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ORIGINAL ARTICLE **OPEN ACCESS**

Phytoconstituent Analysis of *Nigella Sativa* Seeds using Analytical Techniques

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ABSTRACT
Plants are very precious source in the synthesis of new drugs. India is a "Botanical Garden of the world" and around 2200 species of medicinal plants were identified. In English, the Nigella sativa seed is also called as fennel flower, nutmeg flower, black caraway, Roman coriander. In the present study, analytical technique like GC-MS, FT-IR was applied in analyzing the phyto-compounds, functional groups present in Nigella sativa. From the identified compounds, the literature was collected for its medicinal, therapeutic applications. According to GC-MS study, the compounds having higher peak area percent was 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (43.53%), Hexadecanoic acid, ethyl ester (36.08%), cis-11,14-Eicosadienoic acid, methyl ester (10.88%). Compounds having moderate peak are: Heptadecanoic acid, ethyl ester (1.07%), 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.10%), 8,11-Eicosadienoic acid, methyl ester (1.31%), Thymoquinone (1.33%), Glycerol 1-palmitate(1.66%). Compounds having peak area percent at trace level was: Lupan-3-ol, acetate (0.21%), Campesterol (0.35%), Cholesta-22, 24-dien-5-ol, 4,4-dimethyl- (0.35%), Butyl 9,12-octadecadienoate (0.38%), Tetradecanoic acid, ethyl ester (0.38%), Erucic acid (0.29%), Eicosanoic acid, ethyl ester (0.01%), Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1α,3α,4β,6α)- (0.21%). These compounds exhibit wide applications in the field of medicine, pharmacy, industry. The results of FT-IR shows the presence of carbonyl C=O, C=C group, C-H stretching. The compounds identified were further subjected to NMR prediction studies using software to know about their proton, carbon chemical shifts. Thus, these analytical techniques provide a valid information on the phytocompounds present in Nigella sativa seeds a base for future work.
Keywords: *Nigella sativa, phytocompounds*

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INTRODUCTION

Nigella sativa belonging to a family Ranunculaceae is a annual herbaceous, bushy, self branching plant with white, pale to dark blue flowers. *Nigella sativa* is famous in Muslim communities as they believe it cures diseases except death. The book titled "The cannon of medicine" written by Ibn-sina during 980-1037 exposed historical importance of Black seeds i. e how it stimulates the body's energy, help in the recovery from fatigue [1,2]. *N. sativa* seeds are used as a spice, condiment in different recipes for its aroma, bitter, peppery taste [3]. Oil extracted from *N. sativa* seeds are more beneficial for human health. It acts as a flavoring agent in making bread and pickles. Middle East and very few Asian countries uses *N. sativa* seeds to promote good health against common ailments like fever, common cold, headache, asthma, rheumatoid arthrities, microbial infections, to drive out worms from the intestine [4,5]. *N. sativa* seeds are applied in the treatment of immune, endocrine systems gastro-intestinal disturbances, skin, respiratory, cardiovascular disorders [6-9]. The seeds of *N. sativa comprised* of calcium, iron, and potassium [10]. Black cumin finds its use in dietary modifications, and added as an ingredient in cereal based products [11] to fight against oxidative stress, hyperglycemia, and hypercholesterolemia. Now it's the responsibility of the nutritionists to pay attention towards their health claims and their safety issues [12, 13]. Considering the significance and importance of *N. sativa* seeds it was planned to study the phyto-constituents present in it through GC-MS and also its functional group was assessed through Fourier transform infrared spectrum analysis, chemical shifts of carbon and proton via NMR predictions.

MATERIAL AND METHODS

Gas Chromatography-Mass Spectrophotometry and **Fourier transform infrared analysis.**

Sample preparation

Nigella sativa seed was purchased from local shop at Salem. 25grams of powdered seed was extracted with ethanol. The extracted sample was used for phytochemical analysis through Gas Chromatography-Mass Spectrophotometry . The seed purchased was identified for its botanical name through online sources. Gas Chromatography-Mass Spectrophotometry was performed on a Scion 436-GC Bruker carrying Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl95% Dimethyl poly siloxane), 30m x 0.25mm ID x 0.25m df. The column oven temperature program was as follows: 80°C hold for 2 min, Up to 160°C at the rate of 20°C/min-No hold, Up to 280°C at the rate of 5°C / min-No hold, Up to 300°C at the rate of 20°C/min-10 min hold, Injector temperature 280°C, Total GC running time was 36min. The inlet temperature was set at 280°C, source temperature 250°C; ionization mode, ionization at 70-eV ionization energy; For single scan analysis, the scan range was set from m/z 40 to 600; Solvent Delay: 0-3.5 min; and the injection volume was 2µl. The GC-MS/MS was performed by Institute of crop processing technology, Tanjavur.

For FT-IR analysis, the sample was ground in a mortar to reduce the average particle size to 1 to 2 microns. About 0.1mg of finely ground sample was mixed with ground potassium bromide . This mixture was then placed onto the face of a KBr plate, and the second window is placed on top. With a gentle circular and back-and-forth rubbing motion of the two windows, evenly distribute the mixture between the plates. The mixture should appear slightly translucent, when properly prepared. Place the sandwiched plates in the spectrometer and obtain a spectrum. The Fourier transform infrared spectrum was recorded using Bruker Tensor 27 spectrometer in the wavelength range 400-4000 cm^{-1} by potassium bromide pellet technique with a resolution and scanning speed of 4 cm^{-1} and 2 mm/sec respectively.

NMR prediction studies

^{1}H NMR, ^{13}C NMR was studied using chem Ultra software 12.0.

