Review Article

A Review on Spectroscopic Analysis of Phytopharmaceuticals

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ABSTRACT

Phytopharmaceuticals are pharmaceutical products composed of natural compounds derived from botanicals instead of chemicals. As phytopharmaceuticals are complex mixture of many components, therefore it is not easy to do analysis of each component. Thus for proper analysis it is very important to understand chemistry of phytochemicals present in herbal products. Based on its characteristics isolation, structural identification and quantification need to be planned accordingly. Hence, first need to look at chromatographic techniques which isolate various types of components. Once the components are separated they should be analysed by spectroscopic methods for identification and quantification. The review highlights various spectroscopic techniques such as UV-Visible, Infrared, Nuclear magnetic resonance and Mass spectroscopy which are applied for analysis of phytochemicals present in herbal formulations.

Keywords: Phytopharmaceuticals, spectroscopic analysis, UV-Visible spectroscopy, IR spectroscopy, FTIR, NMR spectroscopy, Mass spectroscopy.

INTRODUCTION

Phytopharmaceuticals are produced from fresh or dried plants, or part of plants, by expression, distillation and other operations. Natural ingredients present in herbal medicines are more easily and more readily metabolised by the body. Therefore, they produce fewer or no side effects however pharmaceuticals made from chemical compounds are prone to adverse side effects.[1]

Phytopharmaceuticals should always contain the active principles together with coexisting materials from the source plant, these additional materials having a greater or lesser beneficial influence upon the activity of the drug. A phytopharmaceuticals may frequently not represent the final dosage form administered to the patient. Dry extract is further processed to produce powder mixtures, tablets, suppositories and other dosage forms. They are also considered to be intermediate or semi-finished products whose technical qualities are conductive to further processing. For example, de-enzymized gum acacia and gum tragacanth are also considered as phytopharmaceuticals.[2]

Analysis is the application of a process or series of processes in order to identify and/or quantify a substance, components of a solution or mixture, or the determination of the structure of chemical compounds. There are mainly two types of analysis: Qualitative analysis which provides information about the identity of atomic or molecular species or functional groups in the sample and Quantitative analysis which gives numerical information about the quantity of one or more of this component.[3, 4]

I) Different methods of Analysis[4,5]

Refer Table No 1: Methods of analysis

II) Need of phytopharmaceutical analysis[6]

i. Identity - The Condition of being specific herb.
ii. Purity - The condition of being free from contaminants or adulterant.
iii. Content - The amount of the active constituents present within the defined Limit.

III) Difficulties in phytopharmaceutical analysis[7]

i. Herbal drugs are usually mixtures of many constituents.
ii. The active principles are in most cases unknown.
iii. Selective analytical methods or reference compounds may not be available commercially.
iv. Plant materials are chemically and naturally variable.
v. Chemo-varieties and chemo cultivars exist.
vi. The source and quality of the raw material are variable.
vii. The methods of harvesting, drying, storage, transportation and process (mode of extraction and extracting solvent polarity, instability of constituents, etc.) have an effect.

IV) Steps involved in phytopharmaceutical analysis[8]

i. Sample Preparation
ii. Isolation and Purification of analyte
iii. Identification of analyte
iv. Quantification of analyte

Spectroscopy

Spectroscopy is the investigation of the interaction

between electromagnetic radiation and matter. The most important consequence of such interactions is that energy is absorbed or emitted by the matter in discrete amount is called quanta. The absorption or emission processes are known throughout the electromagnetic spectrum ranging from the gamma region to the radio region. When the measurement of radiation frequency is done experimentally, it gives value for the change of energy involved and from this one may draw the conclusion about the set of possible discrete energy levels of the matter.[9]

