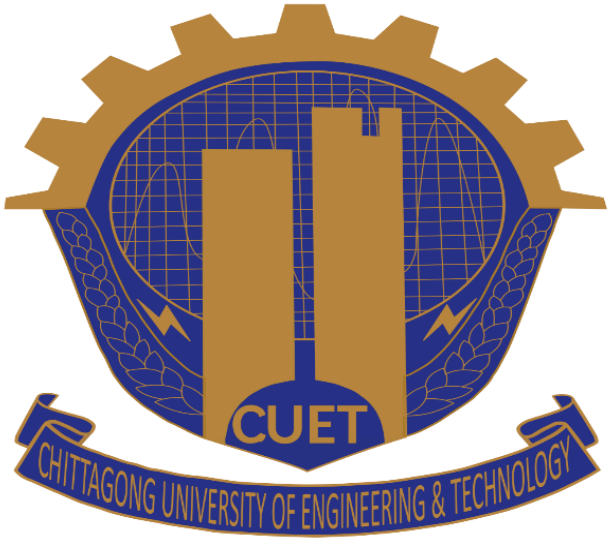
CHEMICAL AND BIOLOGICAL STUDIES ON THE POLAR FRACTION OF THE FRUITS OF *Cassia fistula* Linn. (Sonalu)

by

PROSHANTA SHIL

MASTER OF PHILOSOPHY IN CHEMISTRY

DEPARTMENT OF CHEMISTRY



CHITTAGONG UNIVERSITY OF ENGINEERING AND TECHNOLOGY

JUNE, 2024

CANDIDATE'S DECLARATION

It is hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any Degree or Diploma. To the best of my knowledge, no materials previously published or written by another of this thesis. Furthermore, the thesis complies with the PLAGIARISM and ACADEMIC INTEGRITY regulations of CUET.

Proshanta Shil
17MCHEM003P
Department Of Chemistry
Chittagong University of Engineering and Technology

Copyright Declaration

I hereby declare that I am the sole author of this thesis titled **"CHEMICAL AND BIOLOGICAL STUDIES ON THE POLAR FRACTION OF THE FRUIT OF *Cassia fistula* Linn"** (Sonalu). A soft copy is submitted along with the hard copy which is accepted by the board of examination.

I hereby release my copyright for uploading the electronic version as Digital Repository (Institutional Repository) of CUET after one year from the day of submission. Additionally, this copyright will be granted for research work, Open Learning Resource Policy and Procedures of Chittagong University of Engineering and Technology.

I acknowledge the delivering of copyright to Chittagong University of Engineering and Technology for any purpose of research work.

Signature of Author

Declaration by the Supervisor

This is to certify that Proshanta Shil has carried out this research work under my supervision. He has fulfilled the relevant Academic Ordinance of the Chittagong University of Engineering and Technology and now he is qualified to submit the following thesis in the application for the degree of MASTER OF PHILOSOPHY IN CHEMISTRY. Moreover, the thesis complies with the PLAGLARISM and ACADEMIC INTEGRITY regulation of Chittagong University of Engineering and Technology.

Dr. Ranajit Kumar Sutradhar
Professor
Department of Chemistry
Chittagong University of Engineering and Technology

Dedication

I would like to dedicate this Thesis to my daughter

Progga Chowdhury who is the heartbeat of my life.

Acknowledgement

Foremost, I want to offer this endeavor to almighty for the wisdom he bestowed upon me, the strength, peace of mind and good health in order to finish this research.

I would like to express my sincere gratitude to my honorable supervisor **Dr. Ranajit Kumar Sutradhar,** Professor, Department of Chemistry, Chittagong University of Engineering and Technology for the continuous support of my M.Phil. Study and research for his patience, motivation, enthusiasm and immense knowledge.

I am very much grateful to the Authority of Chittagong University of Engineering and Technology, Chittagong, Bangladesh for giving the necessary permission, research fund and technical support to complete this research work.

I am thankful to all respected Teachers, Department of Chemistry, Chittagong University of Engineering and Technology, Chittagong, Bangladesh for imparting their knowledge and expertise in this study.

I want to express my sincere thanks to the authority of Omargani M.E.S. College for permission and my colleagues for their valuable encouragements and advice in this research work.

I am also thankful with deep gratitude to my parents, teachers, siblings, friends, neighbors, relatives and all of my well-wishers.

Finally, I wish to express my heartfelt gratitude to my Family members because of their generous co-operation, love and moral support during this work.

June, 2024

Proshanta Shil
The Author

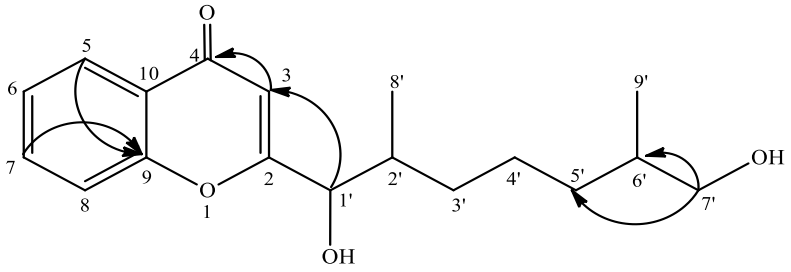
Abstract

The plant *Cassia fistula* is one the important medicinal plants of Bangladesh. It belongs to the family *Fabaceae* and genus *Cassia*. The plant *Cassia fistula* is locally known as Sonalu. It is a medium sized deciduous or semi-deciduous tree, 10 to 15 m tall with a straight trunk to 5 m in height and 1 m in diameter. The leaves are deep green, flowers are bright yellow in color. The fruit is a pendulous and cylindrical. It is black, glabrous and many seeded. Seeds are ovate, imbedded horizontally, dark colored pulp. This plant is used for the treatment of several diseases like skin diseases, abdomen pain, fever and leprosy etc.

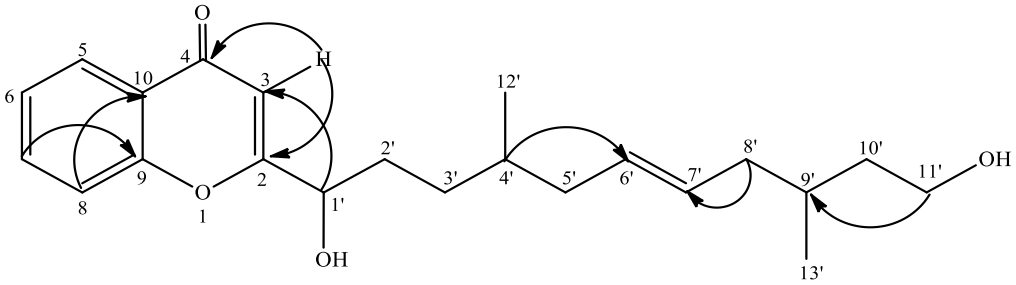
Different types of compounds like flavonoids, glycosides, alkaloids, steroids and amino acids have been reported from the plant of the genus C*assia*. Anthraquinone glycosides, kaempferol, tannins and chromen were reported from *Cassia fistula*. The purpose of the present research work was a phytochemical investigation on the fruits of *Cassia fistula* for the isolation of bioactive secondary metabolites like alkaloid, flavonoid, and chromen as well as to determine their molecular structure.

Fresh and matured fruits of *Cassia fistula* were collected from Sitakunda-Kumira hill region of Chittagong district during the month of December 2020. The dried powdered fruits (3 kg) were successively extracted with dichloromethane (DCM) and rectified sprite (RS). RS extract was concentrated to 250 ml and partitioned with $CHCl_3$, EtOAC and *n*- butanol respectively. On the removal of the solvent $CHCl_3$ extract gave a green Mass 31 g.

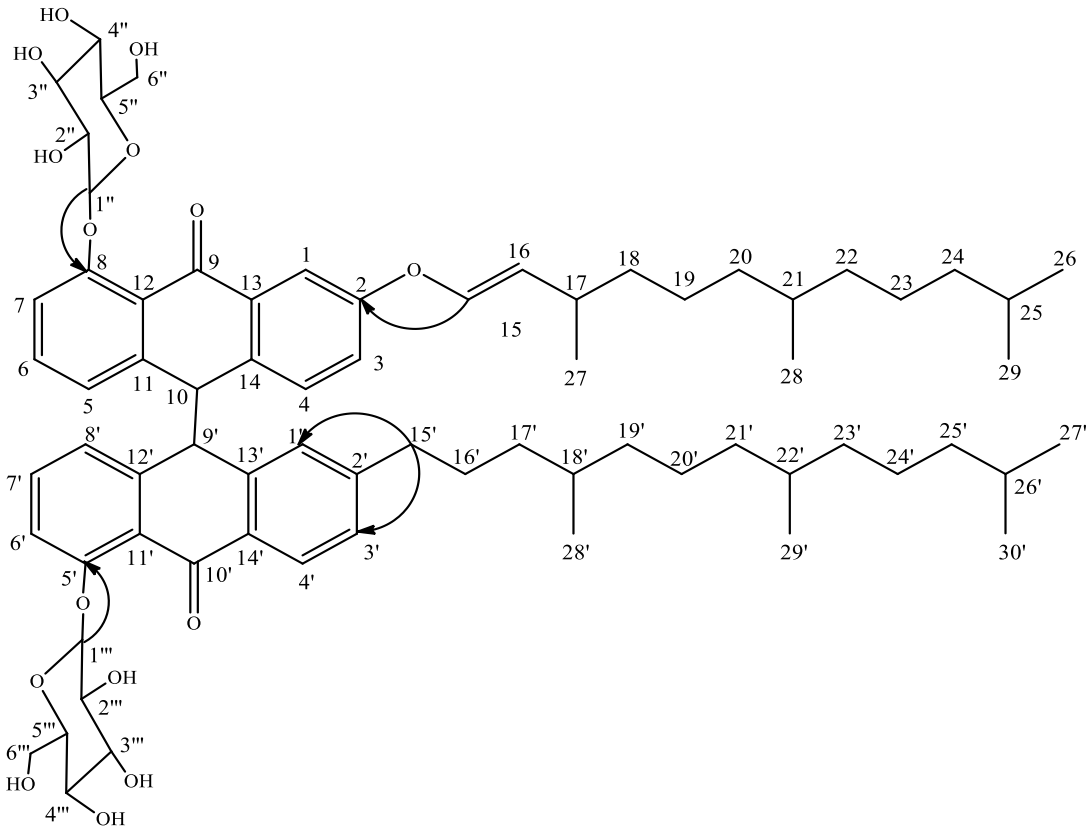
Fractionation and purification of $CHCl_3$ extract afforded three pure compounds, named as **P1**, **P2** and **P3**. Compounds **P1** and **P2** showed remarkable antibacterial activities against gram-positive and gram-negative bacteria and compounds **P3** exhibited significant antifungal activities against the fungi *Aspergillus niger*. Their structure of compounds were established as 2-(1′, 7′ - dihydroxy-2′, 6′ - dimethylheptyl)-4H- chromen-4-one **(P1),** (E)-2-(1′, 11′ -dihy droxy-4′, 9′ -dimethylundee-6′ -en-1′ -yl)-4H-chromen-4-one **(P2)** and 5″[{(2R,3S,5R,6S)-3″,4″,5″-trihydroxy-6″(hydroxymethyl)tetrahydro-2H-pyran-2-yl}oxy]-5‴[{(2S, 3R, 5S,6R)-3‴,4‴,5‴-trihydroxy-6‴ (hydroxymethyl) tetrahydro-2H-pyran-2-yl}oxy]-17-[{(E)-17,21,25 -trimethyldodee-15-en-15-yl}oxy]-2′-(28′,29′,30′-trimethlytridecyl)--9,10′-bianthracene]-9, 10′ (9′H,10H)-dione **(P3)**



P1



P2



P3

সারাংশ

ক্যাসিয়া ফিস্টুলা বাংলাদেশের একটি গুরুত্বপূর্ণ ঔষধি গাছ। এটি *Fabaceae* এবং *Cassia* বংশের অন্তর্গত। ক্যাসিয়া ফিস্টুলা উদ্ভিদ স্থানীয়ভাবে সোনালু নামে পরিচিত। এটি একটি মাঝারি আকারের পর্ণমোচী বা অর্ধ-পর্ণমোচী গাছ, উচ্চতা ১০ থেকে ১৫ মিটার যার একটি সোজা কাণ্ড 5 মিটার লম্বা এবং ব্যাস 1 মিটার হয়। পাতার রং গাড় সবুজ ও ফুল হয় উজ্জ্বল হলুদ বর্ণের। ফল গুলো লটকানো এবং নলাকার হয়। এইগুলো কালো, চকচকে এবং অনেক বীজযুক্ত হয়। বীজ ডিম্বাকার, অনুভূমিকভাবে বাঁধা, গাঢ় রঙের সজ্জার হয়। এই উদ্ভিদটি চর্মরোগ, পেটে ব্যথা, জ্বর এবং কুষ্ঠরোগের মতো বিভিন্ন রোগের চিকিৎসায় ব্যবহৃত হয়।

ফ্ল্যাভোনয়েড, গ্লাইকোসাইড, অ্যালকালয়েড, স্টেরয়েড এবং অ্যামিনো অ্যাসিডের মতো বিভিন্ন ধরণের যৌগগুলি *Cassia* বংশের উদ্ভিদ থেকে পাওয়া গেছে। ক্যাসিয়া ফিস্টুলা থেকে অ্যানথ্রাকুইনোন গ্লাইকোসাইড, রেইন, ফরমিক অ্যাসিড, কেম্পফেরল, উদ্বায়ী তেল, ট্যানিন এবং ক্রোমেন পাওয়া গেছে। বর্তমান কাজের উদ্দেশ্য ছিল সেকেন্ডারি মেটাবোলাইট প্রধানত অ্যালকালয়েড, ফ্ল্যাভোনয়েড, গ্লাইকোসাইড এবং ক্রোমেনের বিচ্ছিন্নকরণের পাশাপাশি তাদের আণবিক গঠন নির্ধারণের জন্য ক্যাসিয়া ফিস্টুলার ফলের উপর একটি ফাইটোকেমিক্যাল ইনভেস্টিগেশন।

২০২০ সালের ডিসেম্বর মাসে চট্টগ্রাম জেলার সীতাকুণ্ড-কুমিরা পার্বত্য অঞ্চল থেকে ক্যাসিয়া ফিস্টুলার তাজা এবং পরিপক্ক ফল সংগ্রহ করা হয়েছিল। এই পরিপক্ক ফলের শুকনো গুঁড়ো (৩ কেজি) পর্যায়ক্রমে RS এবং $CHCl_3$ দিয়ে বের করা হয়েছিল। দ্রাবক $CHCl_3$ নির্যাস অপসারণ করে (RS পার্টিশন থেকে) 8.7492 গ্রাম একটি গাড়ো সবুজ নির্যাস পাওয়া যায়।

$CHCl_3$ নির্যাসের ভগ্নাংশ এবং বিশুদ্ধকরণের মাধ্যমে প্রাপ্ত তিনটি বিশুদ্ধ যৌগের নাম দেওয়া হয়েছিল P1, P2 এবং P3 । P1 এবং P2 যৌগগুলি গ্রাম-পজিটিভ এবং গ্রাম-নেগেটিভ ব্যাকটেরিয়ার বিরুদ্ধে উল্লেখযোগ্য অ্যান্টিব্যাকটেরিয়াল কার্যকলাপ দেখিয়েছে এবং P3 যৌগ *Aspergillus niger* ছত্রাকের বিরুদ্ধে উল্লেখযোগ্য অ্যান্টিফাঙ্গাল কার্যকলাপ প্রদর্শন করেছে।

Table of Contents **Page No.**

CHAPTER – I

1. INTRODUCTION 1-12

CHAPTER-II

2. EXPERIMENTAL 13-21

List of figures:

List of Tables:

List of Schemes:

Abbreviation

TLC	Thin layer chromatography
TMS	Tetra Methyl Saline
UV	Ultraviolet
WHO	World Health Organization
UNCTAD	United Nations Conference on Trade and Development
VLC	Vacuum Liquid Chromatography
GLC	Gas Liquid Chromatography
g	Gram
HMBC	Hetero-Nuclear Multiple Bonds Correlation
HPLC	High Performance Liquid Chromatography
h	Hour
HIV	Human Immune Deficiency Virus
IR	Infrared
m.p	Melting Point
Mol.wt	Molecular Weight
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
ppt.	Precipitate
PC	Paper Chromatography
PPC	Preparative Paper Chromatography
PTLC	Preparative Thin Layer Chromatography
AMP	Aminopyrine
BNH	Bangladesh National Herbarium
CNS	Central Nervous System
CC	Column Chromatography
COSY	Correlated Spectroscopy
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulphoxide
FTIR	Fourier-transform Infrared Spectroscopy
GC	Gas Chromatography
GCMS	Combined Gas Chromatography and Mass Spectrometry

CHAPTER- I
INTRODUCTION
PAGE No. (01-12)

1. Introduction

1.1 General:

From the known prehistoric period, plants leaves, barks etc. have been used in many ways of medicinal purposes treating various diseases. Ancient Greek manuscripts called Egyptian papyri and various Chinese writings describe the uses of herbs. There is evidence that it has been used medicinally for over four thousand years by Indian Vaids, Unani Hakims, Mediterranean and European cultures. The World Health Organization reports that nearly eighty percent of people worldwide rely directly on herbal medicine for their initial health care and disease preventions.

According to pharmacology, plants are mainly divided into two groups- (a) poisonous i.e. harmful plants and (b) beneficial i.e. medicinal plants. Among these two main groups medicinal plants occupy an important place in human life those are also called medicinal herbs and since prehistoric periods, it has been discovered, practiced and used as traditional medicine. Hundreds of medicinal compounds synthesized from these herbs are used for various functions including defense and protection against fungi, viruses, insects, pathogenic microorganisms and various species of herbivorous mammals.

Due to the presence of medicinal molecules in medicinal plants, the plants are considered as main sources of natural compounds that are beneficial to treat human diseases and due to the presence of phytochemicals play a vital role in treating various diseases.

Many medicinal plants are traditionally used for various seasonal ailments. To save human's lives and preserved the trees, it should be propagated. In the last two decades the world has witnessed an unprecedented explosion of interest in the potential uses of these plant medicines in healthcare and this has sparked a renaissance in pharmacognosy.

However, Bangladesh is also looking forward to develop this herbal sector because there remains a wide range of acceptance among general people for having a common belief that herbal medicine is free of all side effects. Through exploiting this acceptance & maintaining proper quality control herbal sector has a huge scope for being established in medicinal practices in Bangladesh.