RESULTS AND DISCUSSION

Table.1. Components in the ethanol extract of *Nigella sativa* seeds

S.No	RT	Name of the compound	Molecular Fomulae	MW	Peak area %
1	4.30	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1α,3α,4β,6α)-	$C_{10}H_{18}O$	154	0.21
2	6.30	Thymoquinone	$C_{10}H_{12}O_2$	164	1.33
3	7.02	Thymol	$C_{10}H_{14}O$	150	0.31
4	8.85	Longifolene	$C_{15}H_{24}$	204	0.35
5	10.60	p-tert-Butyl catechol	$C_{10}H_{14}O_2$	166	0.21
6	13.49	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.38
7	16.01	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	36.08
8	18.55	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	43.53
9	21.78	cis-11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	10.88
10	22.16	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298	1.07
11	23.82	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330	1.66
12	24.49	Erucic acid	$C_{22}H_{42}O_2$	338	0.29
13	24.90	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340	0.01
14	25.83	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354	1.10
15	26.49	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336	0.38
16	29.08	8,11-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	1.31
17	33.41	Lupan-3-ol, acetate	$C_{32}H_{54}O_2$	470	0.21
18	35.13	Campesterol	$C_{28}H_{48}O$	400	0.35
19	35.83	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	$C_{29}H_{48}O$	412	0.35

MF- Molecular Formulae, MW- Molecular Weight, PA-Peak Area percent

Totally 19 compounds were identified using gas chromatography and mass spectrophotometry analysis. The retention time is the time it takes for a compound to travel from the injection port to the detector. Efficiency in separation of compounds depends on the compounds passing through the column at

different rates. The total retention time of the *Nigella sativa* was 35.13. The identified compounds were classified according to their peaks. The peaks were found to be high, moderate, low, very low for certain compounds. The following identified compounds were found to have higher peaks: 9,12-Octadecadienoic acid (Z,Z)-, methyl ester having peak value of 43.53% and its retention time was 18.55. The next compound having higher peak was Hexadecanoic acid, ethyl ester with 36.08% as peak area percent, retention time of 16.01. The compound with moderate peak value was cis-11,14-Eicosadienoic acid, methyl ester 10.88% showing retention time of 21.78. The compounds such as Heptadecanoic acid, ethyl ester (1.07%), 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.10), 8,11-Eicosadienoic acid, methyl ester (1.31), Thymoquinone (1.33), Glycerol 1-palmitate (1.66) show peak area percent in the range of 1.07 to 1.66. The remaining compounds show peak area percent in very low levels i.e in trace amounts. They are as follows: Erucic acid (0.29), Lupan-3-ol, acetate (0.21%), p-tert-Butyl catechol (0.21%), Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1α,3α,4β,6α)- (0.21%), Thymol (0.31 %), Tetradecanoic acid, ethyl ester(0.38%), Eicosanoic acid, ethyl ester (0.01%), Butyl 9,12-octadecadienoate (0.38%), Campesterol (0.35%), Cholesta-22,24-dien-5-ol, 4,4-dimethyl- (0.35%).

The molecular formulae and molecular weight of the identified compound are: Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1α,3α,4β,6α)-$C_{10}H_{18}O$-154, Thymoquinone - $C_{10}H_{12}O_2$- 164, Thymol- $C_{10}H_{14}O$-150, Longifolene - $C_{15}H_{24}$-204, p-tert-Butyl catechol- $C_{10}H_{14}O_2$-166, Tetradecanoic acid, ethyl ester - $C_{16}H_{32}O_2$-256, Hexadecanoic acid, ethyl ester- $C_{18}H_{36}O_2$- 284, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester- $C_{19}H_{34}O_2$-294, cis-11,14-Eicosadienoic acid, methyl ester - $C_{21}H_{38}O_2$-322, Heptadecanoic acid, ethyl ester- $C_{19}H_{38}O_2$-298, Glycerol 1-palmitate - $C_{19}H_{38}O_4$- 330, Erucic acid - $C_{22}H_{42}O_2$-338, Eicosanoic acid, ethyl ester - $C_{22}H_{44}O_2$- 340, 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester - $C_{21}H_{38}O_4$ – 354, Butyl 9,12-octadecadienoate- $C_{22}H_{40}O_2$- 336, 8,11-Eicosadienoic acid, methyl ester - $C_{21}H_{38}O_2$- 322, Lupan-3-ol, acetate - $C_{32}H_{54}O_2$ – 470, Campesterol - $C_{28}H_{48}O$ – 400, Cholesta-22,24-dien-5-ol, 4,4-dimethyl- $C_{29}H_{48}O$ – 412.

The pharmaceutical application of the compounds identified is: Hexadecanoic acid, methyl ester was reported for its antifungal, antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavour, haemolytic, 5-alpha reductase inhibitor, antimicrobial activity [14]. Cholesta-22, 24-dien-5-ol, 4,4-dimethyl was found to be useful in the treatment of antimicrobial anti-inflammatory, anticancer, diuretic, antiarthritic, antiasthmatic diseases [15]. Campesterol have anticarcinogenic activity against prostate [16], lung [17] and breast [18, 19] cancers. Erucic acid commonly called as cis-13-docosenoic acid is a very-longchain monounsaturated fatty acid was used as a raw material in the oleo chemical industry. Likewise, longifolene finds application in making synthetic resins, organic chemicals, perfumes and flotation oils. It is also used to synthesise isolongifolene, isolongifolanone. Thymol is a phenol used as an antiseptic and disinfectant. Thymoquinone forms a very good anti-oxidant, anti-inflammatory, immunomodulatory, anti-histaminic, anti-microbial and anti-tumor agent found to have gastro, hepato, nephro, neuroprotective effect. It is also involved in the treatment of diabetes, reproductive, respiratory, cardiovascular disorders as well as in bone complications, fibrosis.