I) Types of Spectroscopy[10]

i. *Atomic Spectroscopy*

Atomic absorption spectroscopy

Atomic fluorescence spectroscopy

Atomic mass spectroscopy

Atomic X-ray spectroscopy

Atomic emission spectroscopy

ii. *Molecular Spectroscopy*

UV-Visible spectroscopy

Molecular luminescence spectroscopy

Infrared spectroscopy

Raman spectroscopy

Nuclear magnetic resonance spectroscopy

Mass spectroscopy

II) Spectroscopic Methods Used in Phytopharmaceutical Analysis

The advantages of these methods are low time and labour consumption. The precision of these methods is also excellent.[11]

i. **UV-Visible Spectroscopy** - To find out whether the system is conjugated (the coloured compounds such as β-carotene, crocetin are in system of extensively conjugated pi-electrons).

ii. **IR Spectroscopy** - To identify the functional groups that are present in the compound.

iii. **Mass Spectroscopy -** To determine the molecular structure and molecular weight of the compound and to identify the presence of isotopes patterns for Cl and Br.

iv. **NMR Spectroscopy -** It gives idea about structural backbone of compound.

13C-NMR - To identify how many types of carbon atoms are present in the compound.

1H-NMR - To find out how many types of hydrogen atoms are present in the compound and to find out how the hydrogen atoms are connected.[12]

i. *UV-Visible Spectroscopy (UV-Visible)*

The absorption spectra of plant components can be measured in very dilute solution against a solvent blank using a spectrophotometer. For colourless compounds, measurements are made in the range 200-400 nm; for coloured compounds, the range is 200-700nm. The wavelength of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (optical density) at the particular maxima and minima.[13]

Quantitative determination of Glycyrrhizic acid in crude drug and Herbolax capsule[14]

Senthil R. et al developed a simple, precise, accurate and reproducible method for the estimation of glycyrrhizic acid in crude drug and its herbal formulations. Different aliquots of glycyrrhizic acid in distilled water were prepared and absorbances of solutions were measured at 256 nm. The calibration curve was plotted using concentration against absorbance. The amount of glycyrrhizic acid found to be present in crude drug and herbal formulation was found to be 14.526mg/g and 7.819mg/capsule.

The method indicated excellent sensitivity, reproducibility, accuracy and repeatability, which is proved by low percentage relative standard deviation. The results obtained in recovery studies were showing that there is no interference from the excipients used. Thus, it is suggested that the proposed UV spectrophotometric method can be effectively applied for the routine analysis of glycyrrhizic acid in crude drug and herbal formulation in quality control analysis.

Simultaneous estimation of curcumin and quarcetin in Madhujeevanchurna[15]

Patil S. et al estimated the concentration of quarcetin and curcumin present in herbal formulation by simultaneous method using UV-Visible spectroscopy.

From the overlying spectra (fig.2) quarcetin (10µg/ml) and curcumin (10µg/ml), two wavelengths i.e. 256nm of quarcetin and 263nm of curcumin were selected at which both drug showed absorbance for each other. The absorptivity of these two drugs was found at 256nm and 263nm. A set of two simultaneous equations were formed. The concentrations of two drugs in mixtures were calculated by these following equations:

$$Cx = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \quad (1)$$

$$Cy = \frac{A_1\, ax_2 - A_2\, ax_1}{ax_2\, ay_1 - ax_1\, ay_2} \quad (2)$$

Where, Cx and Cy - Concentrations of quarcetin and curcumin in µg/ml

A_1 and A_2 - Absorbances of sample solutions at 256nm and 263nm.

ax_1 and ax_2 – Absorptivity of quarcetin and curcumin at 256nm and 263nm

In Madhujeevanchurna concentration of quarcetin and curcumin found to be 1.082 ± 0.011%w/w and 2.163 ± 0.550%w/w respectively.

