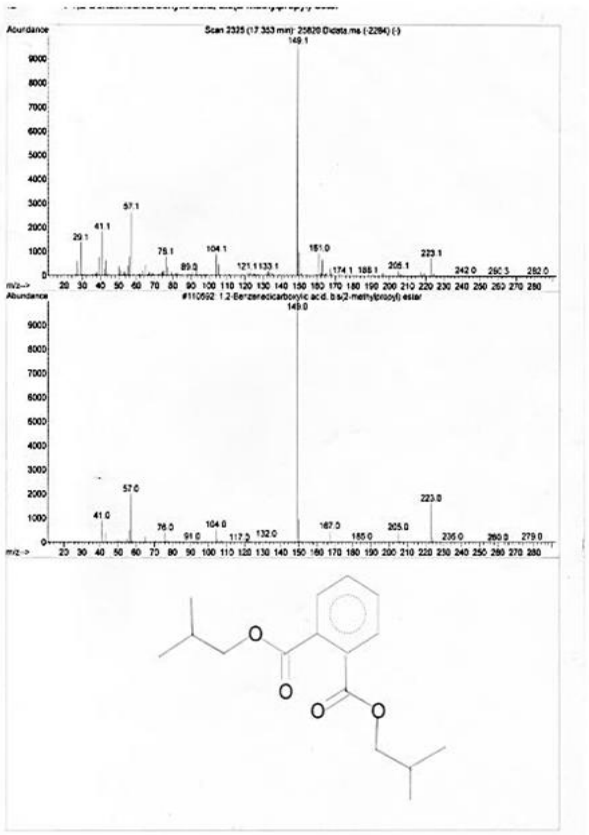

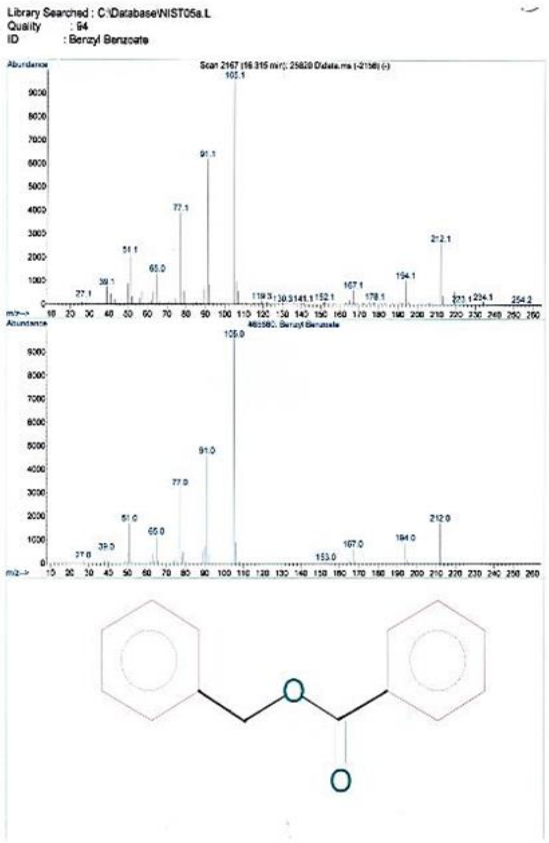
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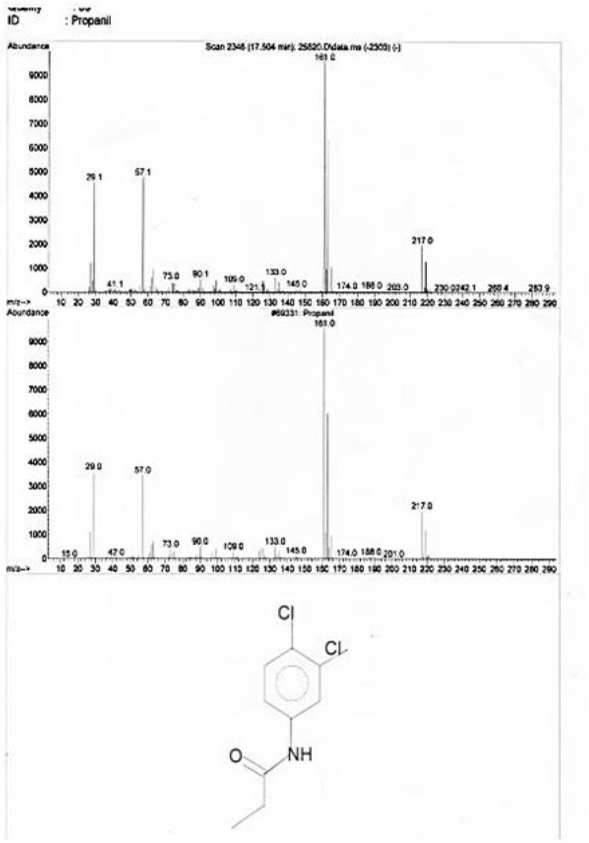
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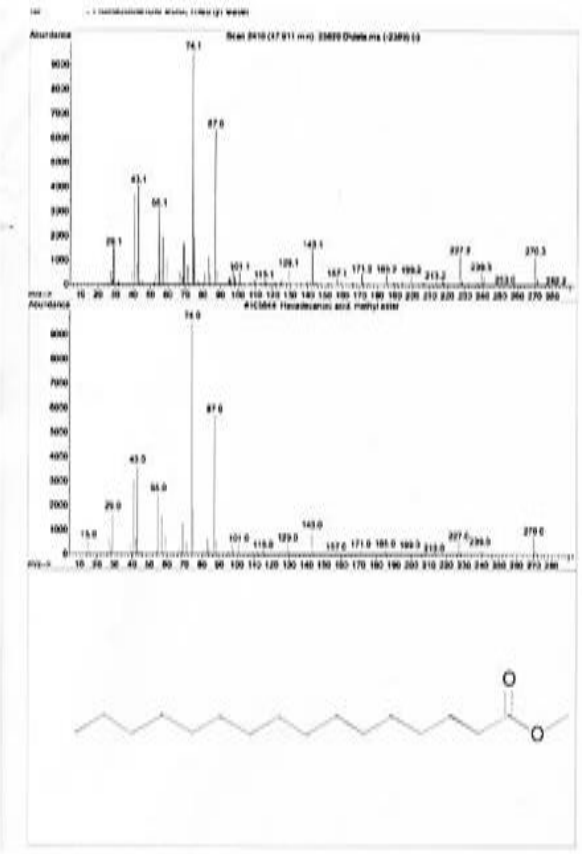