Fig.1 GC-MS Chromatogram

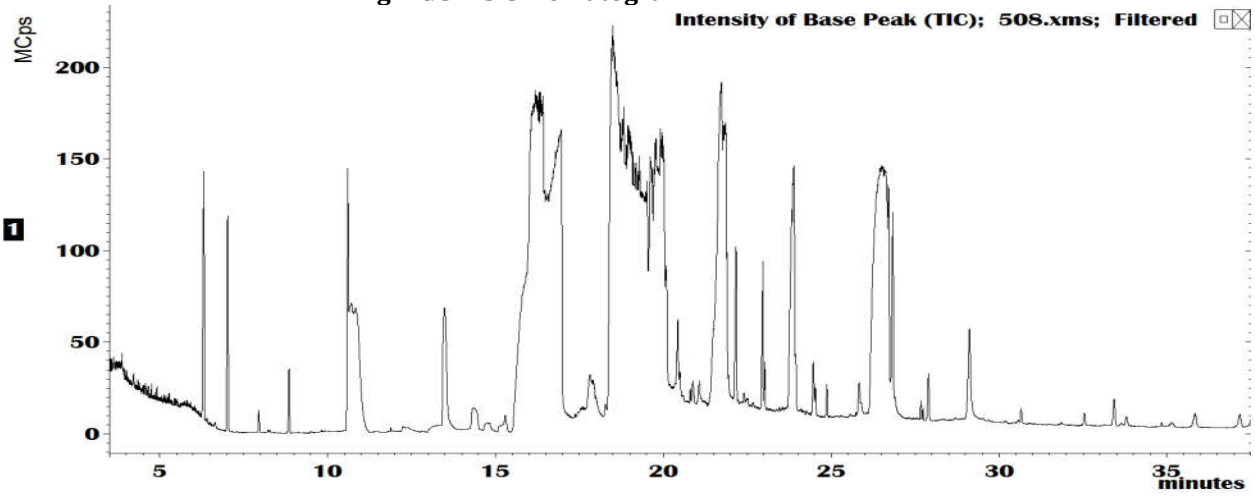
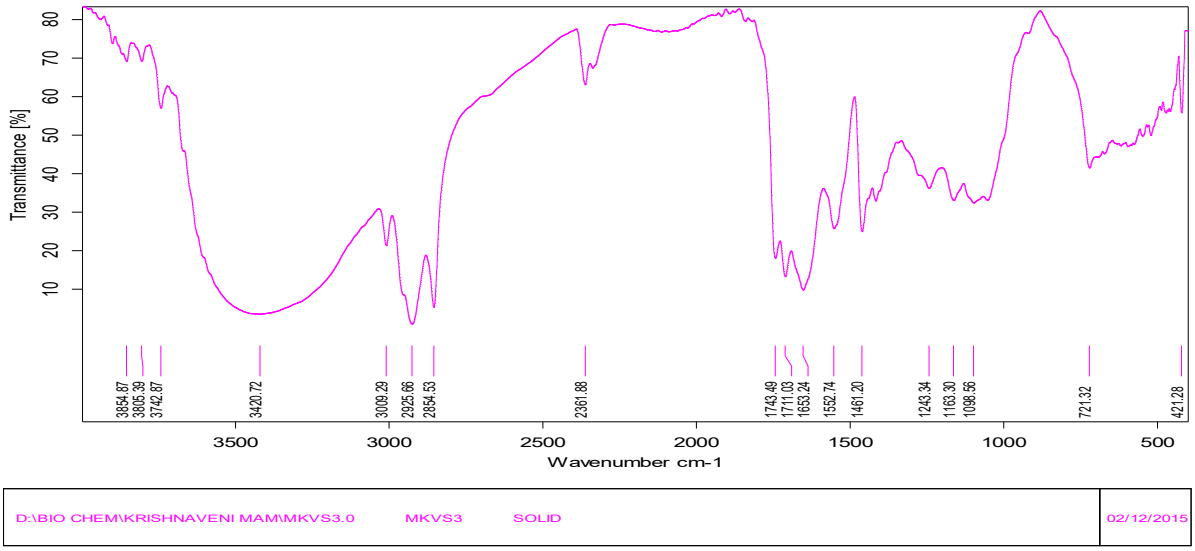


Fig.2 FT-IR Spectrum of *Nigella sativa* seed powder

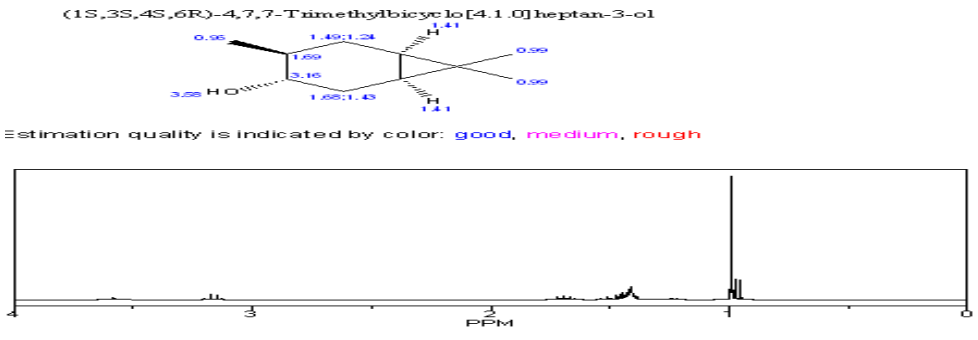


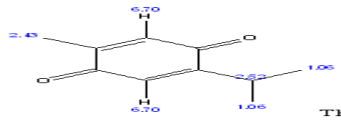
The Fourier Transform Infrared spectroscopy analysis gives significant quantity of compositional, structural information in plants. Infra-Red spectroscopy is fundamentally a vibrational spectrum involving the wavelength measurement and intensity of absorption of mid infrared light by a sample. The principle value of this method speaks about the detection of bands present in organic molecules. As different bands have special vibrational frequency, the occurrence of bands is detected by identifying the typical vibrational frequencies via absorption band in the IR spectrum. The peak at 3742.87 cm^{-1} shows the presence of functional group phenol. The peak at 3009.29 cm^{-1} shows N-H stretching of aminoacids as well as alkenes. likewise peak at 2854.53 cm^{-1} depicts C-H stretching and also alkenes, peak at 2924.94 cm^{-1} shows C-H group, peak at 2361.88 cm^{-1} shows some unknown compound, peak at 1743.49 depicts carbonyl C=O group, the peak at 1711.03cm-1 shows aldehyde, peak at 1653.24 cm^{-1} shows stretching vibration of C-OH bond from proteins and alkene, the peak at 1163.30 cm^{-1} shows nitro compounds. The peak at 1243.34 cm^{-1} shows secondary alcohol. The peak at 1461.20 cm^{-1} shows nitrogen, The bands at 2925.66 cm-1 and 2854.53 cm-1 give –CH2 and C-H stretching mode in alkanes. FT-IR spectroscopy is a well-known time-saving method to characterize and identify functional groups [20].

NMR prediction studies

^{1}H NMR

The ^{1}H NMR spectrum of an organic compound provides information on the structure of unknown organic compounds. Likewise, the ^{13}C NMR spectrum provide information on the carbon skeleton not just the proton attached to it, as 1.1 % of naturally occurring carbon is ^{13}C. The following are the results of ^{1}H NMR spectrum for the 19 compounds identified through GC-MS. In the ^{1}H NMR chemical shift of hydrogen occurs due to the electron distribution in the molecule. The movement of electrons produce a small magnetic field that affect the net field experienced by each hydrogen nucleus. The chemical shift observed in most of the identified compounds are discussed below: The chemical shift ranging from 0.8-1.5 ppm, 1- 5 ppm shows the presence of alkane C-H, alcohol OH amine NH and the chemical shift in higher range of 2.4-4.5 ppm represents more electronegativeatom. Likewise, the chemical shift ranging from 6.5-8.5 ppm shows aromatic nature. Terminal alkyne hydrogens have chemical shifts in 2.3-3.0 ppm range. Similarly, the chemical shift in the range of 4.5-6.5 ppm shows alkene =CH.