Hence UV analysis is most useful in simultaneous estimation target molecules in herbal products.

ii) Infrared Spectroscopy (IR) -

IR spectra may be measured on plant constituents in an IR spectrophotometer either in solution (in chloroform or carbon tetrachloride), as a mull with nujol oil or in the solid state, mixed with potassium bromide. The region in IR spectrum above 1200cm^{-1} shows spectral bands or peaks due to the vibrations of individual bonds or functional groups under examination. The region below 1200 cm^{-1} indicates bands due to the vibrations of the whole molecule and because of its complexity is known as the 'Fingerprint region'. Intensities of the various bands are recorded subjectively on a simple scale as being either strong (S), medium (M) or weak (W).[16]

Determination of organic substances present in Swarnabhasma[17]

Mukharjee B. et al determined the presence of organic substances in Swarnabhasma, which has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and occurrence of early aging. Swarnabhasma has been used by Ayurvedic physicians to treat different diseases like bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders, etc.(fig.3)

An infra-red spectrum of Swarnabhasma was performed in a KBr matrix to assess the presence of organic substances.

The IR spectrum study demonstrated that the trial drug i.e. Swarnabhasma was almost free from any organic compound.

Fourier Transformed Infrared Spectroscopy (FTIR) – It is termed as *'time domain spectrometry'* because the changes in the radiant power are recorded with the changes in time. Time domain spectrum of a source consists of several wavelengths reaching the detector simultaneously. It is special instrument which make use of multiple detectors or channels to obtained information about different parts or elements of the spectrum simultaneously in a small-time period (0.5-1 sec).[18]

The effect of encapsulation on chemical stability of *Piper sarmentosum* was evaluated by FTIR[19]

Eng sheng chan et al studied the effect of encapsulation on chemical stability of herbal extract by FTIR spectroscopy.

The effect of process variables (alginate M/G ratio, alginate concentration, extract concentration, bead size and bead water content) on encapsulation efficiency and biochemical compounds stability were studied.

FT-IR spectra of aqueous extract, encapsulated extract and extract released from alginate beads were generated. The sample first mixed with KBr powder then mixture was laminated by using a pellet mold. The wave number used in the range of 4000-600 cm^{-1}.

The characteristic bands of extract encapsulated within the ca-alginate beads (Fig.4a) and that released from the beads (Fig.4b) were compared to the original extract. In general, the bands remained unaltered and they can be assigned as follows: conjugated C=C or C=O stretching vibrations at 1610cm^{-1}, aromatic ring vibrations at 1500–1600cm^{-1}, methyl group vibrations at 1380cm^{-1}, C-O-C vibrations of esters at 1240cm^{-1}, C-OH stretching vibrations of secondary cyclic alcohols at 1070cm^{-1} and CH out-of-plane bending vibrations at 760 and 625cm^{-1}.

However, the hydrogel material could have minor effects on the signal strength. For example, the absorbance of encapsulated extract at 918 and 1237cm^{-1} are absent or weaker when compared to the original extract (Fig.4a). But the signals reappear in the spectra of the extract released from the hydrogel matrix (Fig.4b). This shows that the signals could have been shielded by the hydrogel matrix. On the other hand, it is significant that several new bands appear at about 813, 900, 1028cm^{-1} in the spectra of the encapsulated extract. These could be the characteristic bands of blank ca-alginate hydrogel beads as it has been reported that the characteristic bands of alginate that correspond to the mannuronic and guluronic acid blocks are in the range between 780 and 1100cm^{-1}.

Hence it demonstrates the potential of hydrogel material for encapsulation of herbal aqueous extract.

iii) Mass Spectroscopy (MS)

The value of the technique is that it requires only microgram amounts of material, that it can provide an accurate molecular weight and that it may yield a complex fragmentation pattern which is often characteristic of that particular compound. The technique works successfully with almost every type of low-molecular weight plant constituent and it has even been used for peptide analysis.[20]

Analysis of Microencapsules containing cinnamon leaf oil and garlic oil in β-cyclodextrin[21]

Fernando A. et al analysed the formulation containing volatile oils by mass spectroscopy.