1.2 Some important plant derived drugs used in modern medicines:

Drug	**Plant Source**	**Pharmacological activity**
Aesculetin	*Frazinus rhychophylla*	Antidysentery
Aescin	*Aesculus hippocastanum*	Antiinflammatory
Anisodamine	*Anisodus tanguticus*	Anticholinergic
Betulinic acid	*Betula alba*	Anticancerous
Bergenin	*Ardisia japonica*	Antitussive
Camphor	*Cinnamomum camphora*	Rubefacient
Caffeine	*Camellia sinensis*	CNS stimulant
Digitalin	*Digitalis purpurea*	Cardiotonic
Danthron	*Cassia species*	Laxative
Etoposide	*Podophyllum peltatum*	Antitumor agent
Glaucine	*Glaucium flavum*	Antitussive
Gitalin	*Digitalis purpurea*	Cardiotonic
Hydrastine	*Hydrastis Canadensis*	Hemostatic
Irinotecan	*Camptotheca acuminate*	Anticancer, antitumor
Kheltin	*Ammi visage*	Bronchodilator
Lapachol	*Tabebuia species*	Anticancer, antitumor
Morphine	*Papaver somniferum*	Analgesic
Menthol	*Mentha species*	Rubefacient
Nicotine	*Nicotiana tabacum*	Insecticide
Papavarine	*Papaver somniferum*	Smooth muscle relaxant
Palmatine	*Coptis japonica*	Antipyretic, detoxicant
Quinine	*Cinchona ledgeriana*	Antimalarial, antipyretic
Rorifone	*Rorippa indica*	Antitussive
Sennosides A, B	*Cassia species*	Laxative
Tetrandrine	*Stephania tetrandra*	Antihypertensive
Vasicine	*Vinca minor*	Cerebral stimulant
Valapotriates	*Valeriana officinalis*	Sedative

1.3 The plant family *Leguminosae (Fabaceae)*:

After *Orchidaceae* and *Asteraceae* plant families, *Leguminosae* is considered as the third-largest containing 740 genera and 19,400 species and is accounted as one of the 12 flowering plants in the plant kingdom. Most of the plants in this family are shrubs and herbaceous plants with essential economic value **(**King and Hickey, 1997). *Leguminous* plants are usually rich in important metal ions i.e. iron, potassium, phosphorus, calcium and also vitamins A, B, C. Due to presence of these beneficial properties, edible legumes are important nutrients for human consumption (Guzmán *et al.* 2003). The high amounts of nutrients in *Leguminosae* also enrich inorganic nitrogen of soil by fixing nitrogen released in the air (Kouas *et al.*, 2009).

The leaves of *Leguminous* plants are usually alternate and compounds those are most often odd or even pinnately compounds. The petals are stamens and small which have long colored filaments. The fruiting ovary usually develops into a legume. Legumes are a common dry fruit that usually dehisces on both sides. A few species of basic leguminous fruit have evolved into samarae, follicles, loments, achenes, indehiscent legumes, drupes, and berries.

1.4 The species *Cassia fistula* and its distribution:

Cassia fistula is a native species in the Indian subcontinent and the nearest area of Southeast Asia. It is generally found as an ornamental species in such regions like tropical and subtropical regions. During hot and dry weather in late spring and early summer, this plant bears profuse flowers, causing the plant to be covered by yellow flowers, often leaving almost no leaves visible. This plant grows better in full sun days in drained soil. The plants are slightly salt-tolerable and relatively drought-tolerable and also light short frosts tolerable, but may be damaged when cold becomes continued.

1.5 Botany of *Cassia fistula*:

Cassia fistula is found as widely grown plant in subtropical and tropical regions due to profuse flowering. This flower is the national symbol of Thailand (Thai Government, 2001). Its genus is *Cassia* and family is *Fabaceae*. In Bangladesh it is better known as 4

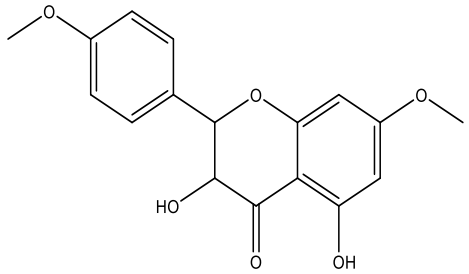
Sonalu. It is a medium-sized deciduous tree with a straight trunk 10 to 15 meters tall and 1 m in diameter and5 m in height. Branches of the trees spread out around forming an open crown. Stems are aerial, woody, erect, cylindrical, branched, glabrous and hard. Leaves are alternates, spirally arranged, peripinnately compounds, 30 to 40 cm long, ovate leaflets. Flowers of *Cassia fistula* are bright yellow, slightly zygomorphic shape and pentamerous. The fruit is an oblong, cylindrical, indehiscent pod, 60 to 100 cm long and 1.5 to 2 cm wide, shiny, many-seeded (up to 100 seeds) and black in color. The seeds are ovate, numerous, dark-colored pulp, imbedded horizontally in a sweet, separated by transverse dispersions called phragmatuses.

1.6 Medicinal uses of *Cassia fistula* Linn:

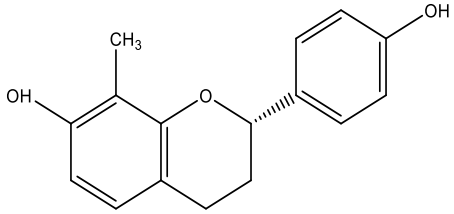
The plant is broadly used in the treatment of traditional medicinal systems (Mohd. Danish et al). Fruits and seeds of the *Cassia fistula* are used to treating fever, skin diseases, stomach problem and leprosy (Perry LM., 1980, Perry LM., 1980). Flower's decoction is prescribed for stomach discomfort (Satyavati *et al.*, 1989). Roots are used for tuberculous glands and various membranous ailments (Alam *et al*., 1990; Asolkar *et al.*,1992).

1.7 Previous phytochemical investigations on the plants of the genus *Cassia*:

Kiplagat *et al.* in 2016 studied on the root of the *Cassia abbreviate* and they isolated 2,3-dihydro-5-hydroxy-8-methoxy-2-(4-methoxyphenyl) chromen-4-one (1) and 3,4-Dihydro-2-(4-hydroxyphenyl)-4-methoxy-2H-chromen-7-ol (2) (Kiplagat *et al*, 2016).

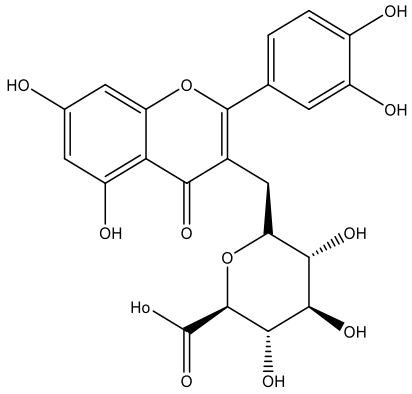


(1)

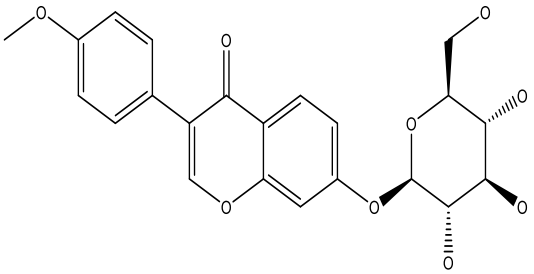


(2)

Vijayalakshmi *et al.* in 2016 investigated on *Cassia tora* leaves and they afforded two compounds, Quercetin-3-O-β-D-glucuronid (3) and Formononetin-7-O-β-D-glucoside (4) (Vijayalakshmi *et al.*, 2016).

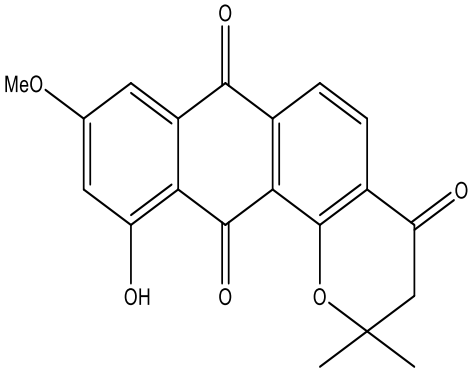


(3)

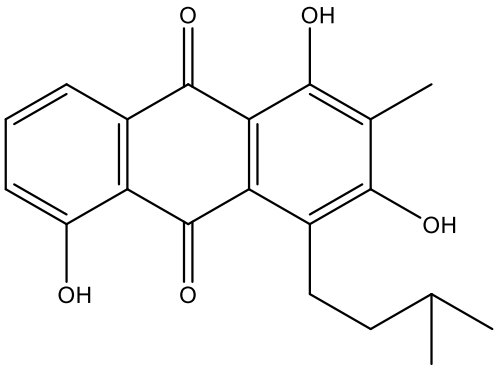


(4)

Ye *et al.* in 2014 studied on the stems of the *Cassia siamea* and reported the presence of Siameaquinones A and B (5) together, Lupinacidin A (6) (Ye *et al.*, 2014).

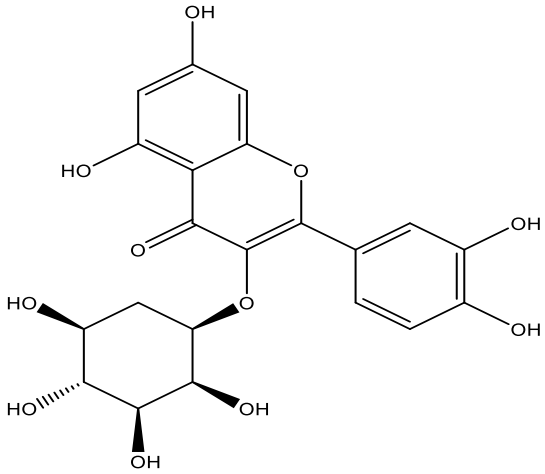


(5)



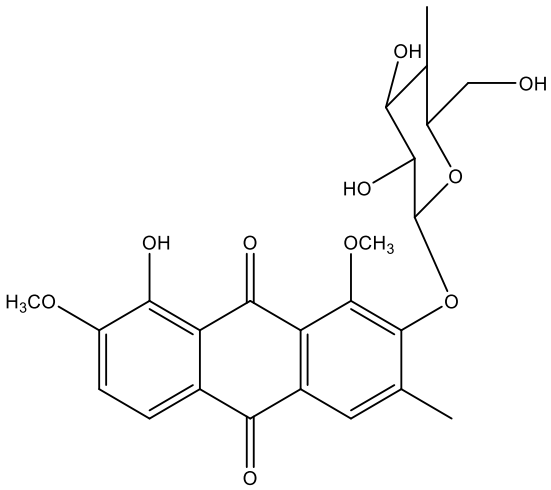
(6)

El-Toumy *et al.* in 2012 investigated on the leaves of the *Cassia roxburghii* and isolated a compound Quercetin 3-O-α-L-rhamnopyranoside (7) (El-Toumy *et al.*, 2012).

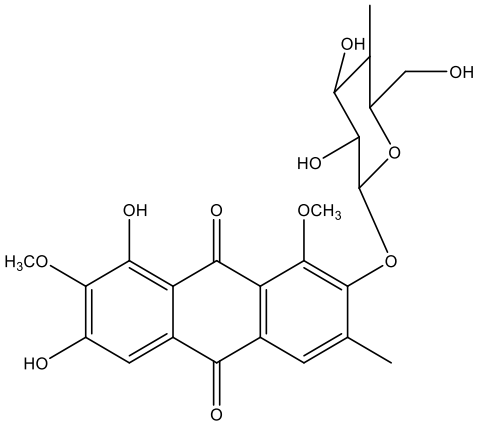


(7)

Tang *et al.* (2008), studied on the seeds of the *Cassia obtusifolia* and they isolated two compounds named of 1-Desmethylaurantio-obtusin-2-O-β-D-Glucopyranoside (8) and Aurantio-obtusin 6-O-β-D-glucopyranoside (9)

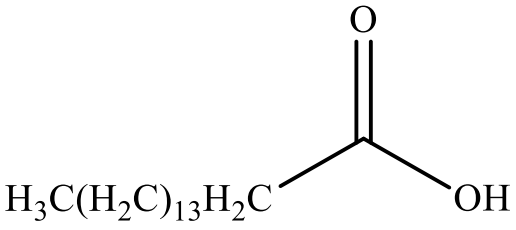


(8)

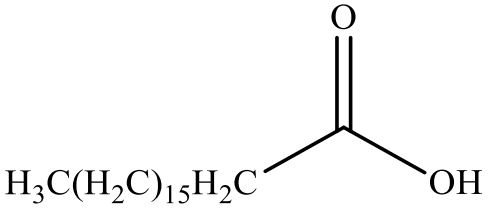


(9)

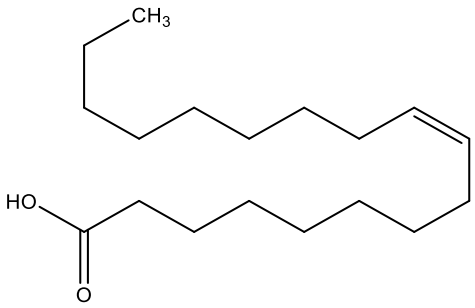
Ledwani and Singh in 2005 investigated on the seeds of the *Cassia reingera* and reported some Miscellaneous compounds named of Palmitic acid (10), Stearic acid (11), Oleic acid (12) and Linoleic acid (13) (Ledwani and Singh, 2005).



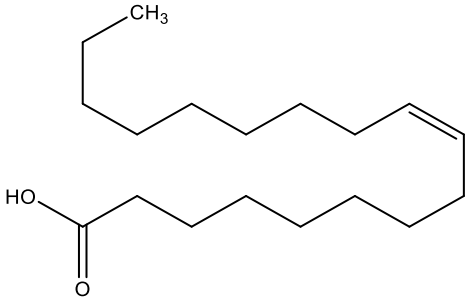
(10)



(11)

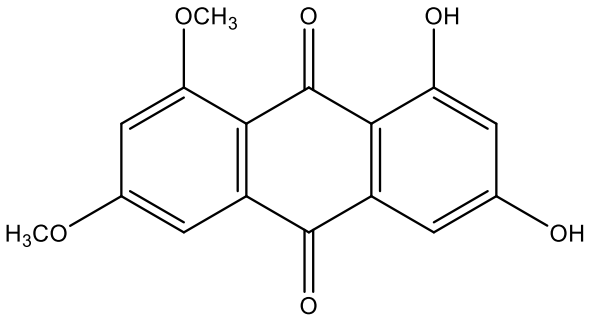


(12)



(13)

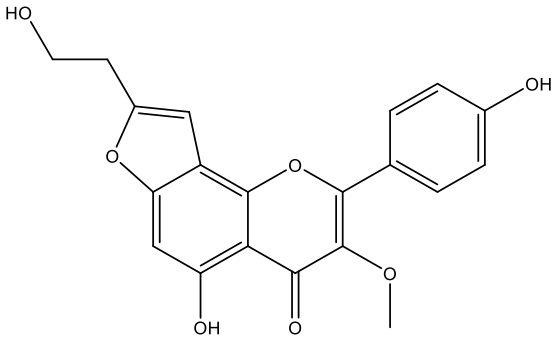
Gupta *et al.*, in 1989 studied on the root of *Cassia marginata* and they isolated 1,3-Dihydroxy-6-8-dimethoxy-2-isoprenylanthraquinone (14) (Gupta *et al.*, 1989).



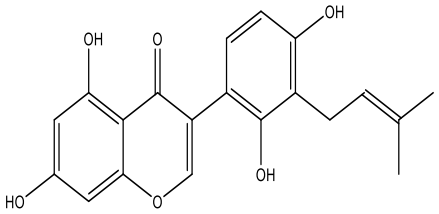
(14)

1.8 Previous phytochemical investigations on the plants of the species *fistula*:

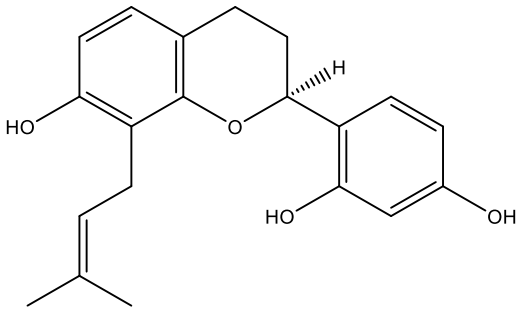
Zhao *et al.* in 2013 carried out phytochemical investigation on the stems of *Cassia fistula* and reported the present of Fistulaflavonoid B (15), Licoisoflavone (16), Morusyunnansins F (17), (3S)-3',7-dihydroxy-2',4',5',8-tetramethoxyisoflavan (18), (3S)-7-hydroxyl - 2',3',4',5',8-pentamethoxyisoflavan (19), (2S)-2',4'-dihydroxy-7- methoxy-8-prenylflavan (20) (Zhao *et al.*, 2013).



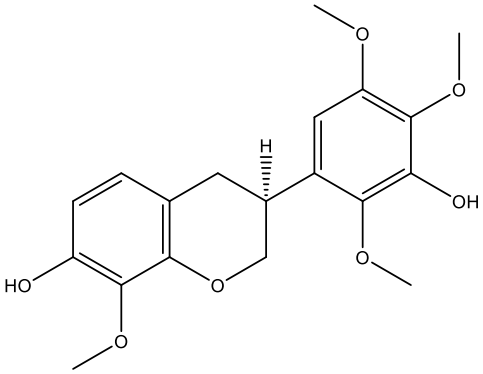
(15)



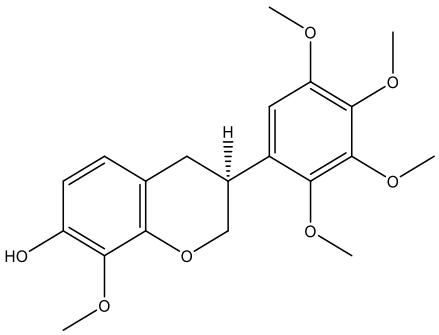
(16)



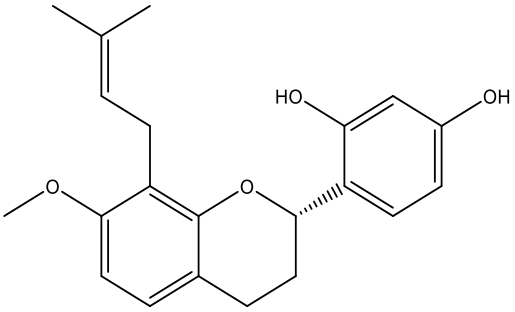
(17)



(18)

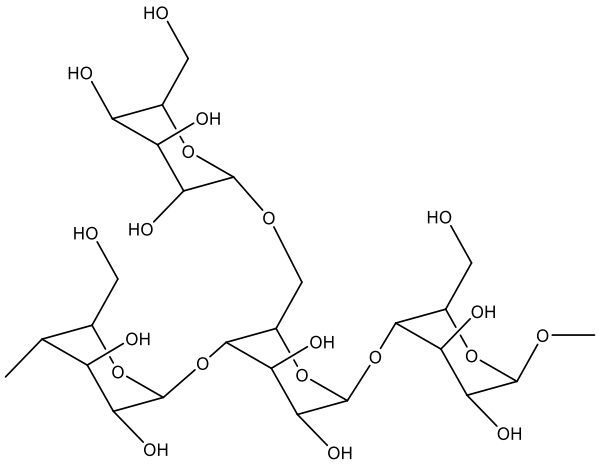


(19)



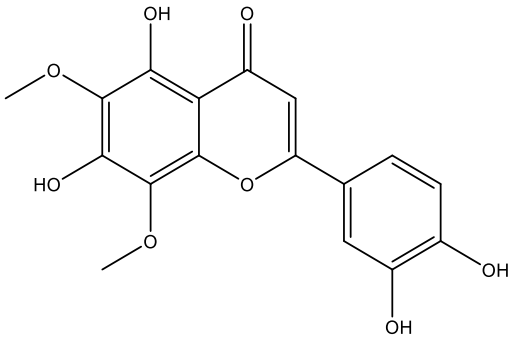
(20)

Agrawal *et al.,* (2012), investigated on the seeds of *Cassia fistula* and isolated one compounds named of Galactomannan (21).