Thymoquinone

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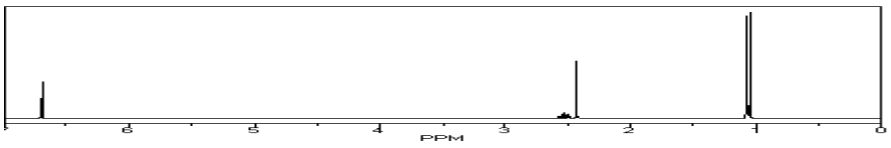

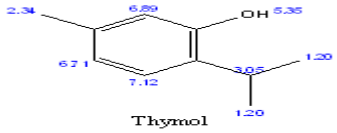

Thymol

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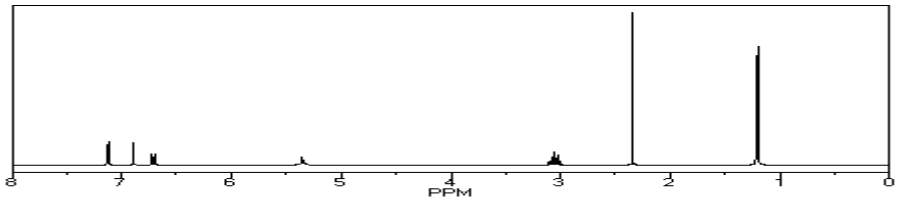
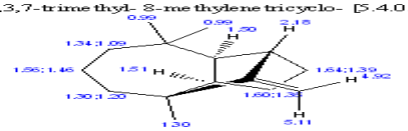


(1R,2S,7S,9S)- 3,3,7-trimethyl- 8-methylene tricyclo- [5.4.0.02,9] undecane



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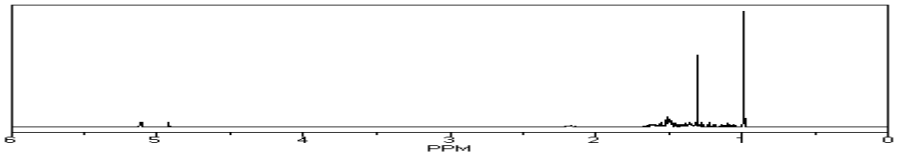
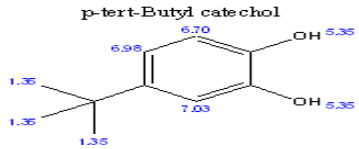


p-tert-Butyl catechol



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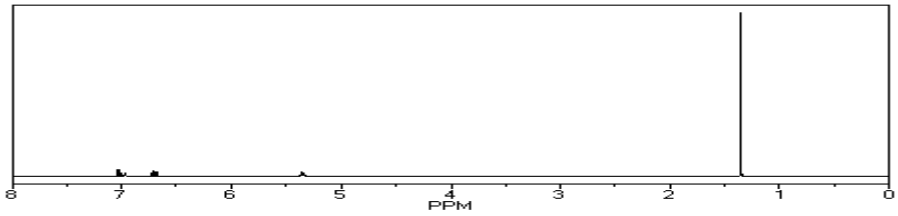

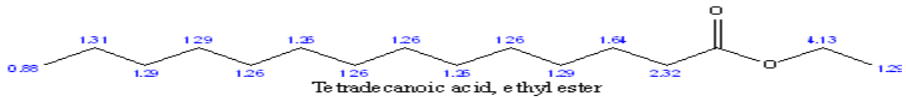

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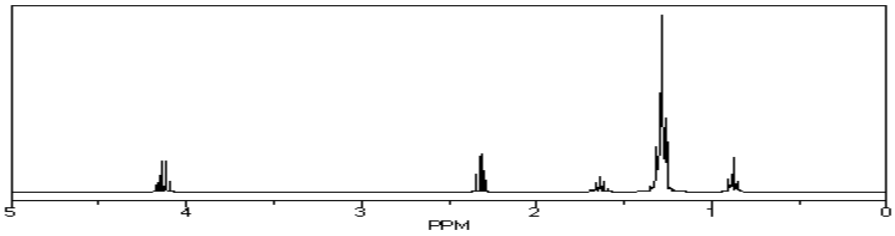

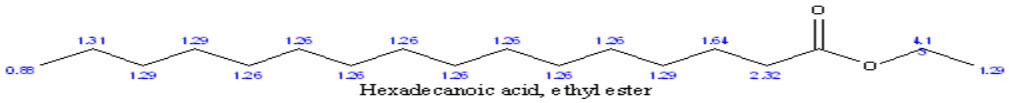

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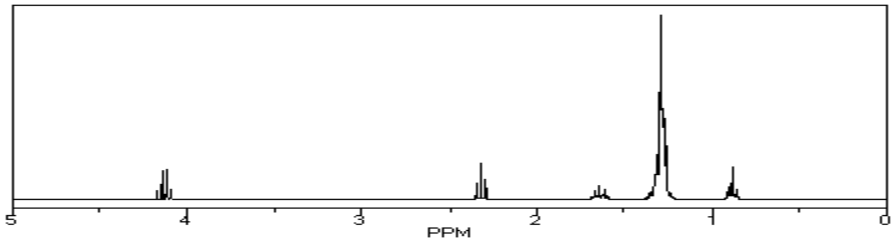

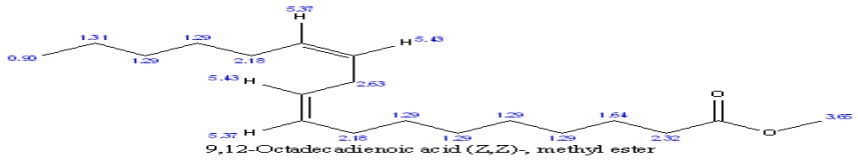

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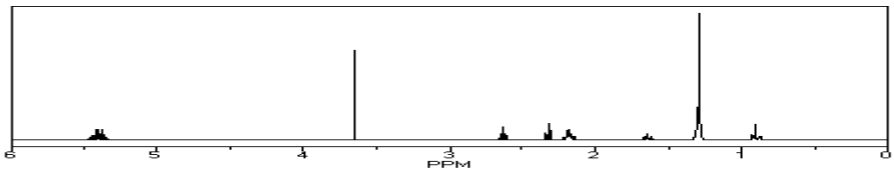