Cinnamon leaf (CLO) and garlic oils (GO) are good antimicrobials, however, their volatility complicates their use as food preservatives. Hence, microencapsulation of CLO and GO with β-cyclodextrin (β-CD) was studied at 4:96, 8:92, 12:88, and 16:84 (oil: β-CD) percent weight ratios.

Analysis of CLO and GO samples before and after microencapsulation in β-Cyclodextrin using GC-MS

system. Identification of the compounds was based on the comparison of their mass spectra. (Fig 5)

Identification of the volatile constituents of CLO (Fig.5) and GO (Fig.6) before (a) and after (b) the microencapsulation process was accomplished by GC-MS analysis. Once identified the major constituent of each microencapsulated oil chromatograms were used to compare the differences among the non-encapsulated oil at the different oil ratios and quantified.

Eugenol was the major constituent detected in the CLO, as shown in (Fig.5a) before and after in (Fig.5b) Eugenol accounts approximately 78% w/w of the total volatiles. Other minor CLO constituents detected were cinnamaldehyde, copaene, and β-caryophyllene.

The main compounds detected by GC-MS in GO (Fig.6) were allyldisulfide, allyltrisulfide and allyltetrasulfide. Fig.6 shows that the presence of the GO compounds was not influenced by the microencapsulation process. However, Allyldisulfide was the major constituent of GO with 21% w/w. From an examination of (Fig.6a) and (Fig.6b) it is possible to see that the concentration of all GO compounds decreased comparing to the non-encapsulated with the encapsulated oil. This effect was more evident for methyl disulfide and allylsulfide.

iv) Nuclear Magnetic Resonance Spectroscopy (NMR)

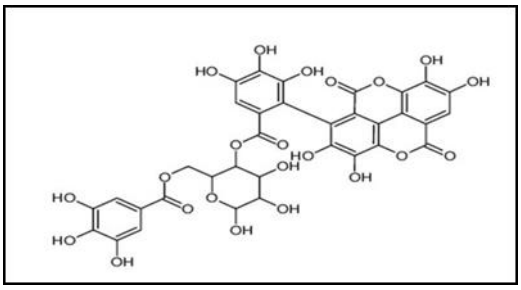
Proton NMR spectroscopy is basically provides a method for determining the structure of an organic compound by measuring the magnetic moments of its hydrogen atoms. In most compounds, hydrogen atoms are attached to different groups (as -CH2-, -CH-, -CHO, -NH2, -CHOH-, etc.) and the proton NMR spectrum provides a record of the number of hydrogen atoms in these different situations. However, it cannot give any direct information on the nature of the carbon skeleton of the molecule; this can only be obtained by carbon 13 NMR spectroscopy.

^{13}C-NMR spectroscopy is complementary to proton NMR and the combination of the two techniques provides a very powerful means of structural elucidation for new terpenoids, alkaloids or flavonoids. It is useful in the analysis of glycosides, in indicating the linkage between sugar moieties and their configurations. Both proton and ^{13}C-NMR measurements have been successfully applied to structural and other analyses of proteins and other macromolecules.[22]

Isolation and Structural identification of Terflavin B from *Terminalia chebula* powder[23]

Angala S. et al isolated and identified the major constituent structure present in drug powder.

Structure of Terflavin B –



The study investigates the phytochemical analysis and structural elucidation of the medicinally active constituents in methanolic extract of *Terminalia chebula* powder. It shows the presence of tannins, alkaloids, proteins, carbohydrates and phytosterols.

The ^{13}C NMR spectrum shows total number of carbons and also confirms the substitution groups available near to that carbon. The data prove that aromatic hydroxyl groups observed at δ 145, 144 ppm and the aldehyde or carboxylic C=O group was confirmed at δ 166 ppm. The aliphatic O-CH3 was confirmed at δ 73 ppm. The aromatic –CH group can be confirmed at δ 116 ppm and Fused aromatic carbons at δ 137 ppm. The data concluded that the structure consists of 34 carbons and it was fused hetero aromatic consisting of polyhydroxyl groups.