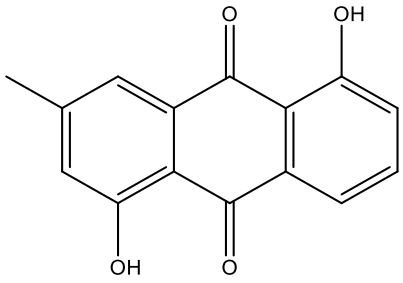
(21)

Danish *et al.* in 2011 studied on the stem bark of *Cassia fistula* and reported the presence of 5,7,3',4'-Tetrahydroxy-6,8-dimethoxyflavone-3-O-α-arabinopyranoside (22) (Danish *et al.*, 2011),.

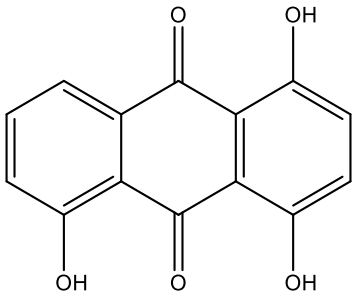


(22)

Lee *et al.* in 2001 investigated the seeds of the *Cassia fistula* and isolated Ziganein (23) and 1,4,5-Trihydroxyanthraquinone (24) (Lee *et al.,* 2001),.

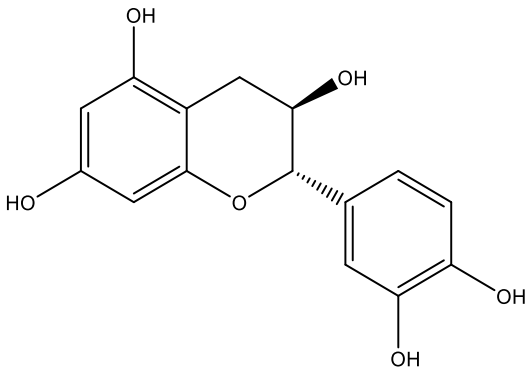


(23)

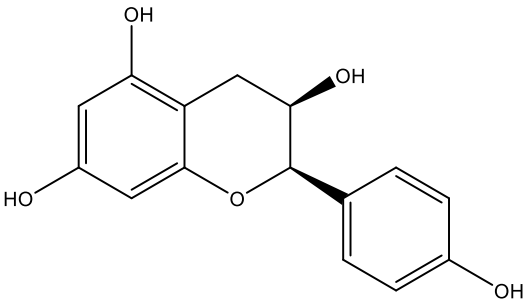


(24)

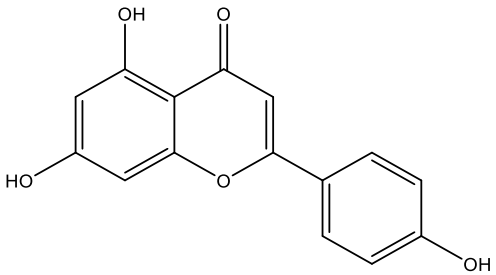
Rastogi and Mehrotra in 1999 studied on the fruits of *cassia fistula* and led to the isolation of (+) Catechin (25), Epi-afzelechin (26), kaempferol (27), Dihydrokaempferol (28) (Rastogi and Mehrotra, 1999).



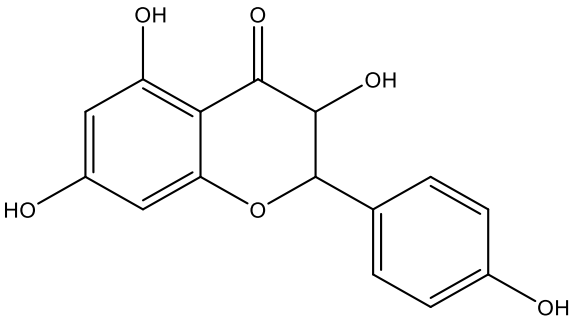
(25)



(26)



(27)



(28)

1.9 Objective with specific aims and possible outcome:

The research project on the fruits of *Cassia fistula* has been undertaken to achieve the following objectives / outcomes.

a) Objectives:

1. Extraction of the fruits of *Cassia fistula* with different organic solvents.
2. Isolation and purification of compounds using physical and chromatographic techniques.
3. Biological studies on the pure compounds with an aim to unfold their biological activities.

b) Possible outcomes:

1. Isolation of secondary metabolites from different extracts.
2. Characterization of isolated secondary metabolites by spectroscopic method.
3. Bioactivity studies of the isolated secondary metabolites.

CHAPTER- II
EXPERIMENTAL
PAGE No. (13-21)

2. Experimental

2.1 General methods:

The following steps are a summary of various methods for extraction, fractionation and purification of compounds during experimental works.

2.1.1 Chemicals and Solvents:

In the experiments, the used chemicals and solvents were procured from BDH (England) or Aldrich (America) or E. Merk (Germany). Before using the solvents for extractions, chromatographic separations or any analytical purposes, all solvents were distilled.

2.1.2 Crystallization and Fractional crystallization methods of samples:

The techniques of crystallization and re-crystallization were used for purifying the column chromatography (CC). Crystallization techniques usually involve choosing a solvent in where the substances or separated crude masses are comparatively less soluble in the solvent. The obtained compounds or crude masses from the separation process were totally dissolved in the minimum amount of solvent for crystallization or fractional crystallization.

2.1.3 Chromatographic techniques:

Chromatography is one technique of the separation process where the analyte is incorporated into a gaseous or liquid mobile phase. To separate the crude extracts into separate pure components from various types of solvent extraction. Various chromatography methods have been developed i.e. thin layer chromatography, preparative thin layer chromatography and column chromatography.

2.1.4 Detection of compounds in chromatography:

At room temperature, the developed chromate-plate was dried and the compounds on the chromate-plate were identified as follows:

(i) Use of UV light: The dried compounds on TLC plates were observed by UV light of different wave lengths such as 350nm and 254nm. It was observed that some compounds were fluoresced and others were dark spots with different and identical colors.

(ii) Uses of Iodine vapor: Detection of compounds by Iodine vapor is very popular and common. Iodine vapor is a versatile reagent that is used for detection and identification of compounds on chromate-plates. In iodine vapor system the developed plates were kept in iodine vapor tank locating the spots.

2.1.5 Column chromatographic technique (CC):

(a) Column development: Glass tubes of several diameters and lengths for example 8cm × 90cm, 3cm × 60cm, and 1cm × 30cm fitted with rotary-flow controlled system those were used for column chromatography separations.

(b) Stationary phase: As stationary phase in column chromatography separation, silica gel G_{60}-G_{254} was used.

(c) Preparation of silica gel column: A burette is usually used as a column in this process. A small amount of cotton is first placed at the bottom of the column so that no solids can escape through the tap. The silica gel (required amount) was swelled into a respective solvent to prepare a specific column. For example, *n*-hexene, ethyl acetate, chloroform or a mixture of several solvents at different ratios were poured into the column while solvent flows continuously. The slurry should be poured carefully so that no air bubbles are formed in the column. The column is equilibrated for homogenous packing with two or three column volume of solvent. Normal separation in column chromatography is usually performed by gravitational flow of solvents.

2.1.6 Steroidal Test:

(a) Salkowski Tests: The reddish color produced when shaking the chloroform solution of extract with conc. H_2SO_4 indicated that steroids are present in the sample.

(b) Lieberman Burchardt tests: Forming reddish ring at the junction of two layers when chloroform solution of extract was mixed with 1 ml of conc. H_2SO_4 and few drops of acetic anhydride indicated that steroids are present in the sample.

2.1.7 Determination of melting points:

All melting points of obtained products were observed and recorded by Fisher John's Electro thermal apparatus (Model no. 1A 9000) and were uncorrected. The heating on the apparatus was done very carefully to maintain steady temperature.

2.1.8 Spectroscopy methods:

(a) Infrared (IR) spectroscopy: IR spectra were recorded on a Shimadzu Spectro photometer, Model FTIR-IRAffinity-1. Samples were run on dry solid KBr. The spectra were run at Wazed Miah Science Research Centre, Jahangirnagar University.

(b) Nuclear magnetic resonance (NMR) spectroscopy: ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker BPX-200 spectrometer, operating at 400 MHz for 'H-NMR and 100 MHz for ^{13}C-NMR in the Wazed Miah Science Research centre, Jahangirnagar University, Savar, Dhaka. Chemical shifts were determined as parts per million (ppm) as compared to tetramethylsilane (TMS).

(c) Mass spectroscopy (MS): Mass spectral data were recorded on Q-TOF mass spectrometer, electrospray ionization (ESI-MS), Department of Chemistry, IIT Madras, India.

2.2.9 Collection of *Cassia fistula* fruits:

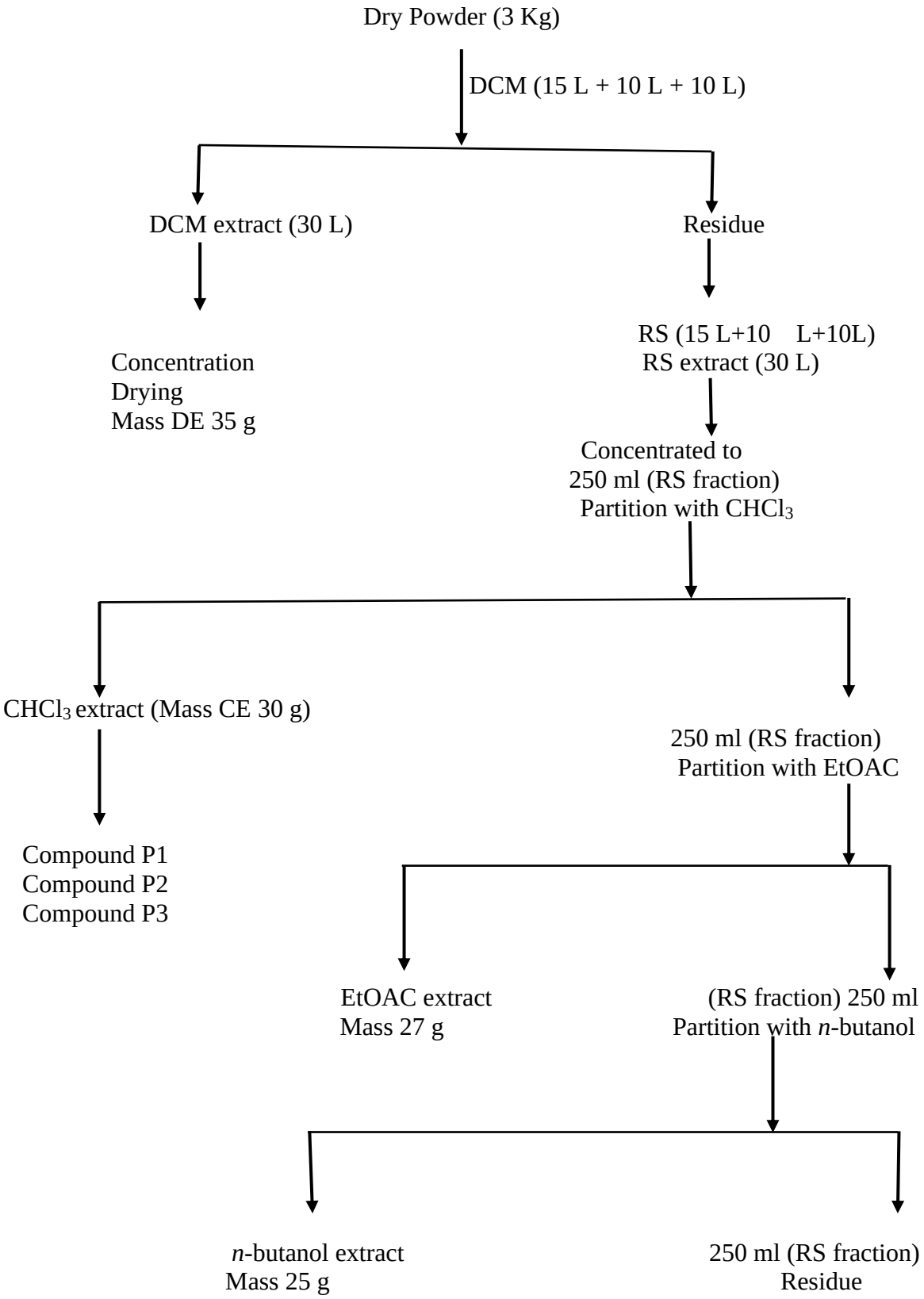
The *Cassia fistula* fruits were collected from Sitakunda-Kumira hill region of Chittagong district, located in the south-eastern region in Bangladesh. Fresh fruits were collected on December 2020.

2.2.10 Drying and grinding of plant materials:

The fruits are dried in the air and cut into very small pieces. Then it was kept dried in absence of sunlight followed by grinding to make powder form using a cyclotec-grinding machine. It was carefully stored in polythene bag until used for experiment.

2.2.11 Extraction process:

Air-dried powder of *cassia fistula* fruits (3 kg) was successively extracted with DCM (3×72 h) and rectified sprit (3×72 h) respectively as described in the fractionation Scheme 2.2.1. On removal of the solvent, the DCM extract gave a green Mass DE (35 g). The rectified sprit (RS) extract was concentrated to 250 ml and partitioned followed by $CHCl_3$, EtOAC and *n*-butanol. On removal of solvent $CHCl_3$ fraction gave a green Mass CE (30.0 g), EtOAC fraction gave a light green Mass EE (27.0 g) and *n*-butanol fraction gave a light yellow Mass BE (25.0 g).



Scheme: 2.2.1 Extraction and fractionation of plant materials

2.2.12 Preliminary examination on the crude of chloroform extract (CE):

The crude mass of chloroform extract was clearly soluble in $CHCl_3$ and partial soluble in *n*-hexene but insoluble in water. It did not give positive color reactions for steroids and flavonoids. It also did not respond to the qualitative tests of alkaloids with Mayer's and Dragendorff reagent. Mass CE was identified by TLC in solvents *n*-C_6H_{14}**:** EtOAc (1**:**1) on silica gel plates. Two spots were observed on TLC plate with R_f value ~ 0.60 and ~ 0.55.

2.2.13 Separation method of the crude of chloroform extract by Colum Chromatography:

Crude Mass CE was dissolved in $CHCl_3$ in small amount that was adsorbed by silica gel and reproduced in the powder form. Then it was placed into a column (90 x 8) cm of silica gel prepared by *n*-hexene. The elution was carried out with *n*-hexene: EtOAC (1:1) solvent system. About 5 ml portions were collected at regular intervals. The eluents were monitored by TLC and pooled into three different fractions CF1, CF2, CF3 according on their TLC behavior.

Table 2.1: Column chromatography separation of Mass CE (5.0 g).

No. of fractions	No. of Test tube	Eluting solvent	Observation	R_f values	Yield
CE1	1-10	*n*-hexene: EtOAC (1:1)	No spot	-	-
CE2	11-20	*n*-hexene: EtOAC (1:1)	One spot	~ 0.60	40 mg
CE3	21-37	*n*-hexene: EtOAC (1:1)	mixture	-	72 mg
CE4	38-50	*n*-hexene: EtOAC (1:1)	One spot	~ 0.55	120 mg
CE5	50-100	CH_3OH	Mixture	-	3.5 g

2.2.14 Isolation of compound P1 and P2 from fraction CE:

Fraction CE2 (40 mg) was a brown color, amorphous solid, soluble in $CHCl_3$ and partial soluble in *n*-hexane. Solid Mass (40 mg as compound P1) was obtained from the *n*-hexane**:** EtOAC (1:1) by purification in where repeated crystallizations were involved. It was observed a single spot on TLC plate with R_f value ~ 0.60 in *n*-hexane: EtOAC (1:1) and was named as **P1.** Fraction CE4 (120 mg) was also a brown amorphous solid. Its R_f value was 0.55 in *n*-hexane: EtOAC (1:1) and was named as **P2.**

2.2.15 Isolation of compound P3 from the fraction CE5:

Fraction CE5 (3.5 mg) was yellow color, amorphous solid, soluble in $CHCl_3$ and EtOAc. Solid Mass (165.0 mg) was obtained from purification and crystallization process of *n*-hexane**:** EtOAc (1**:**2) solvent system. Its R_f value was 0.45 in *n*- hexane**:** EtOAc (1**:**1) and was named as compound **P3.** We could isolate three pure compounds **P1**, **P2** and **P3** from $CHCl_3$ extract (CE)

2.3.1 Physical properties of the compound P1:

White amorphous solid

Soluble in Chloroform

Spectral properties of the compound P1:

IR $\bar{\upsilon}_{max}$ (KBr) cm^{-1}: 3447 cm^{-1} (O-H), 1690 cm^{-1} (>CO)

^{1}H NMR (CD_3OD) δ: ^{1}H NMR spectral data for Compound **P1** are given in the Table 3.1

^{13}C NMR (CD_3OD) δ: ^{13}C NMR spectral data for Compound **P1** are given in the Table 3.1

ESI-MS: *m/z* (M+1) 305.5236, 160, 147, 110, 106, 78, 70 etc.

2.3.2 Physical properties of the compound P2:

Amorphous solid

Soluble in Chloroform

Spectral properties of the compound P2:

IR $\bar{\upsilon}_{max}$ (KBr) cm^{-1}: 3419 cm^{-1} (OH) and 1701 cm^{-1} (>CO)

^{1}H NMR (CD_3OD) δ: ^{1}H NMR spectral data for Compound **P1** are given in the Table 3.2

^{13}C NMR (CD_3OD) δ: ^{13}C NMR spectral data for Compound **P1** are given in the Table 3.2

ESI-MS: *m/z* (M+1), 359.4272 214, 164, 147, 106 (100), 78, 70 etc.

2.3.3 Physical properties of the compound P3:

White crystalline solid

Soluble in MeOH

Spectral properties of the compound P3:

IR $\bar{\upsilon}_{max}$ (KBr) cm^{-1}: 3497 cm^{-1}, 3409 cm^{-1} (OH), 1671 cm^{-1} for >C=O

^{1}H NMR (CD_3OD) δ: ^{1}H NMR spectral data for Compound **P1** are given in the Table 3.3

^{13}C NMR (CD_3OD) δ: ^{13}C NMR spectral data for Compound **P1** are given in the Table 3.3

ESI-MS: *m/z* (M+1) 1191.6936, 597, 373, 227, 226, 164 etc.

2.4 Biological Studies

2.4.1 Antimicrobial assays:

In vitro antimicrobial potencies of the isolated compounds P1, P2 and P3 were determined by the Agar disk diffusion model (ADM) [2]. A continuous monitoring procedure was performed throughout the 24-hours incubation period of the basal media. Sterile cotton bars were used for bacterial and fungal Inoculation. Then the samples' disks were kept carefully on previously inoculated agar plates and incubated at 37°C, twenty four hours for antibacterial incubation and at 26°C, forty eight hours for antifungal incubation. As positive controls, Amphotericin B and Ceftriaxone were used for the antifungal and antibacterial tests, respectively. Dimethyl sulfoxide (DMSO) was utilized for both tests as control.

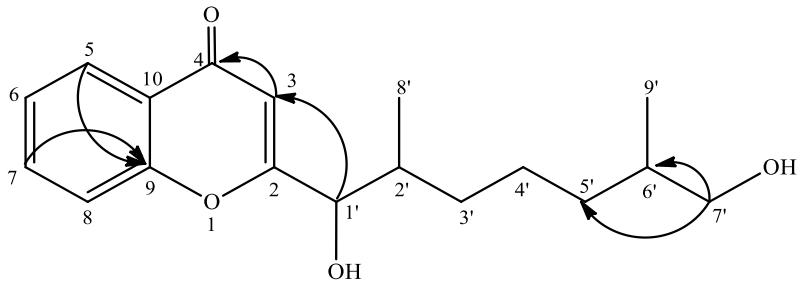
Small amounts of compounds (300 µg) were added to each disk in 25 µL of DMSO sample solution. Furthermore, a positive control disk containing 50 µg of standard Ceftriaxone/Amphotericin B was charged with 10 µL of Ceftriaxone/Amphotericin B solution in DMSO. Again, the dishes were kept undisturbed in an incubator for 24 hours. Finally, using a measuring scale, inhibition zones were measured. In the study, *S. aureus*, *B. subtilis* gram-positive bacteria and *S. typhimurium*, *E. coli* gram-negative bacteria were used. In addition, two antifungal *T. harzianum and A. niger*, strains were used to check antifungal activity.