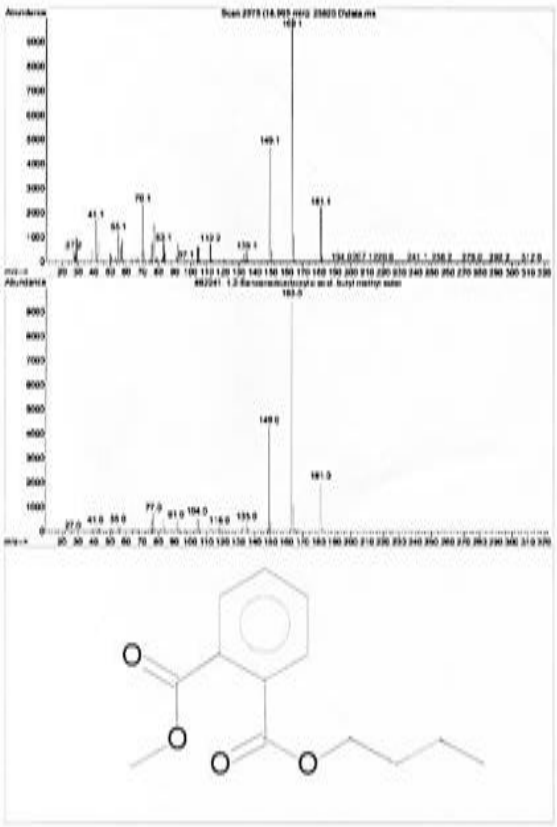

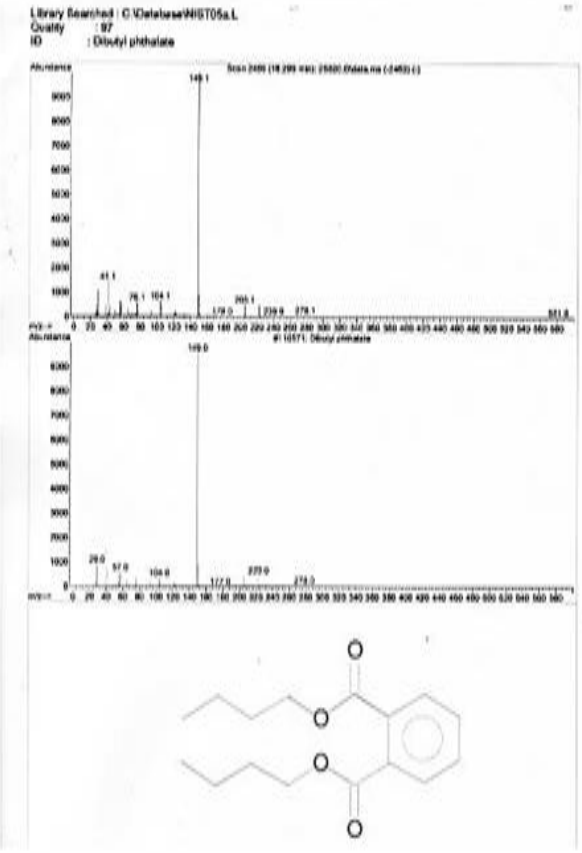
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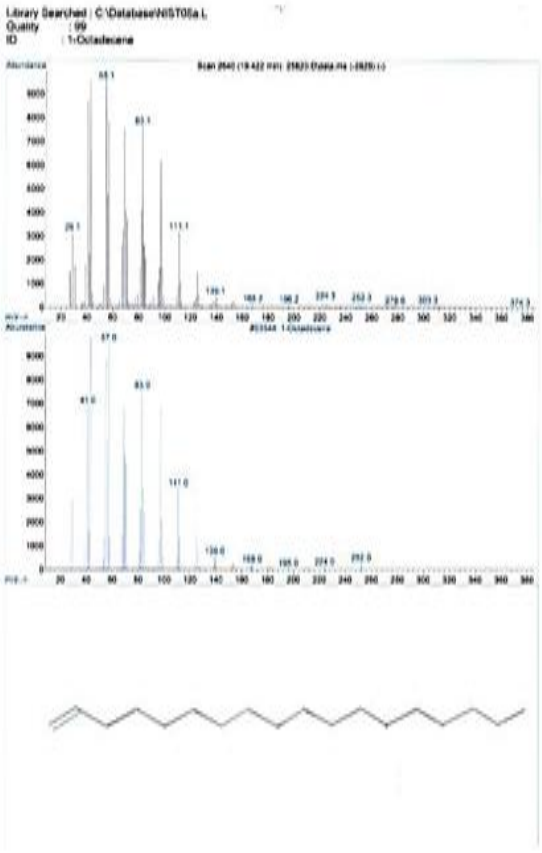
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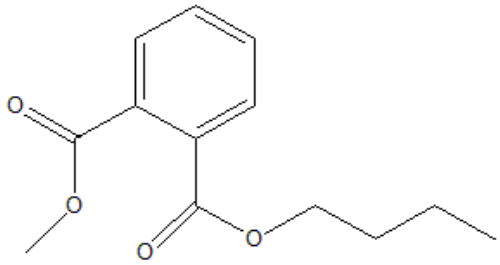
ID : 1-Octadecene

Table 4: Activity of Phyto-Components identified in the flower stalk essential oil of *Samadera indica* by GC-MS Dr.Duke's Phytochemical and Ethnobotanical Databases (Online Database)