The ^{1}H NMR spectra indicated the number of hydrogen and substituted functional groups. As per the data it proved that polyhydroxyl hetero aromatic compound. Thus, isolated compound Terflavin B was confirmed by UV spectroscopy, Infrared spectroscopy, NMR spectroscopy and mass spectroscopy analysis.

CONCLUSION

In the development of new herbal products which contains mixtures other than the pure material, it is necessary to ascertain composition of mixture which shows the optimum characteristics for which the material has been developed.

Phytopharmaceuticals have reached extensive acceptability as therapeutic as well as nutritional agents. Hence, the need for development of authentic analytical methods which can reliably profile the phytochemical composition and quantitative analysis of phytochemicals and testing of marker/bioactive compounds and other major constituents present in the formulation.

Spectroscopic techniques show rapid and precise results in phytopharmaceutical analysis than conventional methods of analysis such as chemical analysis and titrimetric analysis.

Table No 1: Methods of Analysis

i.	**CLASSICAL METHODS**			
	Chemical Tests	**Titration Method**		
ii.	**INSTRUMENTAL METHODS**			
	Electrochemical techniques 1.Potentiometry 2.Voltammetry 3.Amperometry 4.Coulometrty 5.Electrogravimetry 6.Conductance	**Spectroscopic techniques** 1.Fluorescence and Phosphorescence spectrophotometry 2.UV-Visible spectrophotometry 4.Infrared spectroscopy 5. Atomic spectroscopy 6.Raman spectroscopy 7.X-ray spectroscopy 8.Nuclear magnetic resonance spectroscopy 9.Electron spin resonance spectroscopy	**Miscellaneous Techniques** 1.Thermal analysis 2.Mass spectroscopy 3.Kinetic techniques	**Hyphenated Techniques** 1.GC-MS 2.GC-IR 3.ICP-MS 4.LC-MS 5.MS-MS

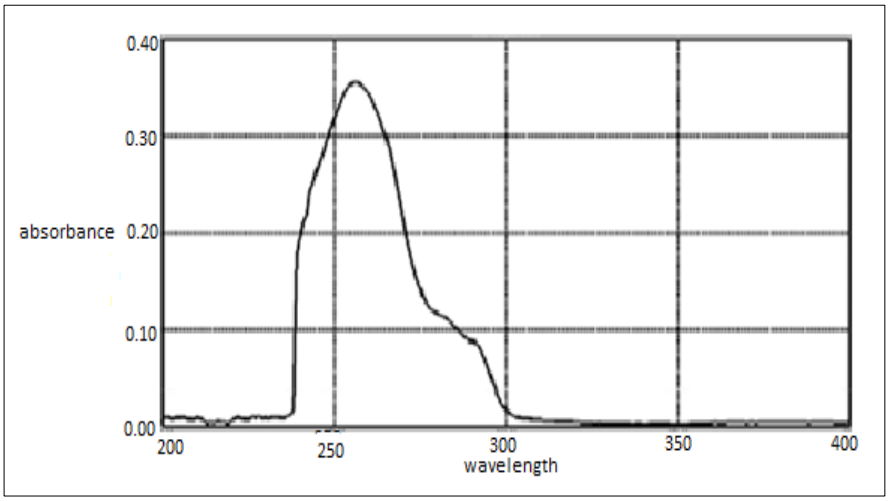


Figure 1: λ_{max} of Glycyrrhizic Acid in Distilled water (256nm)