CHAPTER – III
RESULTS & DISCUSSION
PAGE NO. (22-58)

3. Results & Discussions

3.1 Characterization of compound P1:

Compound **P1** (40 mg) was an amorphous solid of white color and melted at 182-183 °C. The IR spectrum of **P1** (Fig 3.1.2) revealed a broad absorption at $\bar{\upsilon}_{max}$ 3447 cm^{-1} for hydroxyl (OH^-) functional group and sharp absorption was observed at $\bar{\upsilon}_{max}$ 1690 cm^{-1} for carbonyl (=CO) functional group. ^{13}C NMR spectrum of **P1** (Fig.3.1.5) showed that there are 18 carbons present in the compound. Molecular ion peak at *m/z*: 305.5236 [M+1 was observed in mass spectrum of **P1** (Fig.3.1.8) that was corresponding to the molecular formula $C_{18}H_{24}O_4$. Previous phytochemical investigations revealed *Cassia fistula* was a rich source of chromones (Gao *et al*., 2014.). Compound **P1** was to be postulated based on a chromone skeleton along with a long alkyl chain. The ^1H NMR of compound **P1** (Fig.3.1.3) showed the presence of 6 methyl, 8 methylene and 8 methane protons in the molecule (Table 3.1). One olefinic proton appeared at δ 6.79 (s, 1H, H-3) and four aromatic protons attached to C5, C6, C7 and C8 are appeared at δ 7.35 (d, 1H, *J* = 7.6, H-5), 7.79 (t, 1H, *J* = 7.6, H-6), 7.81 (t, 1H, *J* = 7.6, H-7) and 8.34 (d, 1H, *J* = 7.6, H-8) respectively. ^{13}C NMR (DEPT) spectrum of **P1** (Fig.3.1.6) showed that 6 methyl carbons, 8 methine carbons and 8 methylene carbons are present in **P1**. Carbonyl carbon was confirmed by Chemical shift at δ 193.2(C4) from ^{13}C NMR spectrum of **P1**. Important connectivity and H-C correlations observed in the 2D HMBC (Fig. 3.1.9) spectrum are given in the Fig. 3.1. Based on the spectral data obtained from ^1H NMR & ^{13}C NMR, the structure **1** can be suggested as the compound **P1**. Finally compound **P1** is characterized as **2-(1′, 7′ -dihydroxy-2′, 6′ -dimethylheptyl)-4*H*-chromen-4-one. (1)**



(1)

Fig. 3.1: Important HMBC correlations of compound **P1**.

Table 3.1: ^{13}C NMR and ^{1}H NMR data for Compound **P1** (CD_3OD, δ, ppm, *J*/Hz).

C atom	^{13}C, δ	^{1}H, δ	COSY
2	136.9		
3	125.3	6.79 (s)	
4	193.2		
5	120.2	7.35 (d, *J* = 7.6)	H-6
6	123.9	7.79 (t, *J* = 7.6)	H-5,7
7	119.9	7.81 (t, *J* = 7.6)	H-6,8
8	119.2	8.34 (d, *J* = 7.6)	H-7
9	162.2		
10	113.5		
1′	54.5	3.97 (s)	
2′	56.4	3.32 (s)	
3′	31.6	1.30 (s)	
4′	29.3	1.30 (s)	
5′	29.0	1.30 (s)	
6′	32.3	1.9 (m)	
7′	77.4	3.32 (d, *J* = 2.8)	H-6′
8′	21.8	.91 (d, *J* = 4.8)	H-2′
9′	22.1	.91 (d, *J* = 4.8)	H-6′

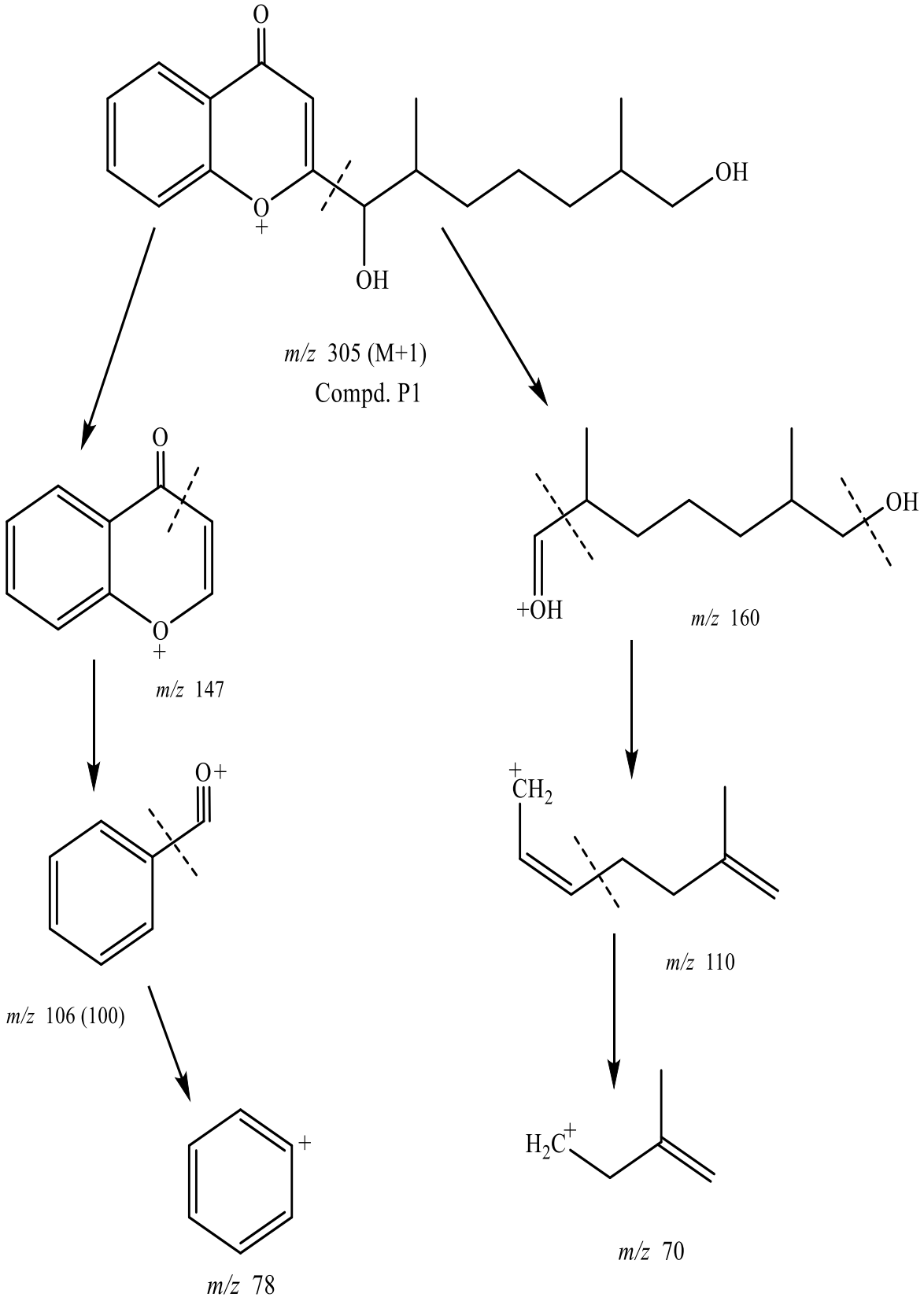


Fig.3.1.1 Mass fragmentations of compound P1.

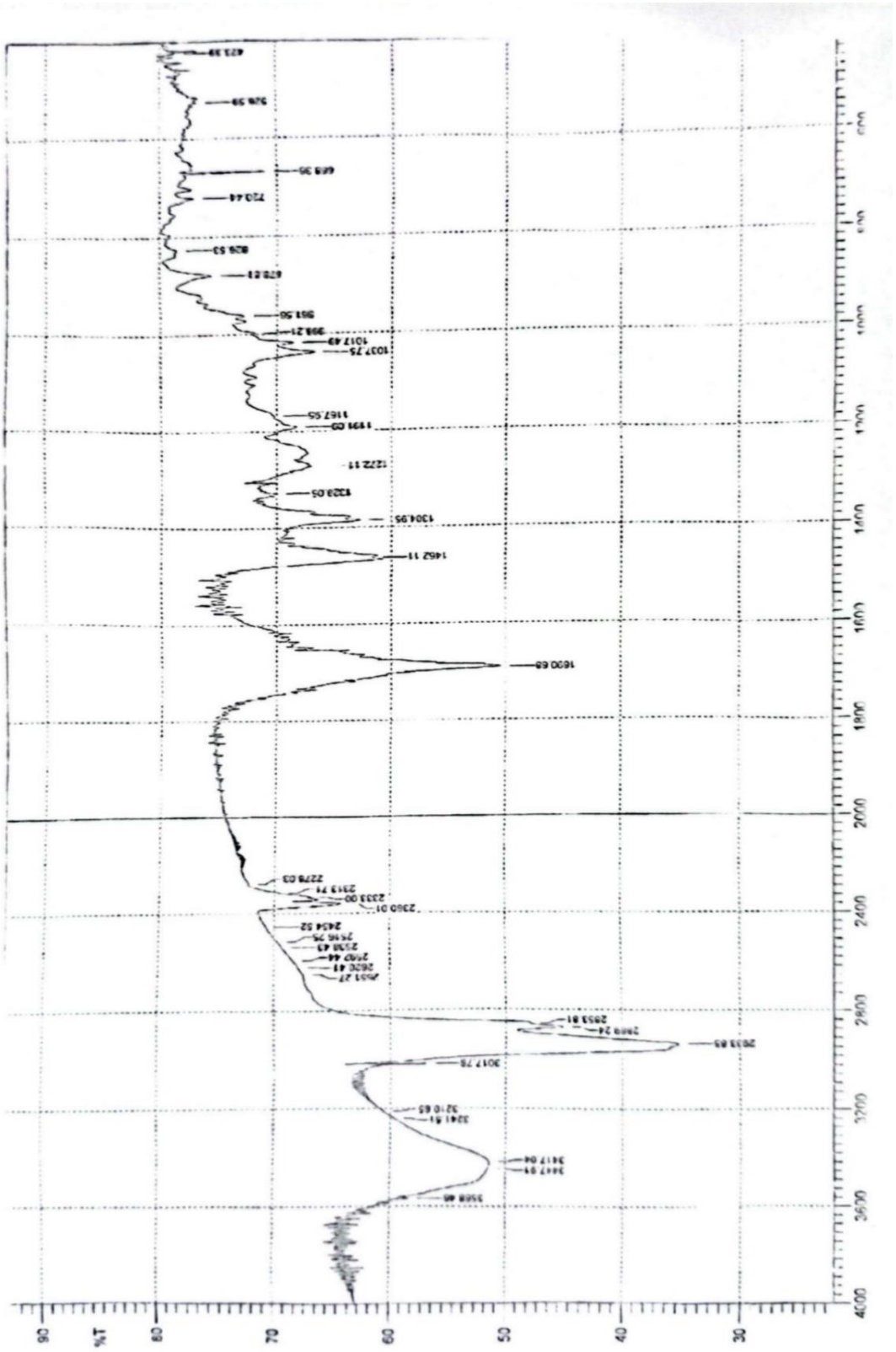
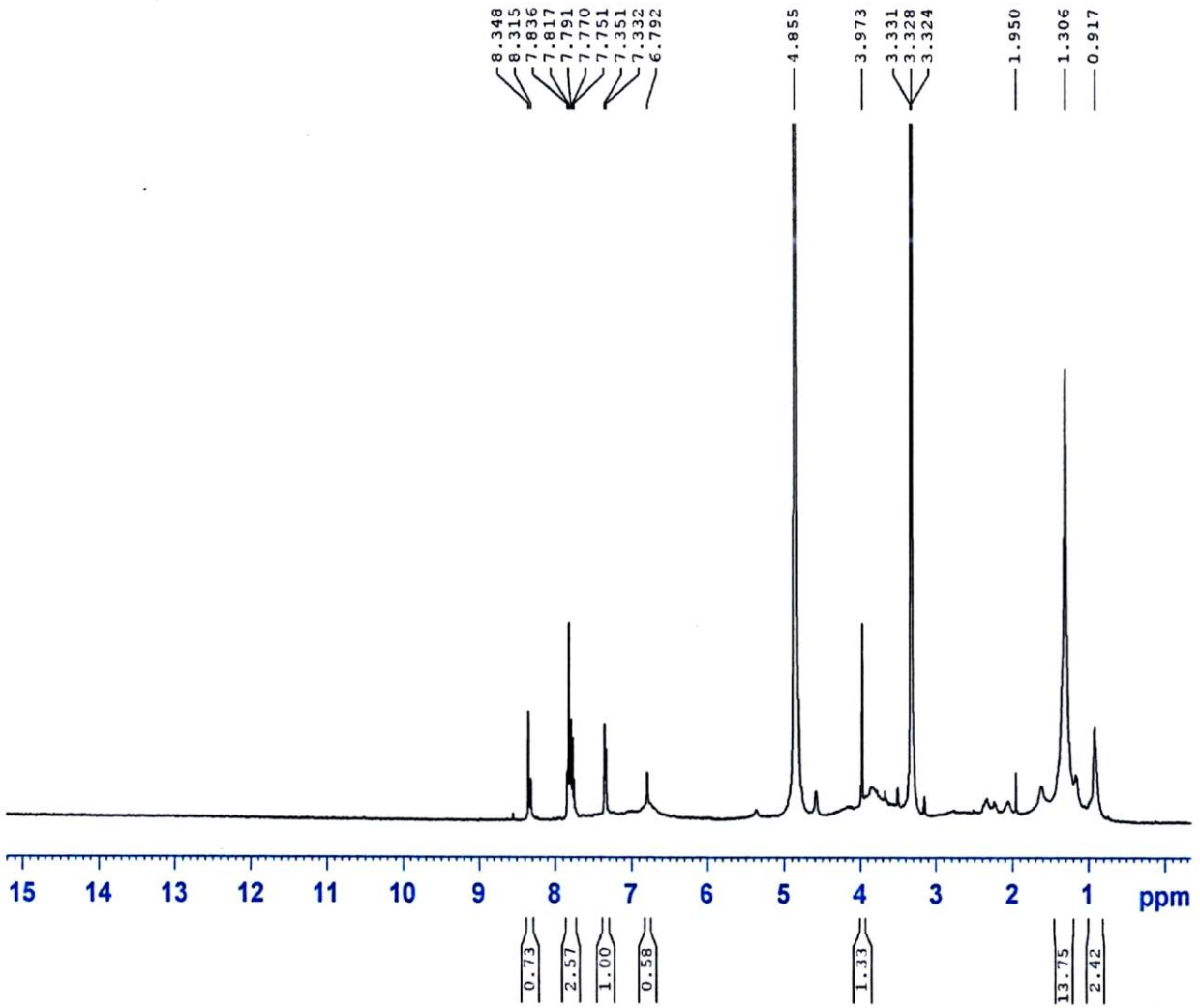
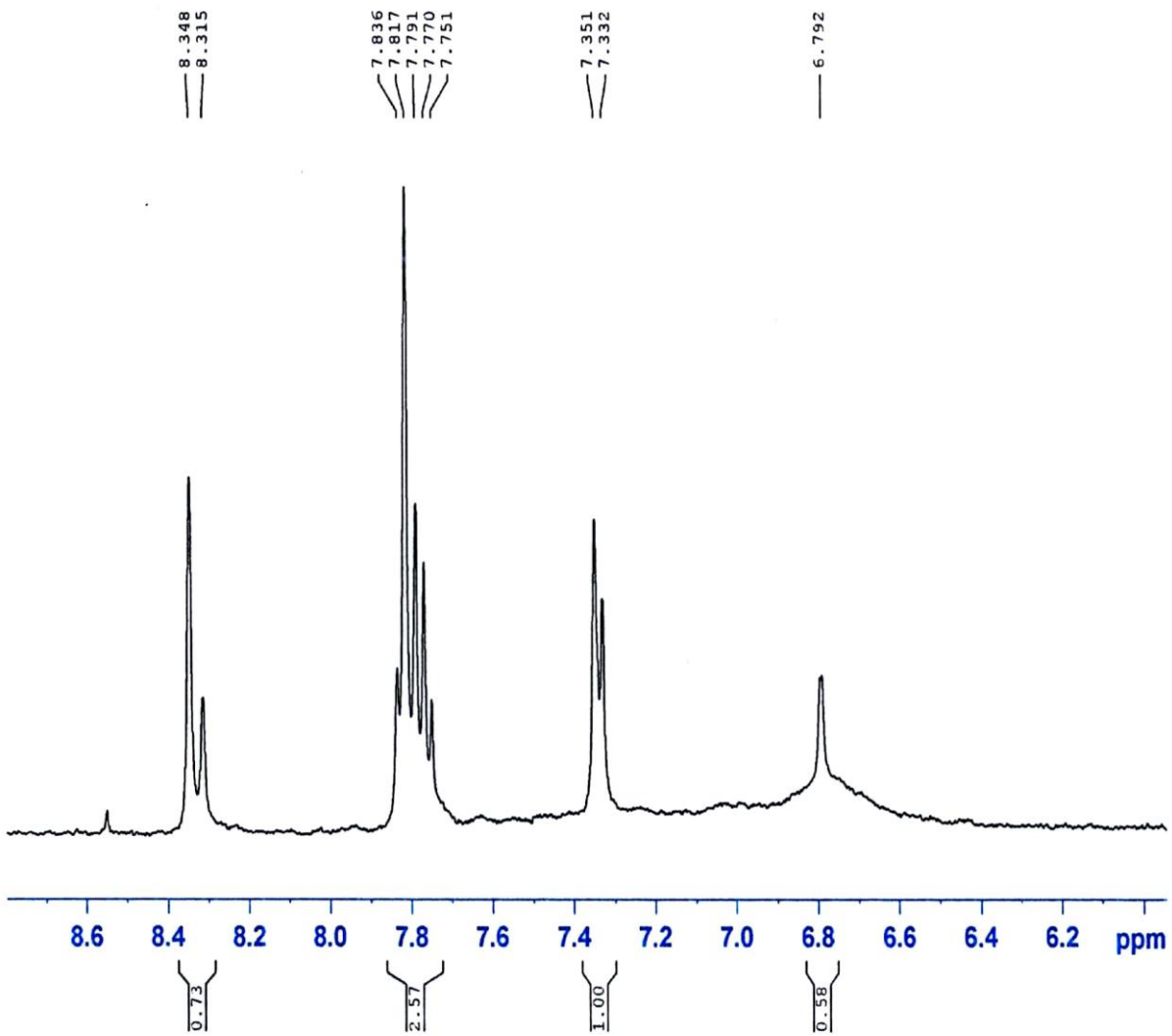