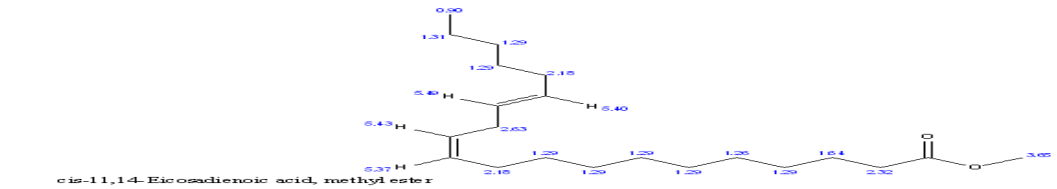

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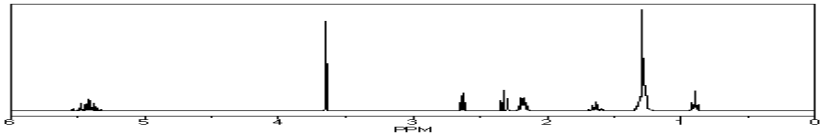

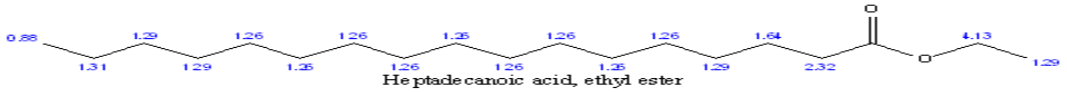

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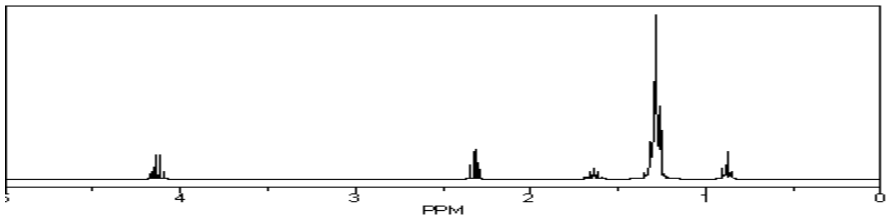

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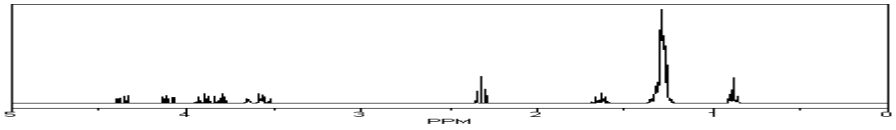

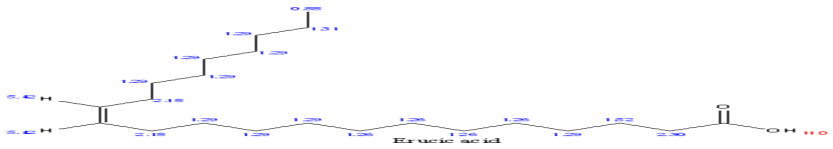

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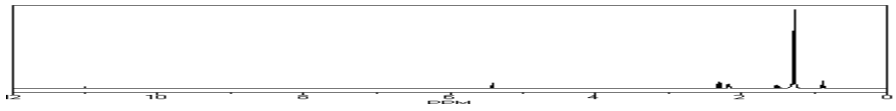

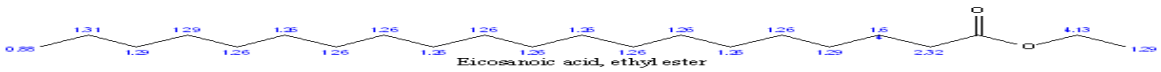

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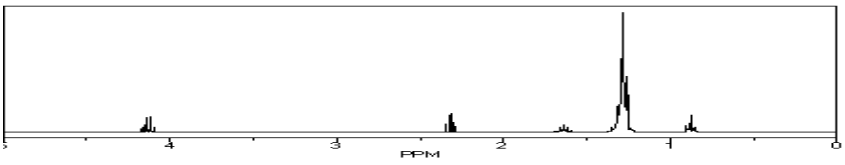

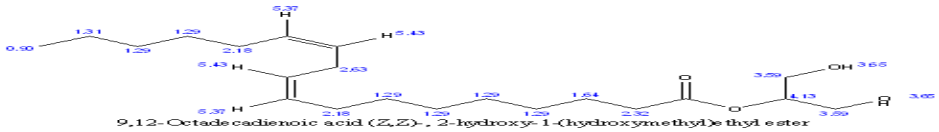

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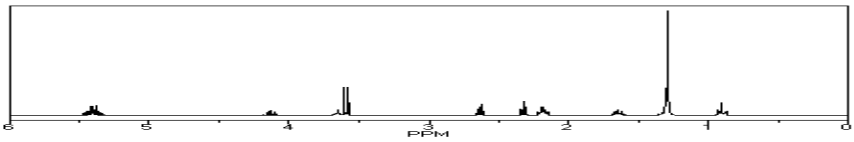

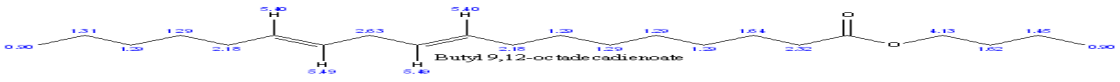

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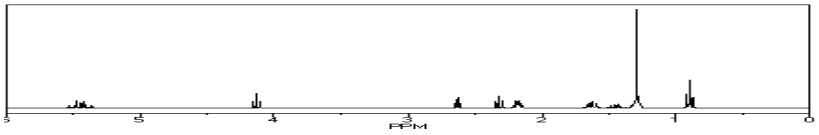

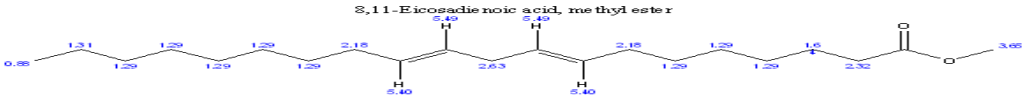

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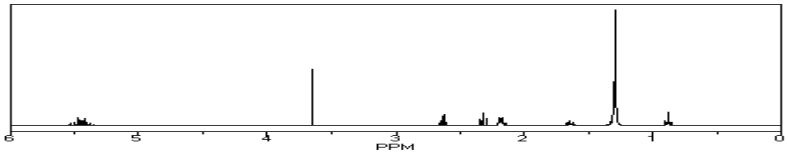