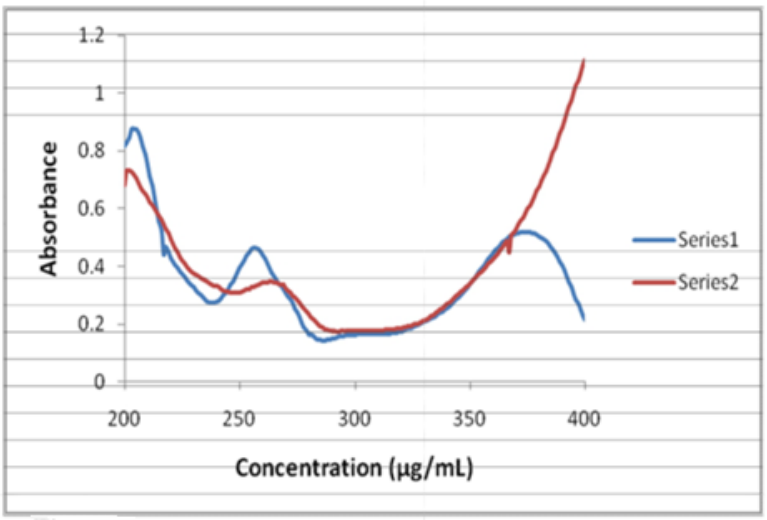


Figure 2: Overlay of Maxima Absorption of Quarcetin and Curcumin on UV Spectrophotometer