Fig 3.1.2: IR Spectrum of compound P1.




```
Current Data Parameters
NAME                  CUET_P1
EXPNO                       1
PROCNO                      1

F2 - Acquisition Paramete
Date_                20230416
Time                    15.24
INSTRUM                 spect
PROBHD   5 mm PABBO BB/
PULPROG                    zg
TD                      65536
SOLVENT                  MeOD
NS                         16
DS                          0
SWH                12019.230 H
FIDRES              0.183399 H
AQ                 2.7262976 s
RG                    142.06
DW                    41.600 u
DE                     6.50 u
TE                    300.9 K
D1                2.00000000 s
TD0                         1

======== CHANNEL f1 =====
SFO1             400.2340023 M
NUC1                      1H
P1                     11.20 u
PLW1             12.00000000 W

F2 - Processing parameter
SI                      131072
SF                 400.2300003 M
WDW                        EM
SSB          0
LB                     1.00 H
GB           0
PC                     1.00
```

Fig 3.1.3: ^{1}H NMR spectrum of compound P1.




```
Current Data Parameters
NAME                  CUET_P1
EXPNO                       1
PROCNO                      1

F2 - Acquisition Paramete
Date_                20230416
Time                    15.24
INSTRUM                 spect
PROBHD   5 mm PABBO BB/
PULPROG                    zg
TD                      65536
SOLVENT                  MeOD
NS                         16
DS                          0
SWH                12019.230 H
FIDRES              0.183399 H
AQ                 2.7262976 s
RG                    142.06
DW                    41.600 u
DE                     6.50 u
TE                     300.9 K
D1                 2.00000000 s
TD0                         1

======== CHANNEL f1 =====
SFO1             400.2340023 M
NUC1                      1H
P1                     11.20 u
PLW1            12.00000000 W

F2 - Processing parameter
SI                     131072
SF               400.2300003 M
WDW                       EM
SSB          0
LB                     1.00 H
GB           0
PC                     1.00
```

Fig 3.1.4 **^{1}H (Expand) spectrum of compound P1.**

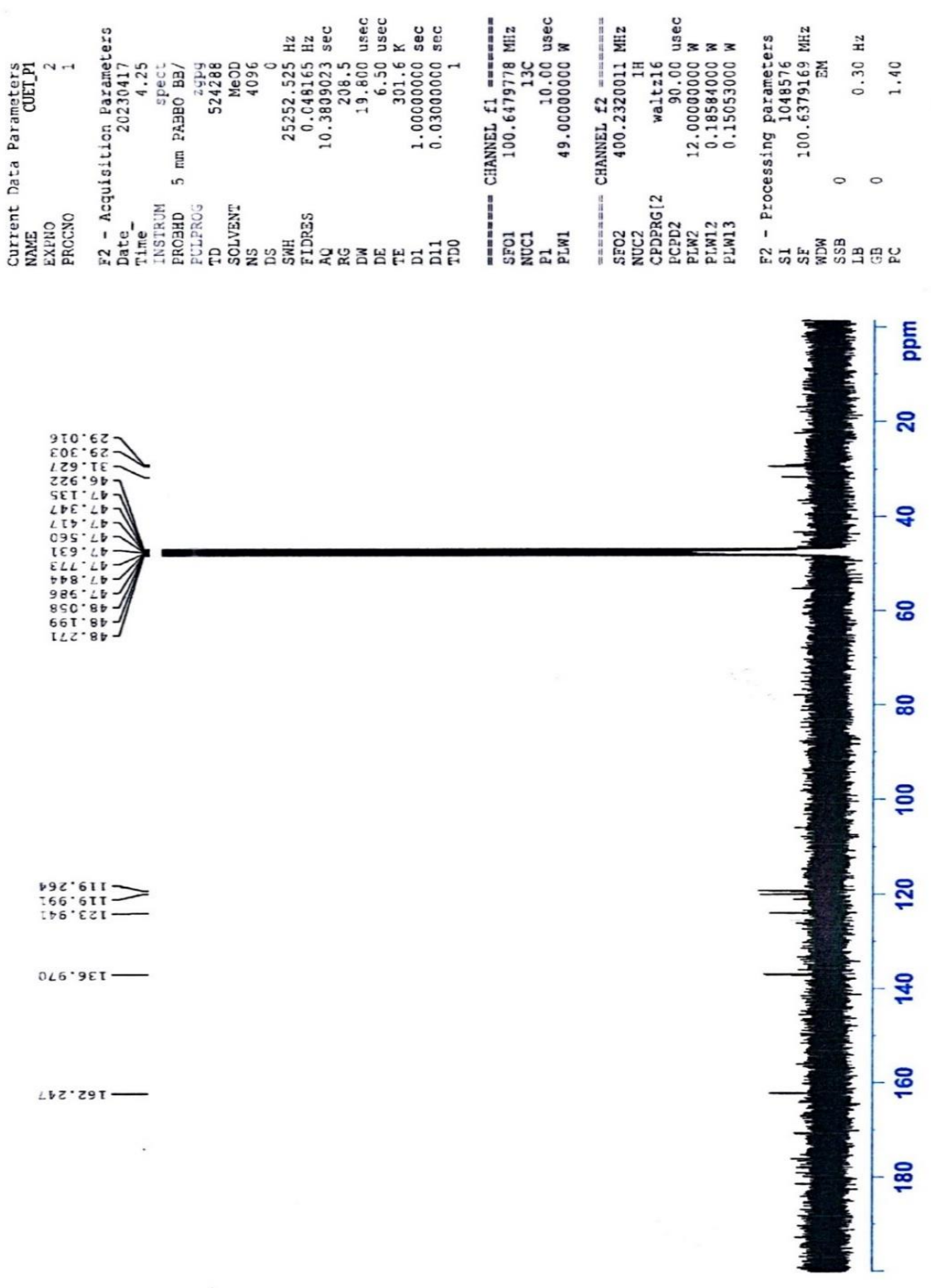
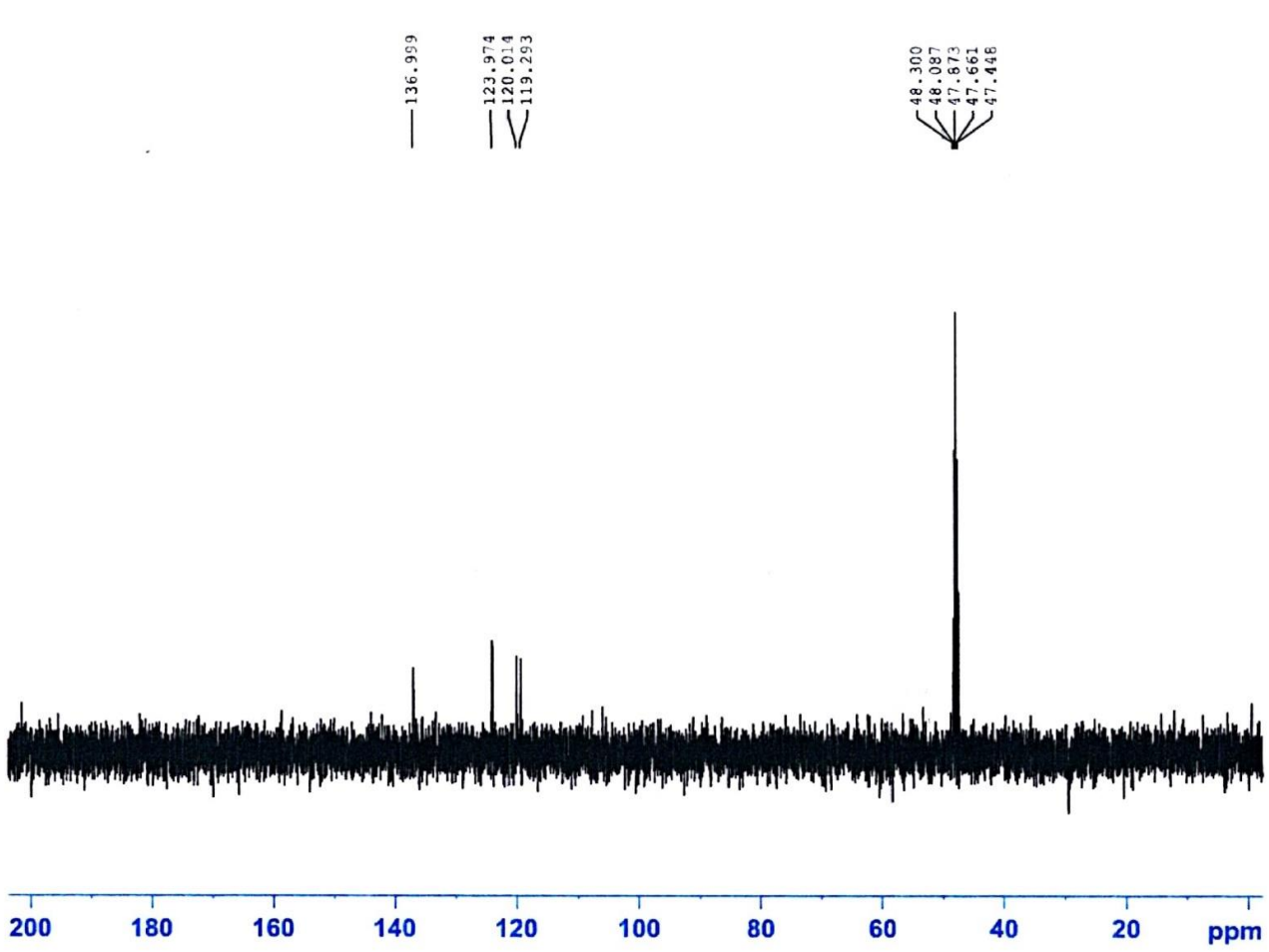
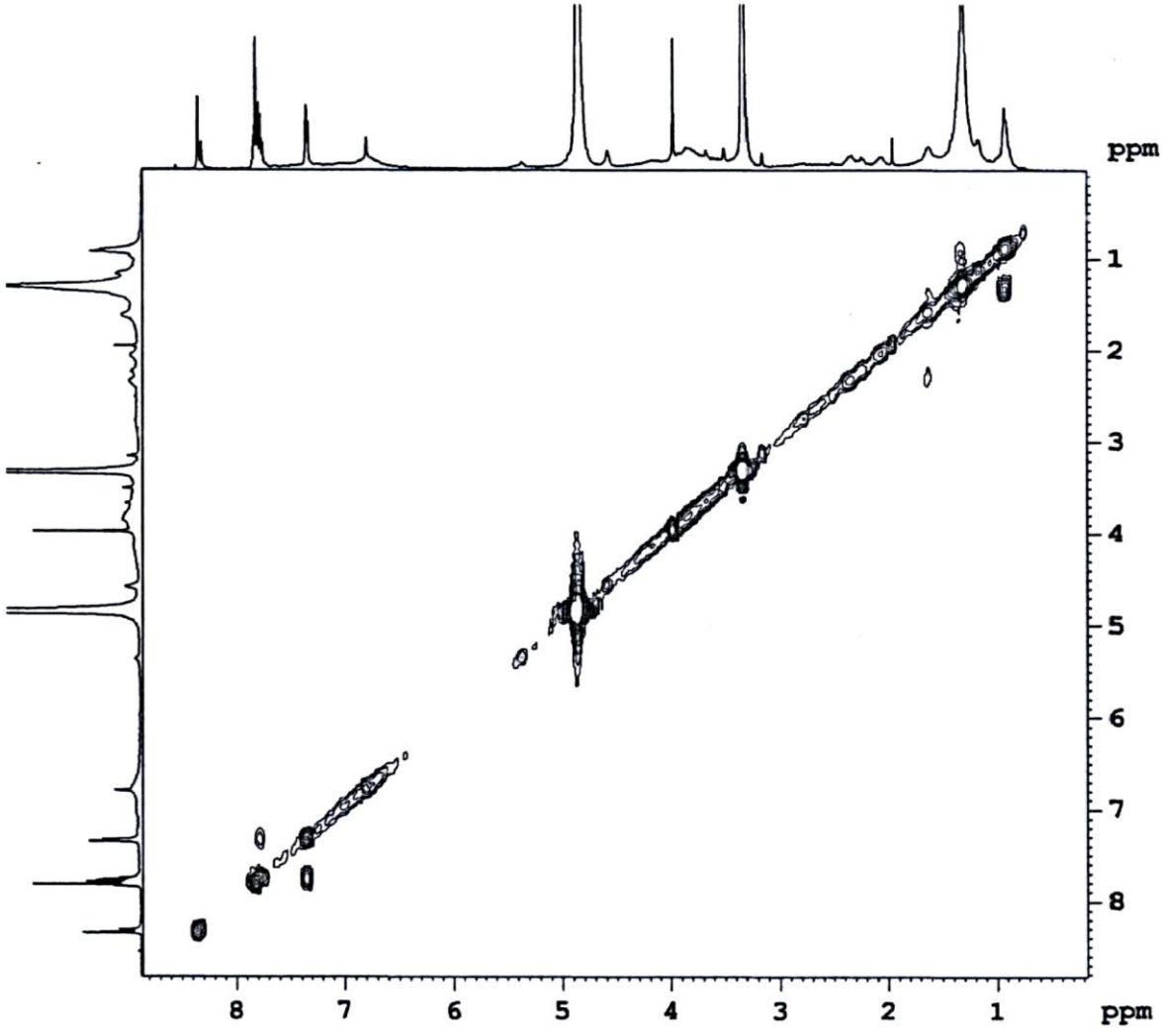


Fig 3.1.5 ^{13}**C NMR spectrum of compound P1.**




```
Current Data Parameters
NAME                  CUET_P1
EXPNO                       3
PROCNO                      1

F2 - Acquisition Parameters
Date_              20230417
Time                    7.05
INSTRUM                spect
PROBHD     5 mm PABBO BB/
PULPROG             deptsp135
TD                      65536
SOLVENT                 MeOD
NS                      4096
DS                         8
SWH                25252.525 Hz
FIDRES              0.385323 Hz
AQ                  1.2976128 sec
RG                      208.5
DW                     19.800 usec
DE                      6.50 usec
TE                     301.0 K
CNST2              145.0000000
D1                   1.00000000 sec
D2                   0.00344828 sec
D12                  0.00002000 sec
TD0                         1

======== CHANNEL f1 ========
SFO1               100.6479773 MHz
NUC1                       13C
P1                       10.00 usec
P13                    2000.00 usec
PLW0        0 W
PLW1              49.00000000 W
SPNAM[5]          Crp60comp.4
SPOAL5                 0.500
SPOFFS5     0 Hz
SPW5               7.48659992 W

======== CHANNEL f2 ========
SFO2               400.2320011 MHz
NUC2                       1H
CPDPRG[2                waltz16
P3                       11.20 usec
P4                       22.40 usec
PCPD2                    90.00 usec
PLW2              12.00000000 W
PLW12              0.18584000 W

F2 - Processing parameters
SI                      32768
SF                 100.6379135 MHz
WDW                        EM
SSB         0
LB                       1.00 Hz
GB          0
PC                       1.40
```

Fig 3.1.6 **^{13}C NMR (DEPT) spectrum of compound P1.**




```
Current Data Parameters
NAME                  CUET_P1
EXPNO                       4
PROCNO                      1

F2 - Acquisition Parameters
Date_               20230417
Time                    7.06
INSTRUM                spect
PROBHD   5 mm PABBO BB/
PULPROG             cosygpqf
TD                      2048
SOLVENT                 MeOD
NS                         4
DS                        16
SWH                12019.230 Hz
FIDRES              5.868765 Hz
AQ                  0.0851968 sec
RG                      208.5
DW                     41.600 usec
DE                      6.50 usec
TE                     300.7 K
D0                 0.00000300 sec
D1                 1.00000000 sec
D13                0.00000400 sec
D16                0.00020000 sec
IN0                0.00008320 sec

======== CHANNEL f1 ========
SFO1             400.2340023 MHz
NUC1                      1H
P0                     11.20 usec
P1                     11.20 usec
PLW1              12.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]          SMSQ10.100
GPZ1                   10.00 %
P16                  1000.00 usec

F1 - Acquisition parameters
TD                        256
SFO1                  400.234 MHz
FIDRES              46.950119 Hz
SW                     30.031 ppm
FnMODE                     QF

F2 - Processing parameters
SI                       2048
SF                400.2299975 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
PC                       1.40

F1 - Processing parameters
SI                       1024
MC2                        QF
SF                400.2300029 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
```

Fig 3.1.7 2D-COSY spectrum of compound P1.