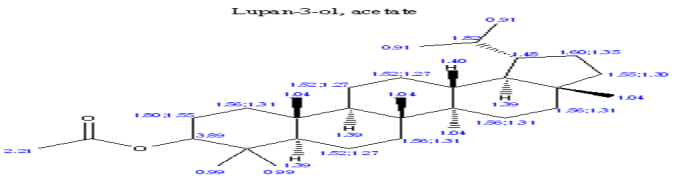

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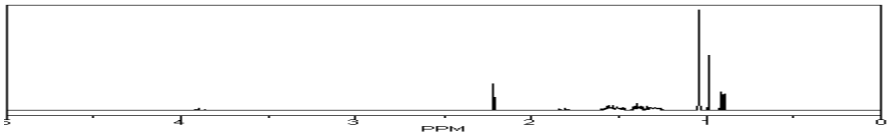

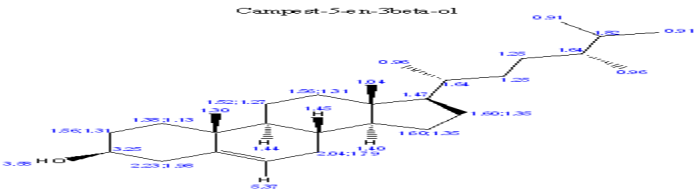

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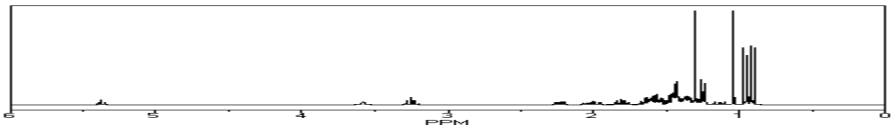

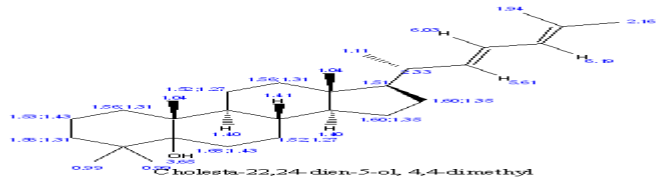

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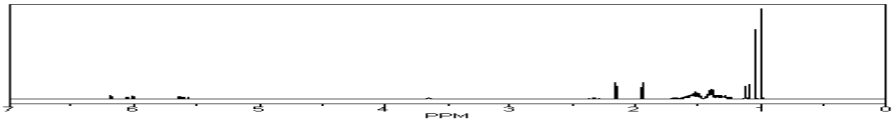
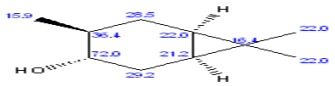


^{13}C NMR

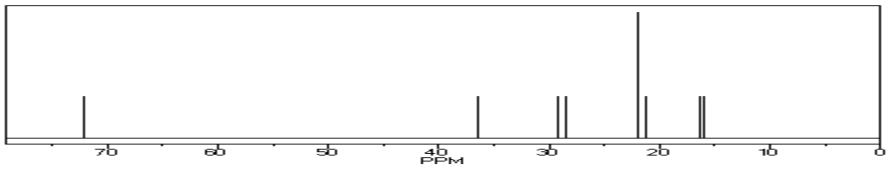
The following are the results of ^{13}C NMR spectrum for the 19 compounds identified through GC-MS. It allows the identification of carbon atoms in an organic molecule just as proton NMR identifies hydrogen

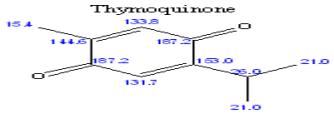
atoms. As such ^{13}C NMR is an important tool in chemical structure elucidation in organic chemistry. ^{13}C NMR detects only the ^{13}C isotope of carbon. Carbon NMR spectra show a single peak for each chemically non-equivalent carbon atom. The 19 compounds identified through GC-MS showed following chemical shifts: The chemical shift ranging from 8- 30 ppm shows R-CH3 carbon, and chemical shift in the range of 115-140 ppm shows alkenes, likewise, chemical shifts in the range of 125-150 ppm shows the presence of aromatic carbon.



(1S,3S,4S,6R)-4,7,7-Trimethylbicyclo[4.1.0]heptan-3-ol

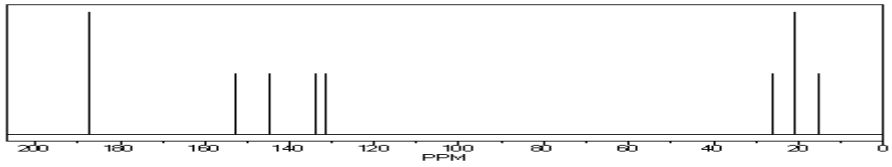
Estimation quality is indicated by color: good, medium, rough

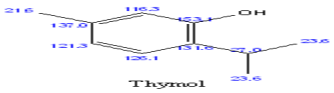




Thymoquinone

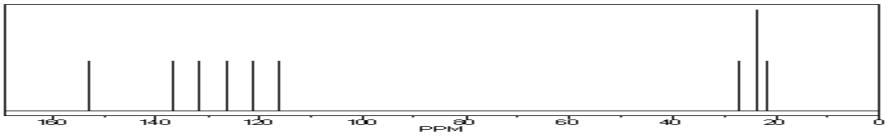
Estimation quality is indicated by color: good, medium, rough



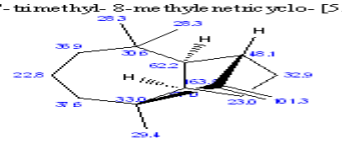


Thymol

Estimation quality is indicated by color: good, medium, rough



(1R,2S,7S,9S)- 3,3,7- trimethyl- 8-methylenetricyclo- [5.4.0.02,9] undecane



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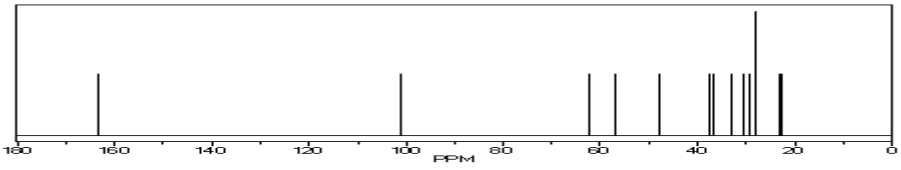