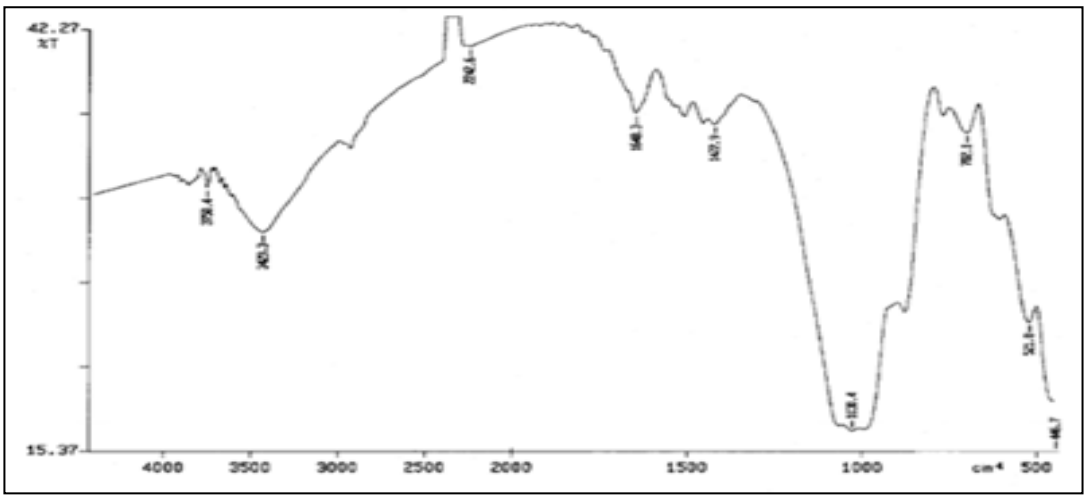


Figure 3: Infrared Spectrum of Swarnabhasma in KBR Matrix

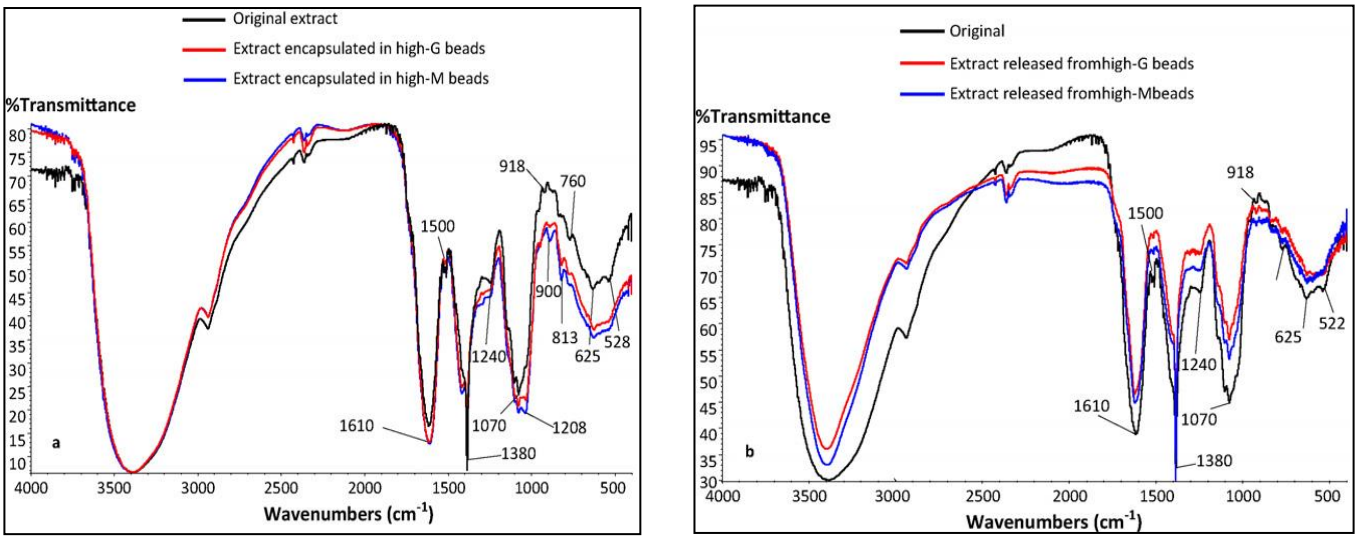


Figure 4: FT-IR Spectra of *P. Sarmentosum* Extract (a) Encapsulated in Beads and (b) Released from Beads.

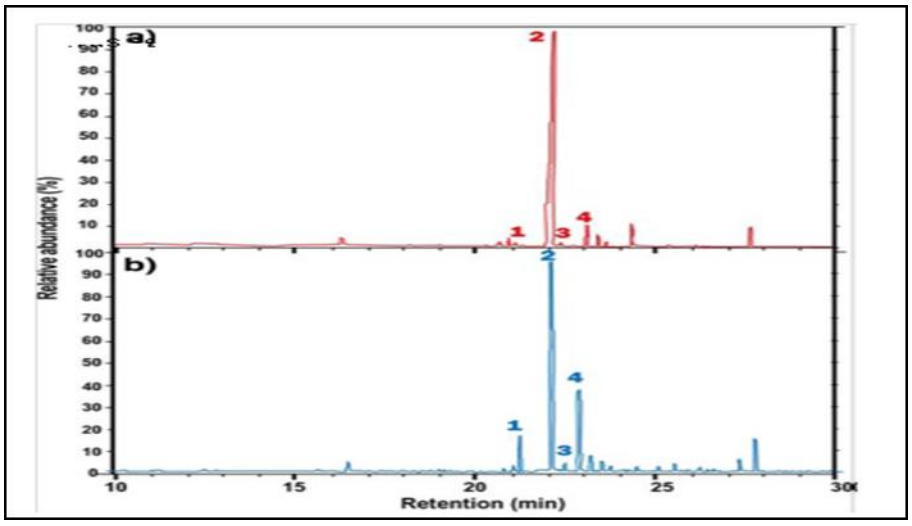


Figure 5: GC-MS Volatile Profile of CLO Before (a) and after (b) Microencapsulation in β-CD. 1: cinnamaldehyde, 2: Eugenol, 3: Copaene, 4: β-caryophyllene.