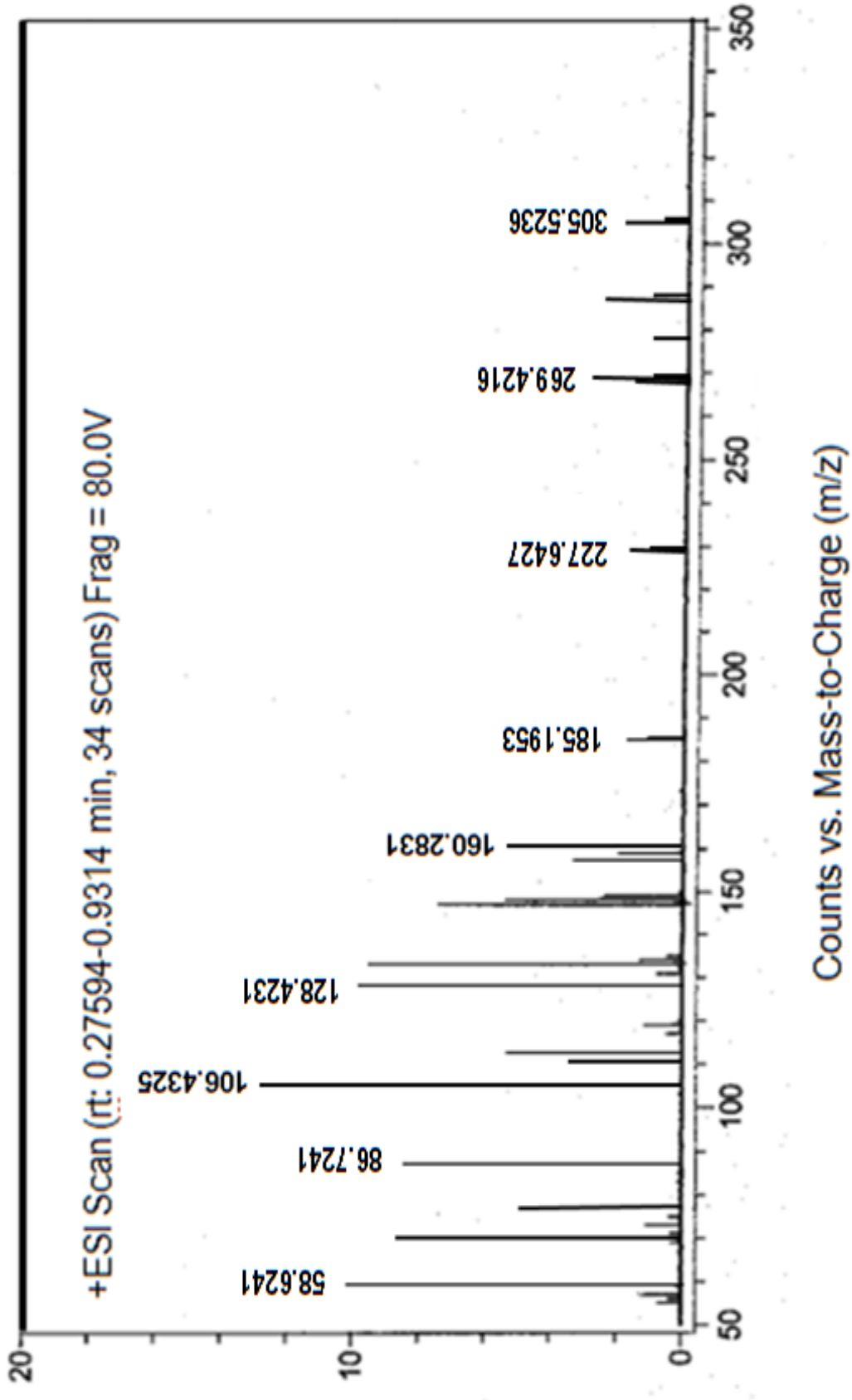
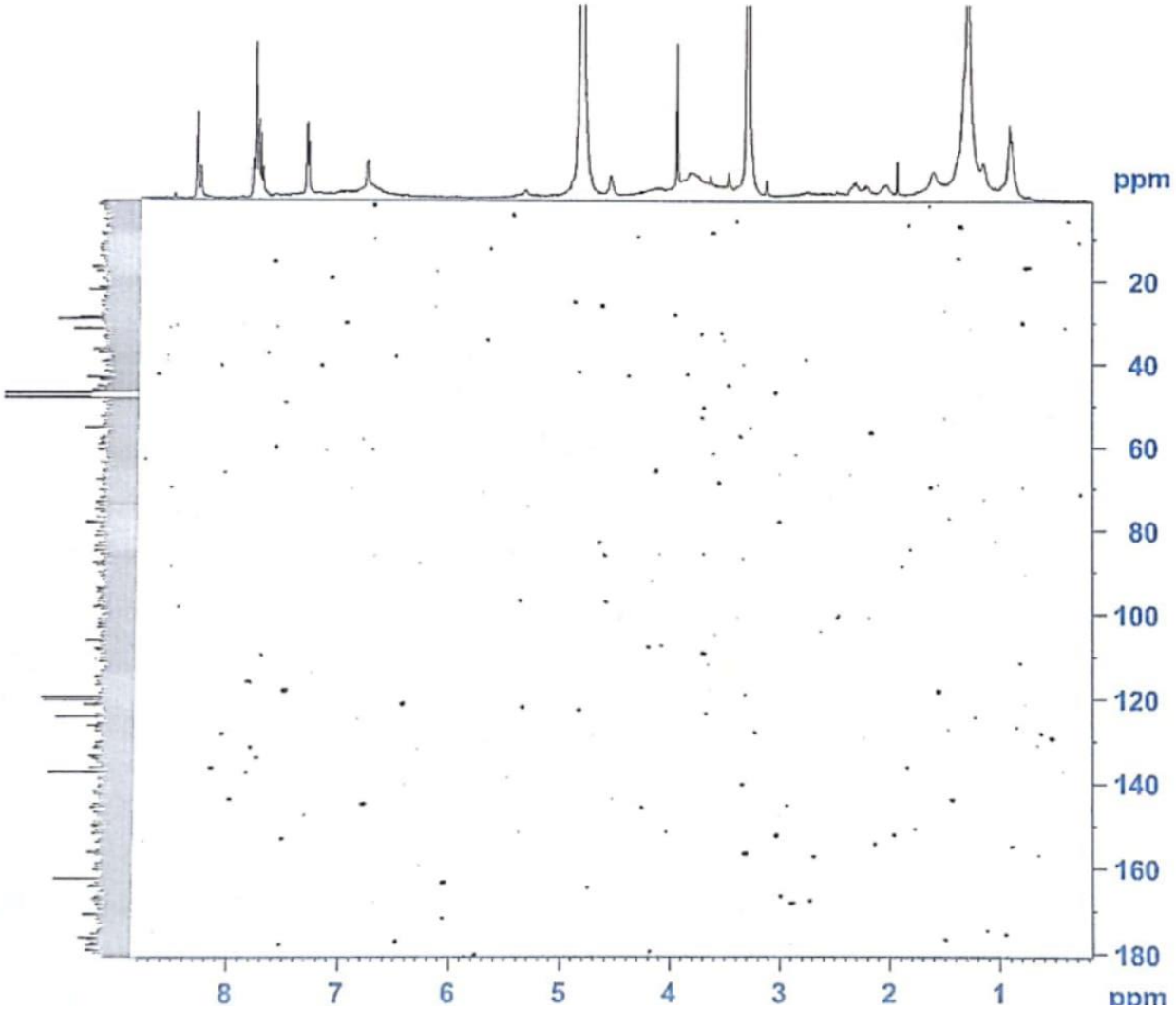


Fig 3.1.8 Mass spectrum of compound P1.

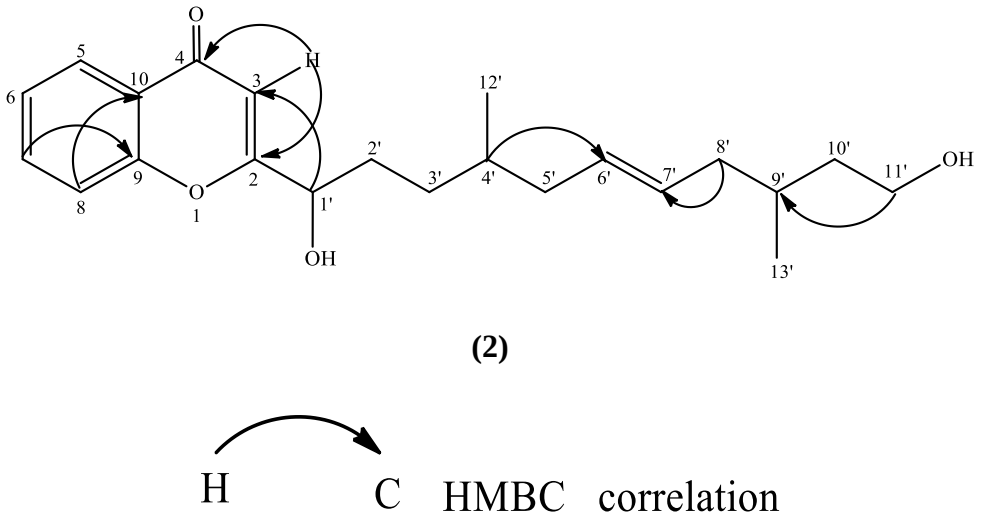



```
Current Data Parameters
NAME                  CUET_P1
EXPNO                       5
PROCNO                      1

F2 - Acquisition Parameters
Date_              20230417
Time                   7.45
INSTRUM                spect
PROBHD     5 mm PABBO BB/
PULPROG    hmbcgplpndqf
TD                     2048
SOLVENT                MeOD
NS                        4
DS                       16
SWH              12019.230 Hz
FIDRES               5.869745 Hz
AQ                 0.0851989 sec
RG                     208.5
DW                  41.600 usec
DE                   6.50 usec
TE                  300.0 K
CNST2            145.0000000
CNST13            10.0000000
D0                 0.00000400 sec
D1                 1.00000000 sec
D2                 0.00344828 sec
D6                 0.05000000 sec
D16                0.00020000 sec
IN0                0.00001980 sec

======== CHANNEL f1 ========
SFO1             400.2340023 MHz
NUC1                      1H
P1                   11.20 usec
P2                   22.40 usec
PLW1            12.00000000 W

======== CHANNEL f2 ========
SFO2             100.6479773 MHz
NUC2                      13C
P3                   10.00 usec
PLW2            49.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]         SMSQ10.100
GPNAM[2]         SMSQ10.100
GPNAM[3]         SMSQ10.100
GPZ1                 50.00 %
GPZ2                 30.00 %
GPZ3                 40.10 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      512
SFO1             100.648 MHz
FIDRES           49.331939 Hz
SW                 250.899 ppm
FnMODE                  QF

F2 - Processing parameters
SI                     2048
SF               400.2300000 MHz
WDW                    SINE
SSB          0
LB           0 Hz
GB           0
PC                     1.40

F1 - Processing parameters
SI                     1024
MC2                    QF
SF               100.6379135 MHz
WDW                    SINE
SSB          0
LB           0 Hz
```

Fig 3.1.9 HMBC spectrum of compound P1.

3.2 Characterization of compound P2:

Compound **P2** (120 mg) was an amorphous solid and melted at 165-166°C. The IR spectrum (Fig. 3.2.2) of P2 showed characteristic absorption band at $\bar{\upsilon}_{max}$ 3419 cm^{-1} for hydroxyl (OH^-) functional group and 1701 cm^{-1} for carbonyl(=CO) functional group. The highest molecular ion peak *m/z* 359.4272 [M+1] of compound **P2** (Fig. 3.2.10) afforded its molecular formula $C_{22}H_{30}O_4$ which was consistent with ^{13}C NMR spectrum of **P2** (Fig.3.2.5). Comparison of the ^1H NMR (Table 3.2) and ^{13}C NMR data (Table 3.2) of **P2** with that of P1 suggested that **P2** has same chromone moiety along with a long alkyl chain additional one double bond in the chain. The ^1H NMR spectrum of compound **P2** (Fig.3.2.3) showed four aromatic proton absorptions at δ 7.35 (d, 1H, *J* = 7.6, H-5), 7.79 (t, 1H, *J* = 7.6, H-6), 7.81 (t, 1H, *J* = 7.6, H-7) and 8.34 (d, 1H, *J* = 7.6, H-8) attached to the carbons C5, C6, C7 and C8 respectively. One olefinic proton appeared at δ 6.79 (s, 1H, H-3), a multiplet at δ 5.36 (m) appeared due to two olefinic protons attached to the carbon C6′ and C7′ in the molecule. Protons of two methyl groups attached to the carbon C12′and C13′ appeared at δ .93 (d,3H, *J* = 6.0, H-12′) and .90 (d, 3H, *J* = 6.0, H-13′) respectively. The presence of 6 methylene carbons in the molecule were confirmed from the ^{13}C NMR (DEPT 135°) spectrum of **P2** (Fig.3.2.7). Absorption at δ 192.6 is due to the presence of one carbonyl carbon (C4) in the molecule. Compound P2 showed 6 aromatic carbon absorptions at δ 120.2(C5), 123.9(C6), 119.9(C7), 119.2(C8), 162.2(C9), 113.5(C10) and four olefinic carbon absorptions at δ 136.9(C2), 125.3(C3), 124.2(C6′) and 124.3(C7′). The connectivity and citation of side chains and functional groups are located analyzing HMBC spectrum (Fig. 3.2.8). Important mass fragments of compound P2 *m/z* 359.4272 [M+1], 214, 164, 147, 106(100), 78, 70 etc. are shown in Fig.3.2.1. On the basis of the above spectral data structure (**2**) has been assigned to compound **P2**. Hence the structure of compound **P2** was assigned as **(*E*)-2-(1′, 11′ -dihydroxy-4′, 9′ -dimethylundee-6′ -en-1′ -yl)-4*H*-chromen-4-one. (2)**



(2)

Fig. 3.2: Important HMBC correlations of compound P2.

Table 3.2: ^{13}C NMR and ^{1}H NMR data for Compound **P2** (CD_3OD, δ, ppm, *J*/Hz).

C atom	^{13}C, δ	^{1}H, δ	COSY
2	136.9		
3	125.3	6.79 (s)	
4	192.6		
5	120.2	7.35 (d, *J* = 7.6)	H-6
6	123.9	7.79 (t, *J* = 7.6)	H-5,7
7	119.9	7.81 (t, *J* = 7.6)	H-6,8
8	119.2	8.34 (d, *J* = 7.6)	H-7
9	162.2		
10	113.5		
1′	54.5	3.79 (t, *J* = 6.0)	H-2′
2′	56.4	3.32 (m)	
3′	31.6	1.30 (m)	
4′	33.5	1.62 (m)	
5′	29.3	2.32 (m)	
6′	124.2	5.36 (m)	
7′	124.3	5.36 (m)	
8′	29.0	2.32 (m)	
9′	32.3	1.62 (m)	
10′	28.8	1.30 (m)	
11′	77.1	3.32 (t, *J* = 3.2)	H-10′
12′	12.8	.93 (d, *J* = 6.0)	H-4′
13′	12.9	.90 (d, *J* = 6.0)	H-9′

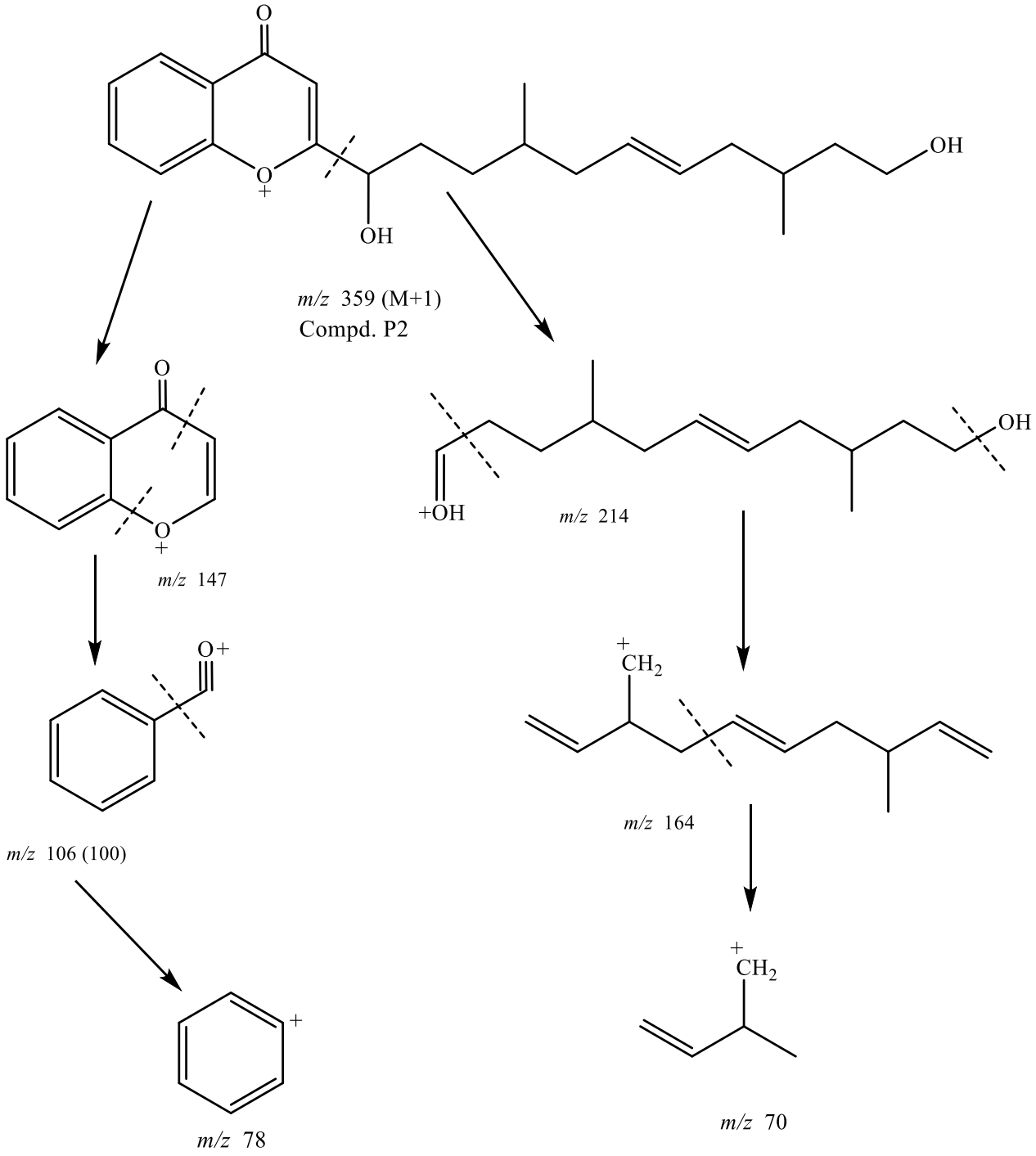


Fig 3.2.1 Mass fragmentation of compound P2.