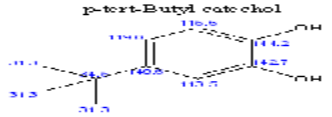

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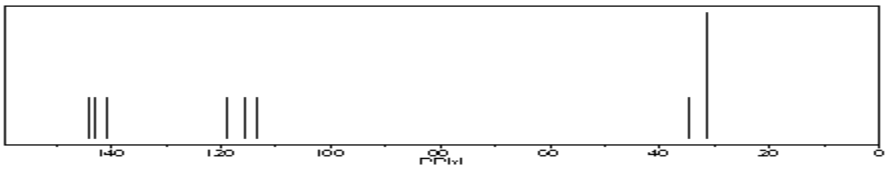

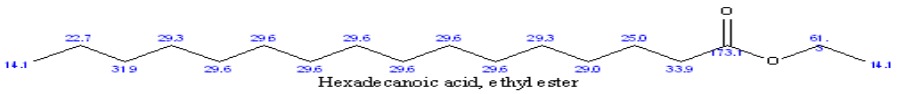

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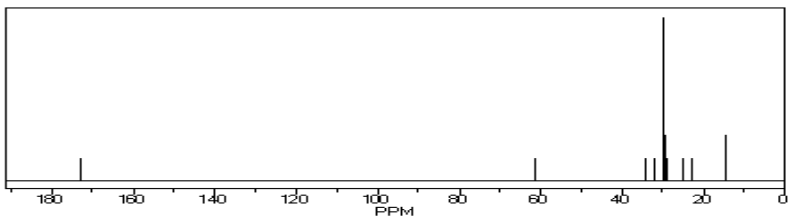

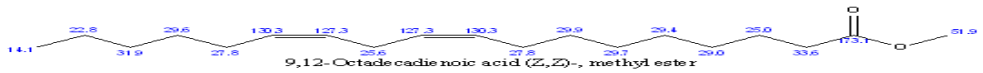

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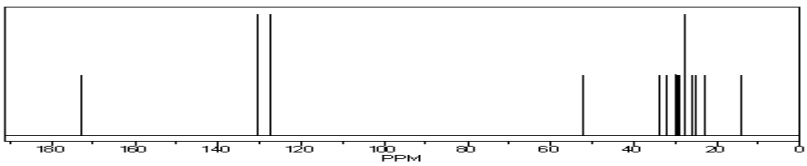

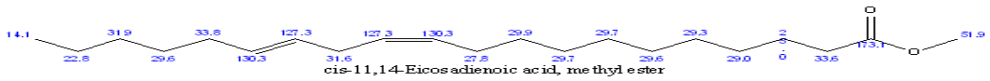

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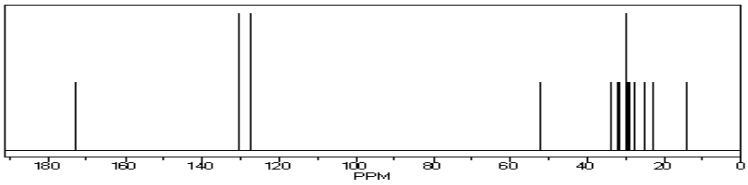

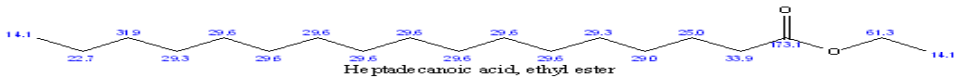

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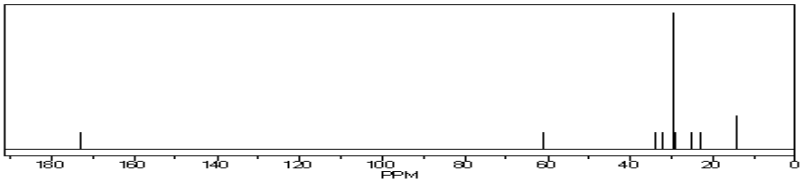
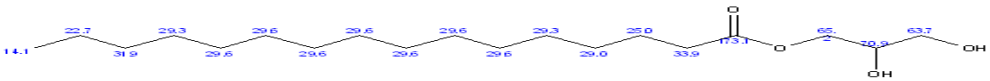
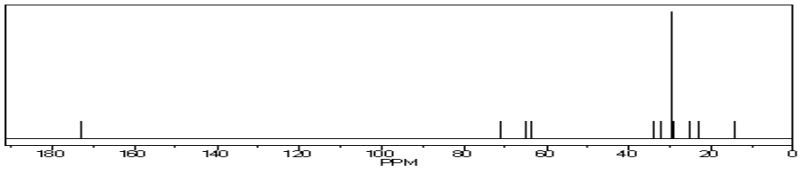
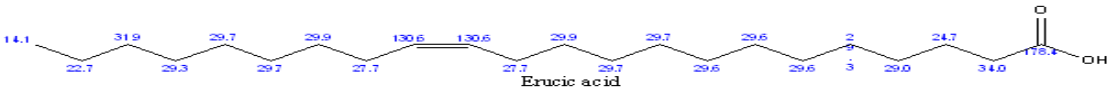




Glycerol 1-palmitate
This name appears to be ambiguous

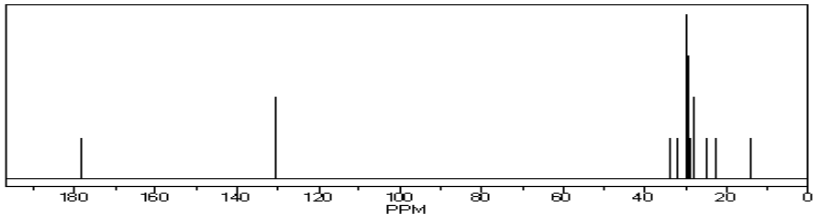
Estimation quality is indicated by color: good, medium, rough

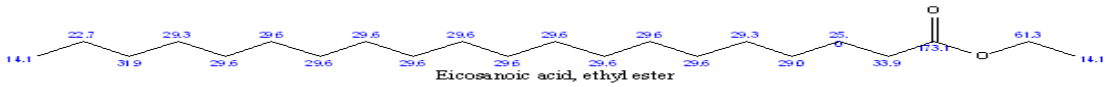




Erucic acid

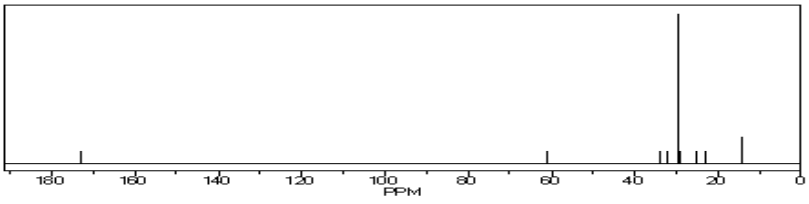
Estimation quality is indicated by color: good, medium, rough