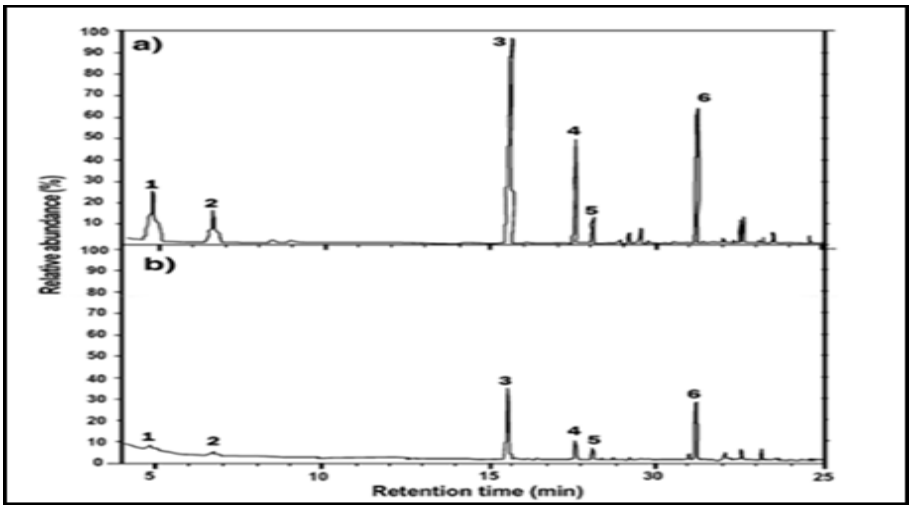


Figure 6: GC-MS Volatile Profile of GO Before (a) and After (b) Microencapsulation in β-CD. 1: Methyl Disulfied, 2: Allyl Sulphide, 3: Allyl Disulfied, 4: Allyl Trisulfied, 5: Trimethylene Trisulfied, 6: Allyl Tetrasulfied.

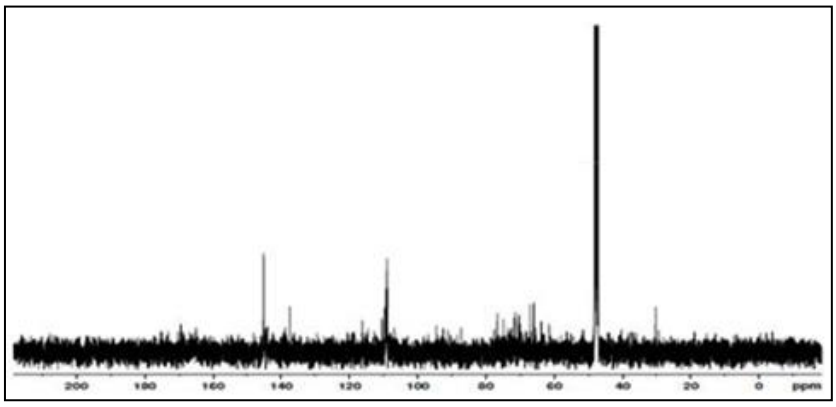


Figure 7: ^{13}C NMR Spectra of Isolated Compound

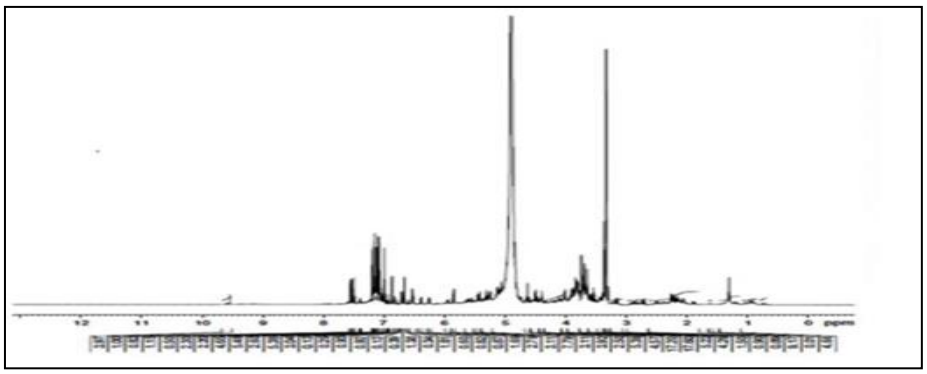


Figure 8: ^{1}H NMR Spectra of Isolated Compound

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