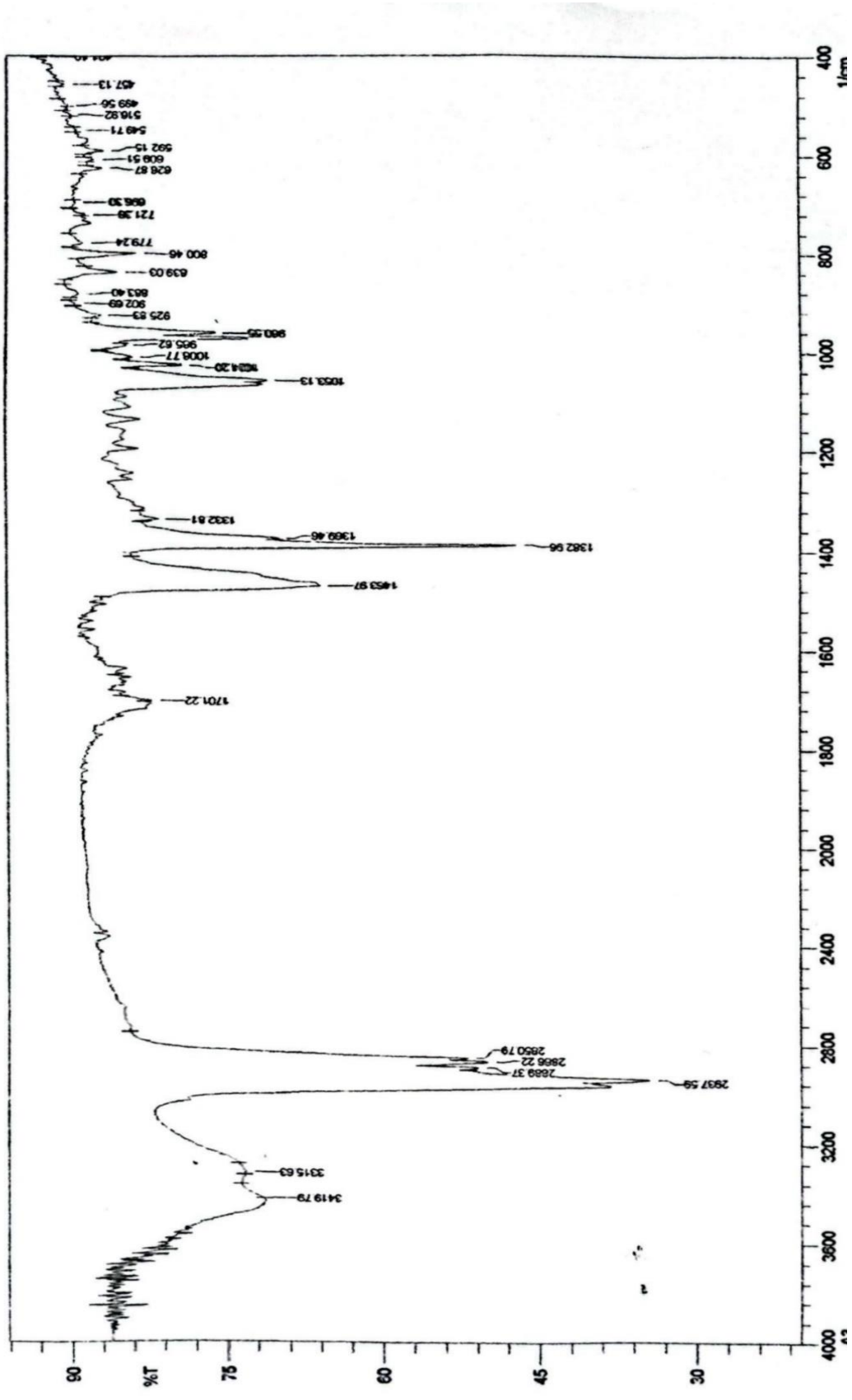
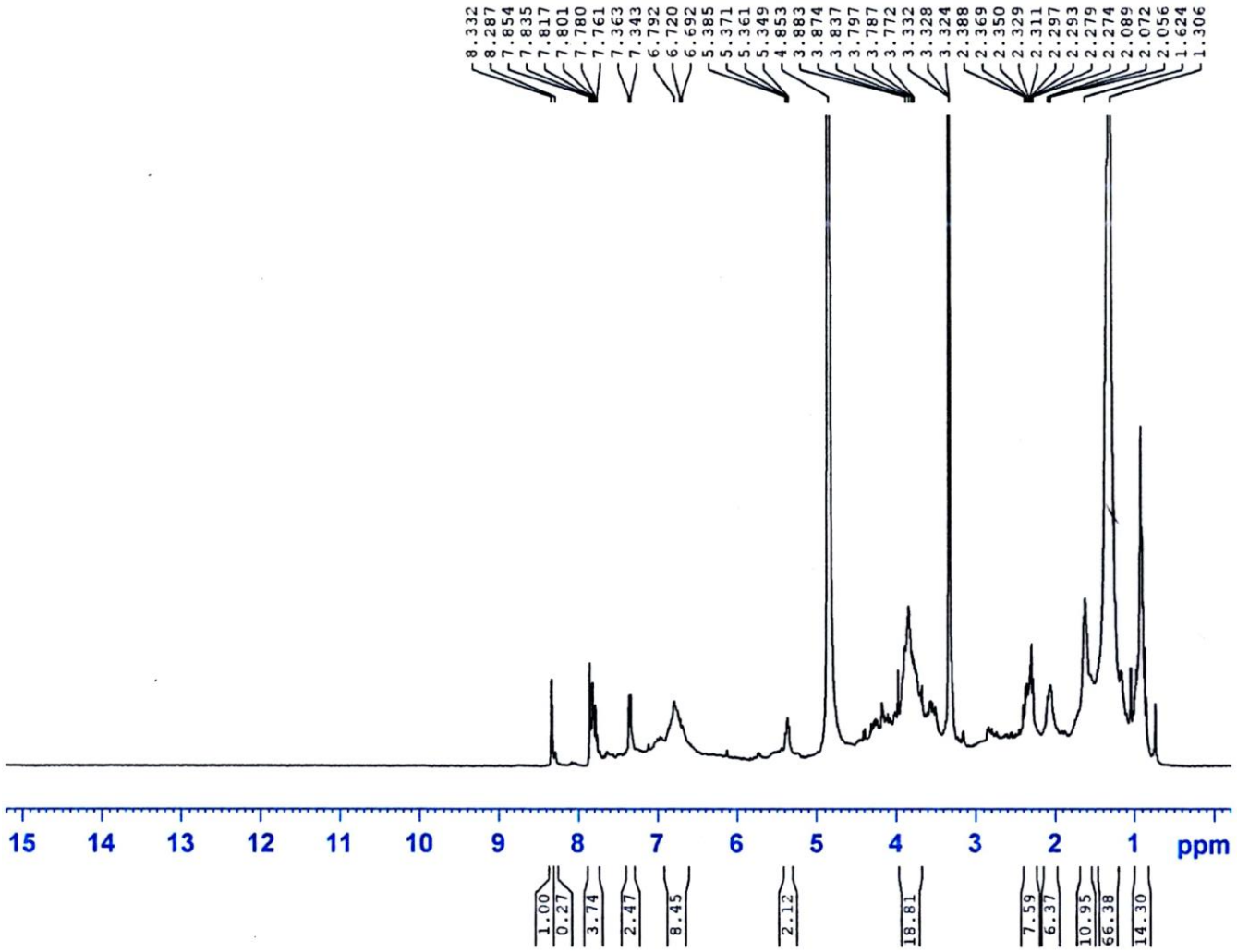
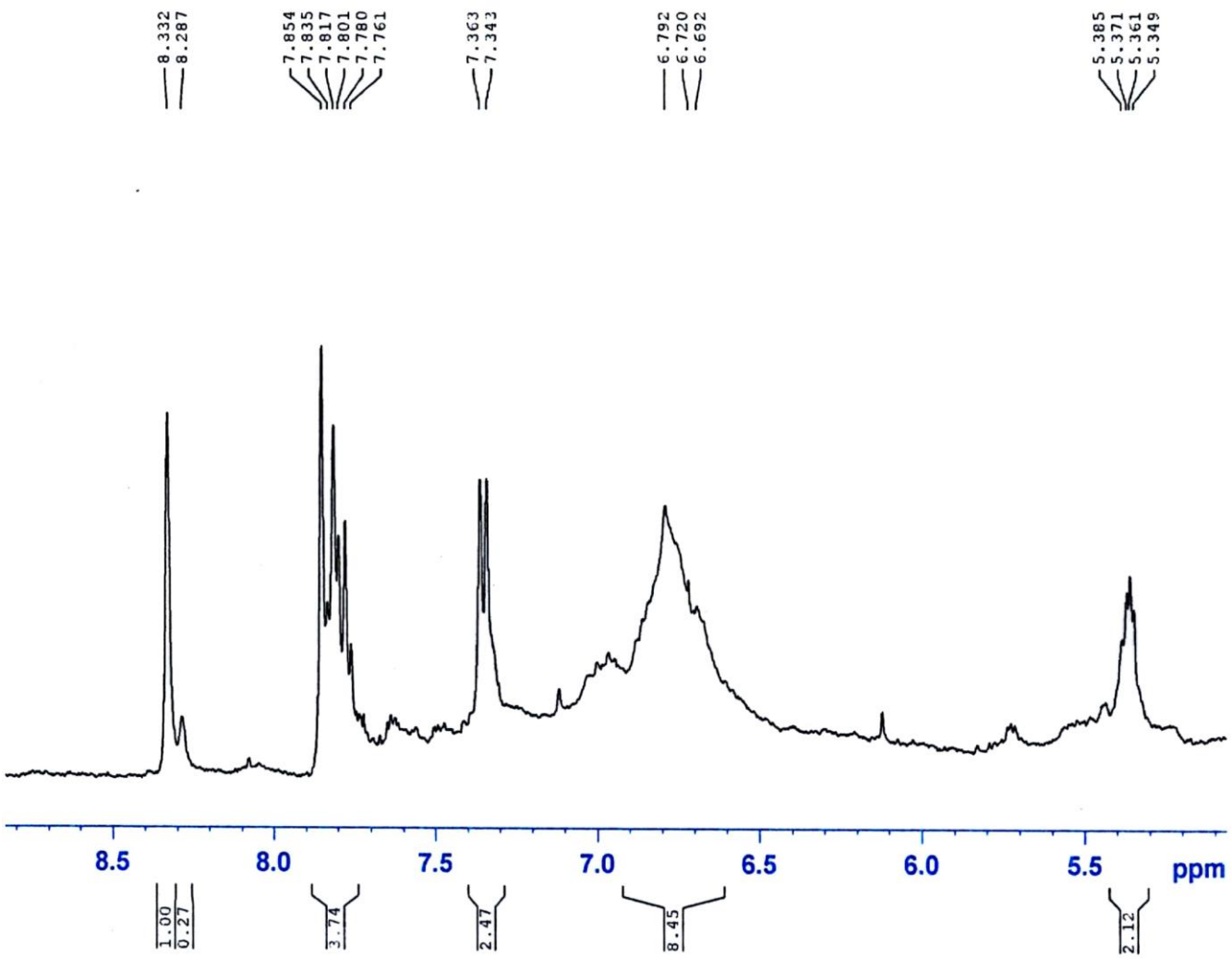


Fig 3.2.2 IR spectrum of compound P2.



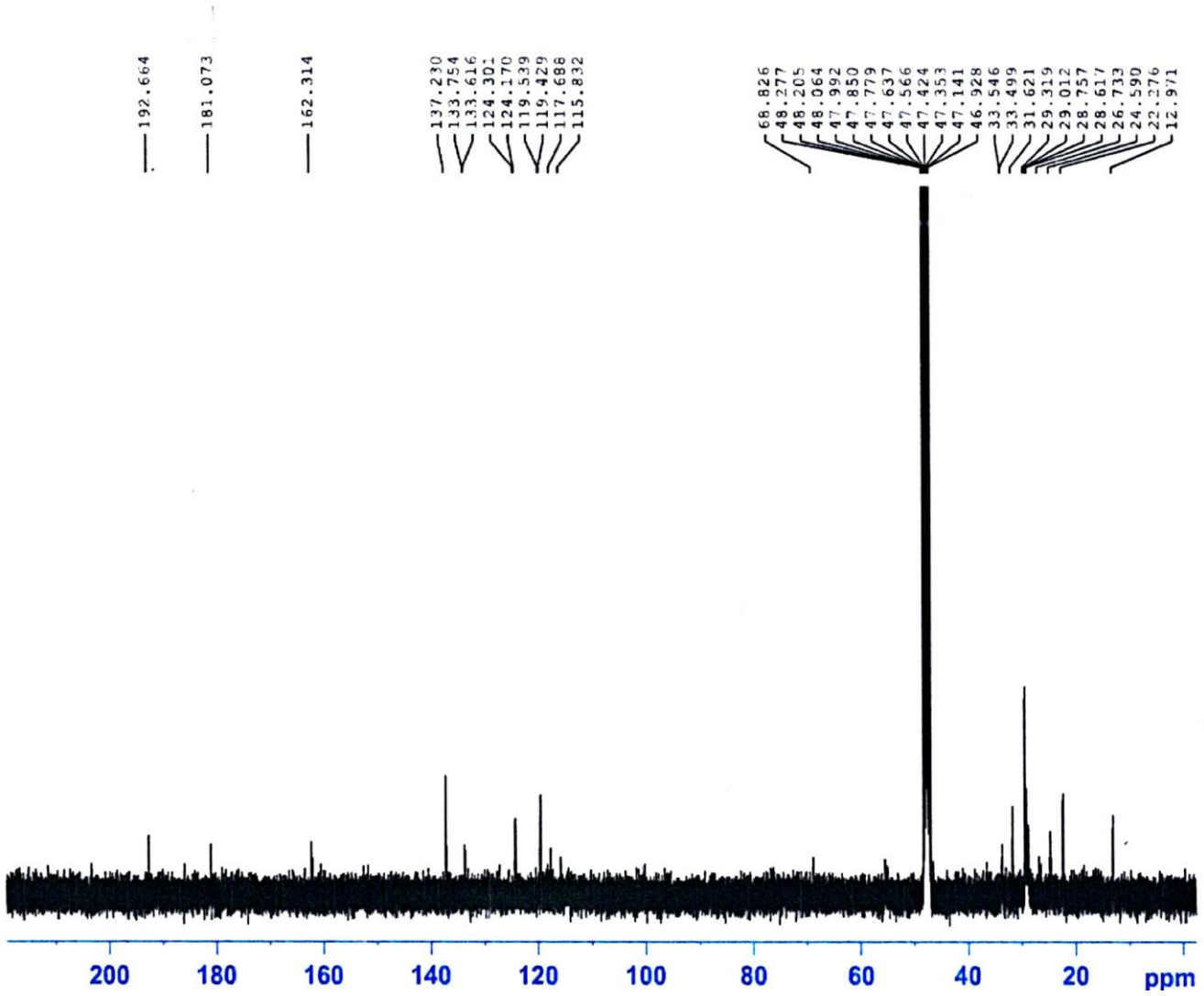

```
Current Data Parameters
NAME                  CUET_P2
EXPNO                       1
PROCNO                      1

F2 - Acquisition Paramete
Date_                20230417
Time                    13.42
INSTRUM                 spect
PROBHD    5 mm PABBO BB/
PULPROG                   zg
TD                     65536
SOLVENT                 MeOD
NS                        16
DS                         0
SWH                12019.230 H
FIDRES              0.183399 H
AQ                  2.7262976 s
RG                     103.88
DW                     41.600 u
DE                      6.50 u
TE                     301.3 K
D1                 2.00000000 s
TD0                         1

======== CHANNEL f1 =====
SFO1            400.2340023 M
NUC1                     1H
P1                    11.20 u
PLW1           12.00000000 W

F2 - Processing parameter
SI                     131072
SF                400.2300003 M
WDW                       EM
SSB          0
LB                     1.00 H
GB           0
PC                     1.00
```

Fig 3.2.3 ^{1}H spectrum of compound P2.



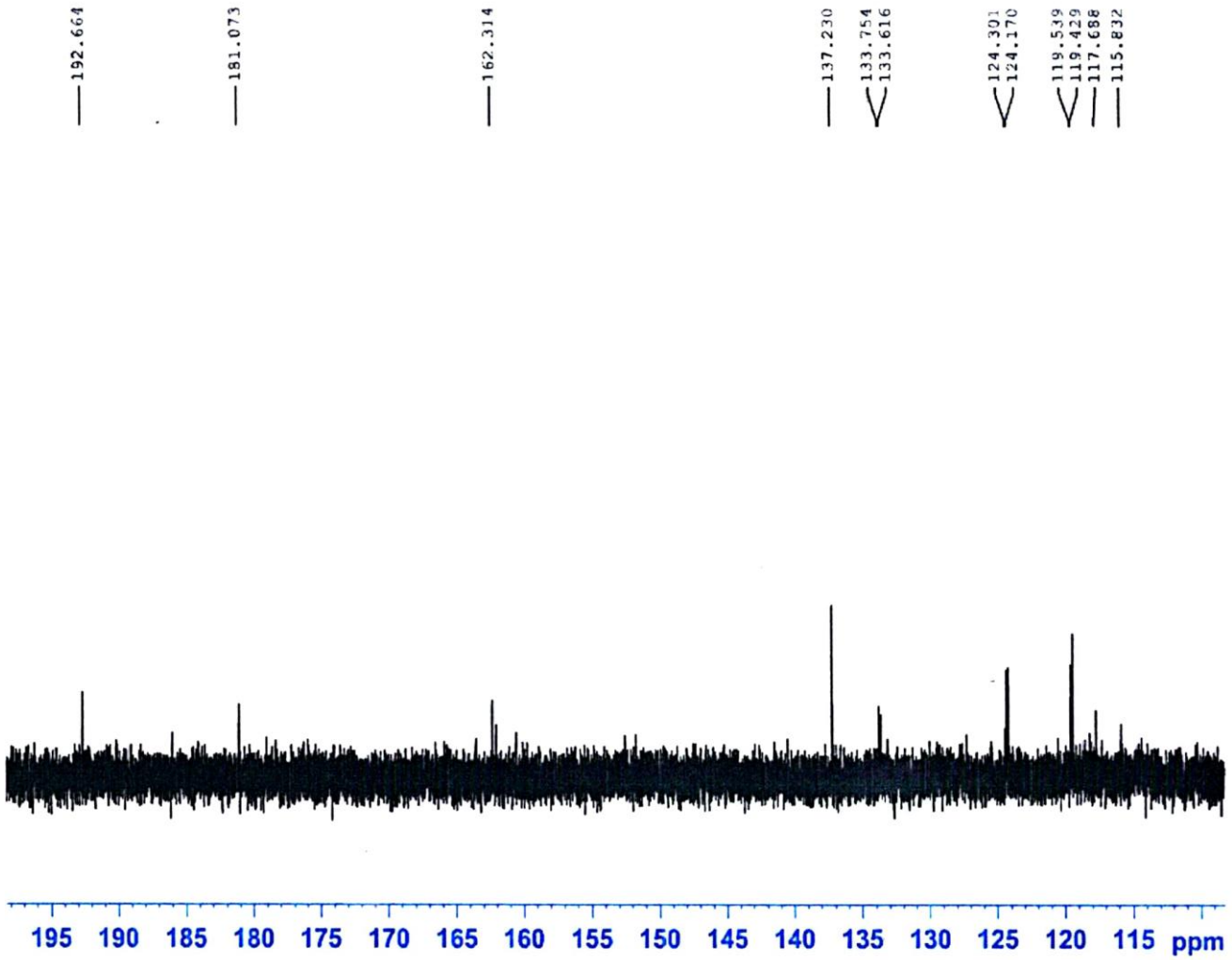

```
Current Data Parameters
NAME                  CUET_P2
EXPNO                       1
PROCNO                      1

F2 - Acquisition Paramete
Date_                20230417
Time                    13.42
INSTRUM                 spect
PROBHD    5 mm PABBO BB/
PULPROG                   zg
TD                     65536
SOLVENT                 MeOD
NS                        16
DS                         0
SWH                12019.230 H
FIDRES              0.183399 H
AQ                 2.7262976 s
RG                    103.88
DW                    41.600 u
DE                     6.50 u
TE                    301.3 K
D1                2.00000000 s
TD0                         1

======== CHANNEL f1 =====
SFO1           400.2340023 M
NUC1                    1H
P1                   11.20 u
PLW1          12.00000000 W

F2 - Processing parameter
SI                     131072
SF                400.2300003 M
WDW                       EM
SSB          0
LB                    1.00 H
GB           0
PC                    1.00
```

Fig 3.2.4 ^{1}H (Expand) spectrum of compound P2.