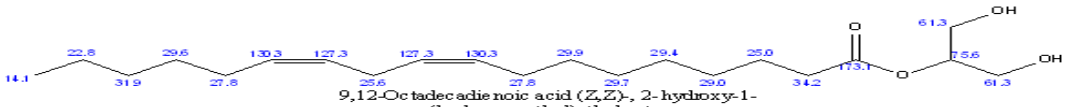




Eicosanoic acid, ethyl ester

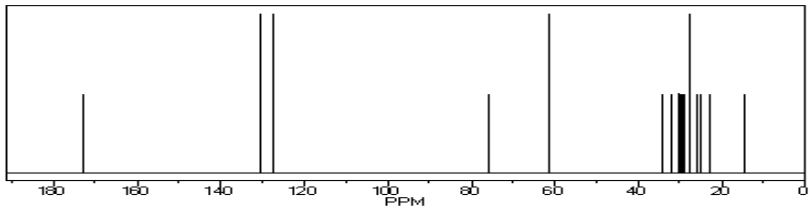
Estimation quality is indicated by color: good, medium, rough

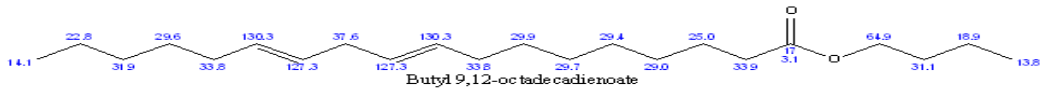




9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester

Estimation quality is indicated by color: good, medium, rough





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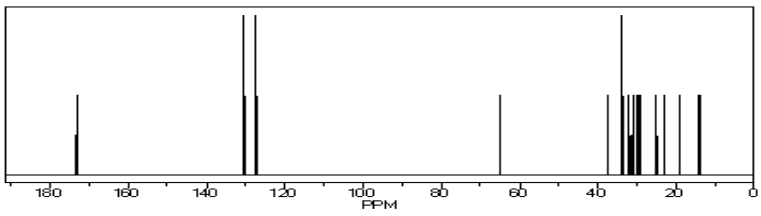

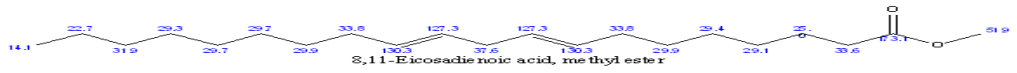

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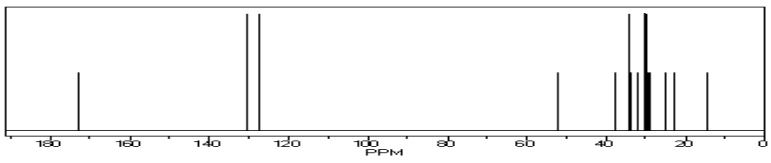
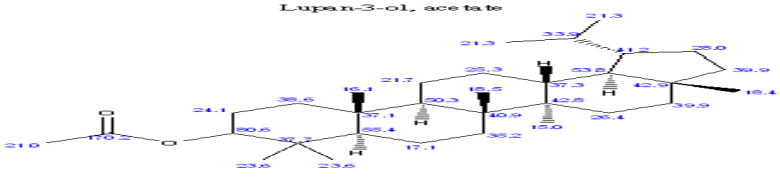


Lupan-3-ol, acetate



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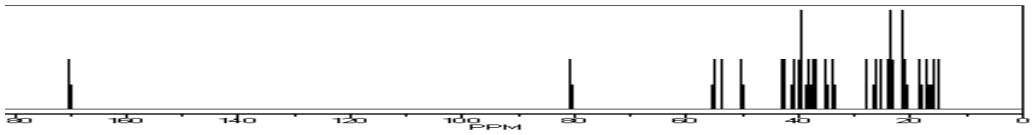

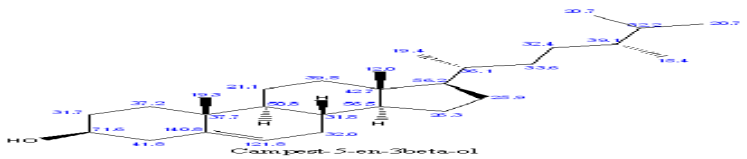

Campest-5-en-3beta-ol

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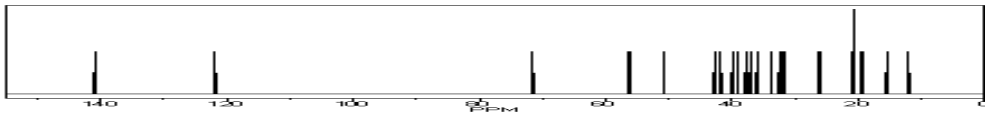
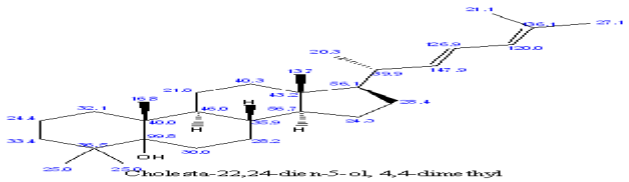
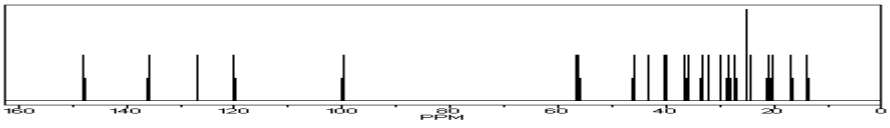


ChemNMR ^{13}C Estimation



Cholesta-22,24-dien-3-ol, 4,4-dimethyl

Estimation quality is indicated by color: good, medium, rough



CONCLUSION

Herbal, medicinal plants are accepted drugs in the process of recovery from diseases i.e alterated physiological system. In order to document the properties of *Nigella sativa* seeds and its identified compounds in the promotion of health, hygiene in an economically cheaper way. Hence, priority has to be given to the analytical research as it provides an effective approach towards the discovery of constructive medicinally active identity. So, the present study was studied analytically using GC-MS to know about the compounds present in it as well as FT-IR to have in depth knowledge on its functional groups and also ^{1}H NMR,^{13}C NMR predicts were done to have a knowledge on its chemical shifts. These observations form a platform for the clinical trials in future.

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CONFLICT OF INTEREST

No conflict of interest.

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