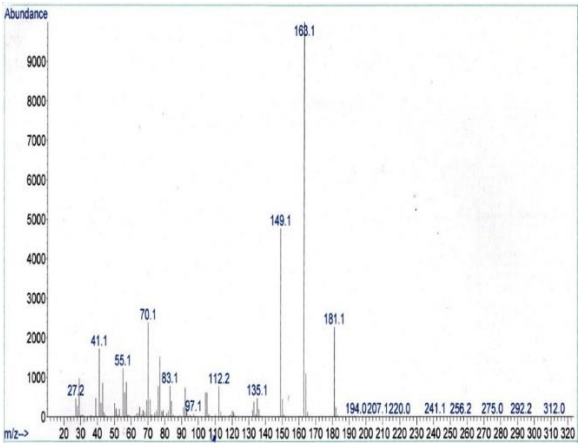
SL.No	Name of compound	Activity
1.	1,2-Benzenedicarboxylic acid, butyl methyl ester	Antimicrobial Antifouling
2.	1,2- Benzenedicarboxylic acid, bis (2-methyl propyl) ester	Antibacterial, Antioxidants
3.	2-ethyl-1-hexanol	fragrant organic compounds, Antibacterial
4.	Phytol	antidepressant, antiseptic, antispasmodic, expectorant

Figure 8: 1,2 – Benzenedicarboxylic acid, butyl methyl ester



1, 2 - Benzenedicarboxylic acid, butyl methyl ester

Figure 9: GCMS for 1,2 – Benzenedicarboxylic acid, butyl methyl ester



CONCLUSION

Phytochemical studies revealed the presence of various secondary metabolites in the flower and flower stalk extracts of *Samadera indica*. The major components of flower essential oil are phytol, 2-Naphthalene methanol,1,2,3,4,4a,5,6,7-octahydro-alpha., alpha., 4a, 8-tetramethyl-(2R-cis)-Hexadecanoic acid,methyl ester,Heptanoic acid, 3-hexenyl ester, (Z)-, n-Hexadecanoic acid, Isophytol, Alpha-Cadinol,Tricosane, 3-Hexen-1-ol, benzoate,(Z). Out of 42 isolated components of flower essential oils, these are the components present in high percentages. It includes esters, phenols as well as terpenoids, which are reported to be antioxidants, antimicrobials and cytotoxic[9,10,11,12]. Samadera *indica* flower stalk essential oil contains 1,2-Benzenedicarboxylic acid butyl methyl ester, 1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester, 2-ethyl-1-Hexanol, phytol and other esters. Out of 17 components identified, these four are the components present in high percentages.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

REFERENCES

[1] K. Husnu Can Baser, Gerhard Buchbauer: Handbook of Essential oils Science, Technology and Applications; Second Edition 2015

[2] Julia Lawless: The Encyclopedia of essential oils; 2013 ISBN 978-1-57324-614-9.

[3] Chien y. Wang, shiow y. Wang, chitsun chen: Increasing Antioxidant Activity and Reducing Decay of Blueberries by Essential Oils. *J. Agric. Food Chem.* 2008, *56*, 3587–3592 3587

[4] Ferrándiz Marcela, Capablanca Lucía, Bou Eva, García David, Bonet Ma Ángeles, Bartolome Luis: Microencapsulation of Essential Oils by Interfacial Polimerization Using Polyurea as a Wall Material. Journal of Encapsulation and Adsorption Sciences, 2015, 5, 165-177

[5] Eristanna Palazzolo, Vito Armando Laudicina and Maria Antonietta Germanà:. Current and Potential Use of Citrus Essential Oils. *Current Organic Chemistry*, 2013, *17*, 3042-3049

[6] Mitsuo Miyazawa, Shinsuke Marumoto, Tomohiro Kobayashi, Shuhei Yoshida and Yuya Utsumi.: Determination of Characteristic Components in Essential Oils from *Wisteria brachybotrys* Using Gas Chromatography - Olfactometry Incremental Dilution Technique Rec. *Nat. Prod*. 5:3 (2011) 221-227

[7] Renan O. Silva, Francisca Beatriz M. Sousa,Samara R.B. Damasceno, Nathalia S. Carvalho, Valdelânia G. Silva, Francisco Rodrigo M.A. Oliveira, Damião P. Sousa, Karoline S. Aragão, André L.R. Barbosa, Rivelilson M. Freitas, Jand Venes R: Medeiros Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundamental & Clinical Pharmacology. Volume 28, Issue 4 August 2014 10.1111/fcp.12049

[8] Sheeja L, D. Lakshmi, Shruthi Bharadwaj and K. Sajidha Parveen: Anticancer activity of phytol purified from *Gracilaria edulis* against human breast cancer cell line (MCF-7), Int j curr sci 2016, 19(4): e 36-46

[9] Camila Carolina de Menezes Patrício Santos, Mirian Stiebbe Salvadori, Vanine Gomes Mota, Luciana Muratori Costa, Antonia Amanda Cardoso de Almeida, Guilherme Antônio Lopes de Oliveira, Jéssica Pereira Costa, Damião Pergentino de Sousa, Rivelilson Mendes de Freitas, and Reinaldo Nóbrega de Almeida: Antinociceptive and Antioxidant Activities of Phytol in Vivoand In Vitro Models. Neuroscience Journal Volume 2013 (2013), Article ID 949452.

[10] Babar Ali1, Naser Ali Al-Wabel, Saiba Shams, Aftab Ahamad, Shah Alam Khan, Firoz Anwar: Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed 2015; 5(8): 601–611.

[11] Cai Y, Luo Q, Sun M and Corke H: Antioxidant activity and Phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences 2004;74, 2157-2184.

[12] Sezik, E., Tumen, G., Kirimer, N., Ozek, T., and Baser, K.H.C: Essential Oil Composition of Four Origanumvulgare Subspecies of Anatolian Origin. J. Ess. Oil Res., 19935: 425-431.