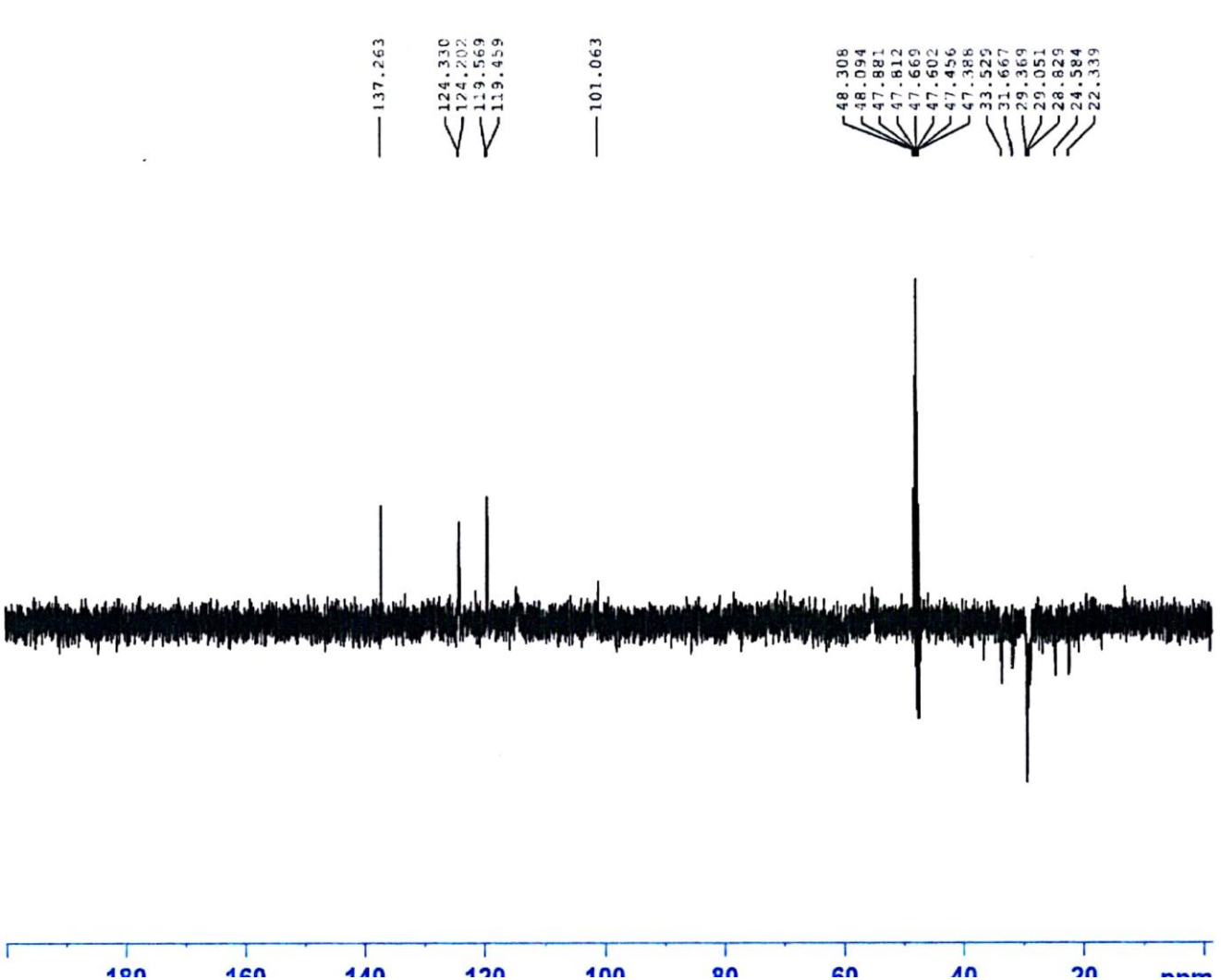

```
Current Data Parameters
NAME                  CUET_P2
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_                20230418
Time                     2.43
INSTRUM                  spect
PROBHD    5 mm PABBO BB/
PULPROG                  zgpg
TD                     524288
SOLVENT                  MeOD
NS                       4096
DS                          0
SWH                25252.525 Hz
FIDRES              0.048165 Hz
AQ                 10.3809023 sec
RG                      208.5
DW                     19.800 usec
DE                      6.50 usec
TE                     302.3 K
D1                 1.00000000 sec
D11                0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
SFO1             100.6479778 MHz
NUC1                     13C
P1                     10.00 usec
PLW1              49.00000000 W

======== CHANNEL f2 ========
SFO2             400.2320011 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                  90.00 usec
PLW2              12.00000000 W
PLW12              0.18584000 W
PLW13              0.15053000 W

F2 - Processing parameters
SI                     1048576
SF               100.6379169 MHz
WDW                         EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.40
```

Fig 3.2.5 **^{13}C NMR spectrum of compound P2.**



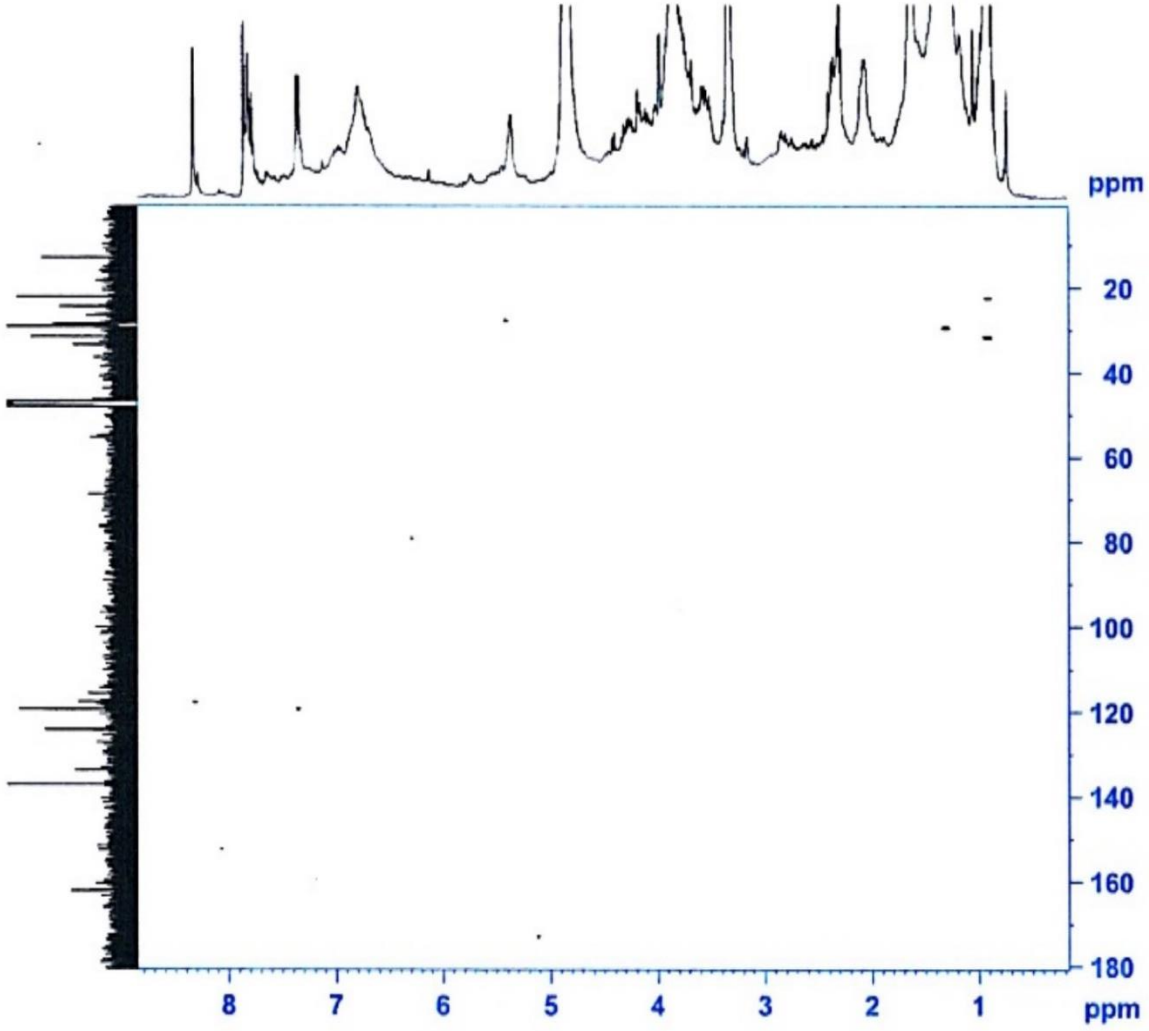

```
Current Data Parameters
NAME                  CUET_P2
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_                20230418
Time                     2.43
INSTRUM                  spect
PROBHD    5 mm PABBO BB/
PULPROG                  zgpg
TD                     524288
SOLVENT                  MeOD
NS                       4096
DS                          0
SWH                25252.525 Hz
FIDRES              0.048165 Hz
AQ                 10.3809023 sec
RG                      208.5
DW                     19.800 usec
DE                      6.50 usec
TE                     302.3 K
D1                 1.00000000 sec
D11                0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
SFO1             100.6479778 MHz
NUC1                     13C
P1                     10.00 usec
PLW1              49.00000000 W

======== CHANNEL f2 ========
SFO2             400.2320011 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                  90.00 usec
PLW2              12.00000000 W
PLW12              0.18584000 W
PLW13              0.15053000 W

F2 - Processing parameters
SI                     1048576
SF               100.6379169 MHz
WDW                         EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.40
```

Fig 3.2.6 ^{13}C NMR (Expand.) spectrum of compound P2 .



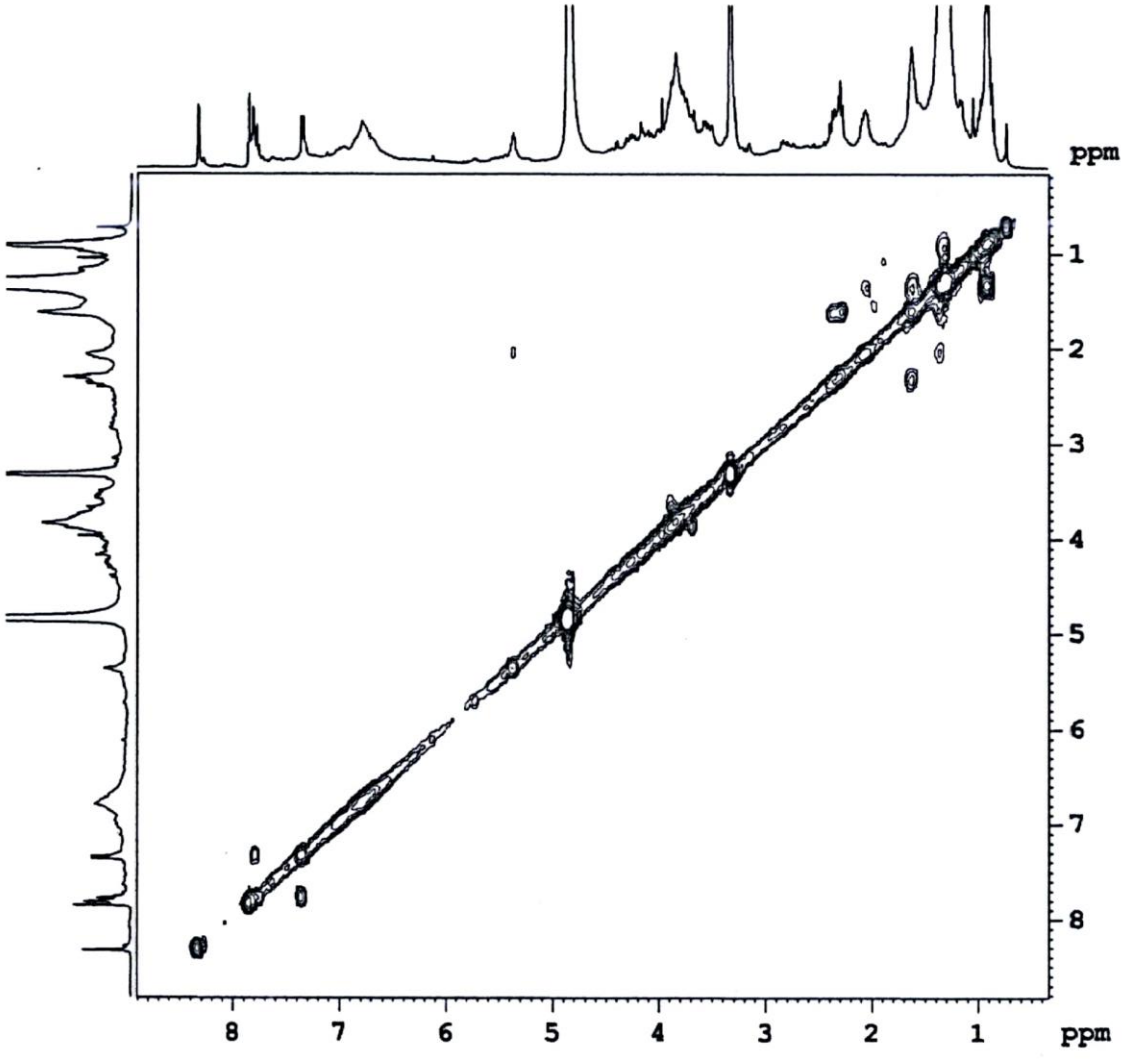

```
Current Data Parameters
NAME                  CUET_P2
EXPNO                       3
PROCNO                      1

F2 - Acquisition Parameters
Date_              20230418
Time                   5.24
INSTRUM               spect
PROBHD    5 mm PABBO BB/
PULPROG          deptsp135
TD                   65536
SOLVENT                MeOD
NS                     4096
DS                        8
SWH               25252.525 Hz
FIDRES             0.385323 Hz
AQ                 1.2976128 sec
RG                     208.5
DW                    19.800 usec
DE                     6.50 usec
TE                    301.5 K
CNST2             145.0000000
D1                 1.00000000 sec
D2                 0.00344828 sec
D12                0.00002000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1              100.6479773 MHz
NUC1                      13C
P1                      10.00 usec
P13                   2000.00 usec
PLW0        0 W
PLW1             49.00000000 W
SPNAM[5]      Crp60comp.4
SPOAL5                 0.500
SPOFFS5     0 Hz
SPW5              7.48659992 W

======== CHANNEL f2 ========
SFO2              400.2320011 MHz
NUC2                      1H
CPDPRG[2               waltz16
P3                      11.20 usec
P4                      22.40 usec
PCPD2                   90.00 usec
PLW2             12.00000000 W
PLW12             0.18584000 W

F2 - Processing parameters
SI                        32768
SF                100.6379135 MHz
WDW                        EM
SSB         0
LB                      1.00 Hz
GB          0
```

Fig 3.2.7 **^{13}C NMR (DEPT) spectrum of compound P2 .**




```
Current Data Parameters
NAME                  CURT_P2
EXPNO                       5
PROCNO                      1

F2 - Acquisition Parameters
Date_               20230418
Time                    6.03
INSTRUM                 spect
PROBHD    5 mm PABBO BB/
PULPROG     hmbcqplpndqf
TD                       2048
SOLVENT                  MeOD
NS                          4
DS                         16
SWH                 12019.230 Hz
FIDRES              5.868765 Hz
AQ                  0.0851968 sec
RG                      208.5
DW                     41.600 usec
DE                      6.50 usec
TE                     301.1 K
CNST2               145.0000000
CNST13               10.0000000
D0                   0.00000400 sec
D1                   1.00000000 sec
D2                   0.00344828 sec
D6                   0.05000000 sec
D16                  0.00020000 sec
IN0                  0.00001980 sec

======== CHANNEL f1 ========
SFO1                400.2340023 MHz
NUC1                        1H
P1                       11.20 usec
P2                       22.40 usec
PLW1                12.00000000 W

======== CHANNEL f2 ========
SFO2                100.6479773 MHz
NUC2                        13C
P3                       10.00 usec
PLW2                49.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]             SMSQ10.100
GPNAM[2]             SMSQ10.100
GPNAM[3]             SMSQ10.100
GPZ1                    50.00 %
GPZ2                    30.00 %
GPZ3                    40.10 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                          512
SFO1                100.648 MHz
FIDRES              49.321339 Hz
SW                    250.899 ppm
FnMODE                     QF

F2 - Processing parameters
SI                         2048
SF                  400.2300000 MHz
WDW                        SINE
SSB         0
LB          0 Hz
GB          0
PC                         1.40

F1 - Processing parameters
SI                         1024
MC2                        QF
SF                  100.6379135 MHz
WDW                        SINE
SSB         0
LB          0 Hz
GB          0
```

Fig 3.2.8 HMBC spectrum of compound P2.




```
Current Data Parameters
NAME                  CUET_P2
EXPNO                       4
PROCNO                      1

F2 - Acquisition Parameters
Date_              20230418
Time                   5.24
INSTRUM                spect
PROBHD   5 mm PABBO BB/
PULPROG             cosygpqf
TD                      2048
SOLVENT                 MeOD
NS                         4
DS                        16
SWH                12019.230 Hz
FIDRES              5.868765 Hz
AQ                  0.0851968 sec
RG                      208.5
DW                     41.600 usec
DE                      6.50 usec
TE                     301.3 K
D0                  0.00000300 sec
D1                  1.00000000 sec
D13                 0.00000400 sec
D16                 0.00020000 sec
IN0                 0.00008320 sec

======== CHANNEL f1 ========
SFO1              400.2340023 MHz
NUC1                       1H
P0                      11.20 usec
P1                      11.20 usec
PLW1             12.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]          SMSQ10.100
GPZ1                   10.00 %
P16                  1000.00 usec

F1 - Acquisition parameters
TD                        256
SFO1                  400.234 MHz
FIDRES               46.950119 Hz
SW                      30.031 ppm
FnMODE                    QF

F2 - Processing parameters
SI                       2048
SF                400.2299975 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
PC                        1.40

F1 - Processing parameters
SI                       1024
MC2                       QF
SF                400.2300029 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
```

Fig 3.2.9 COSY spectrum of compound P2.

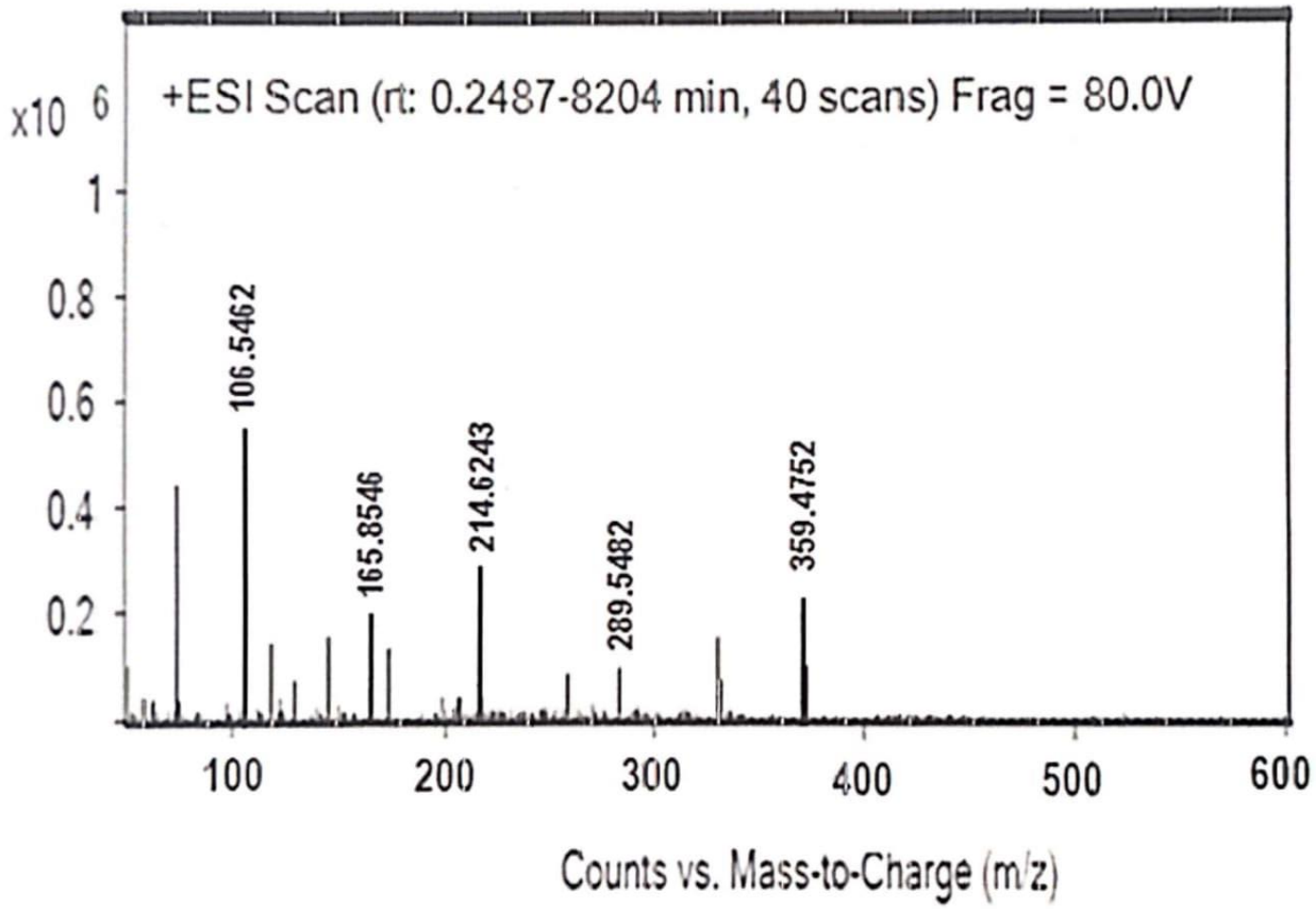
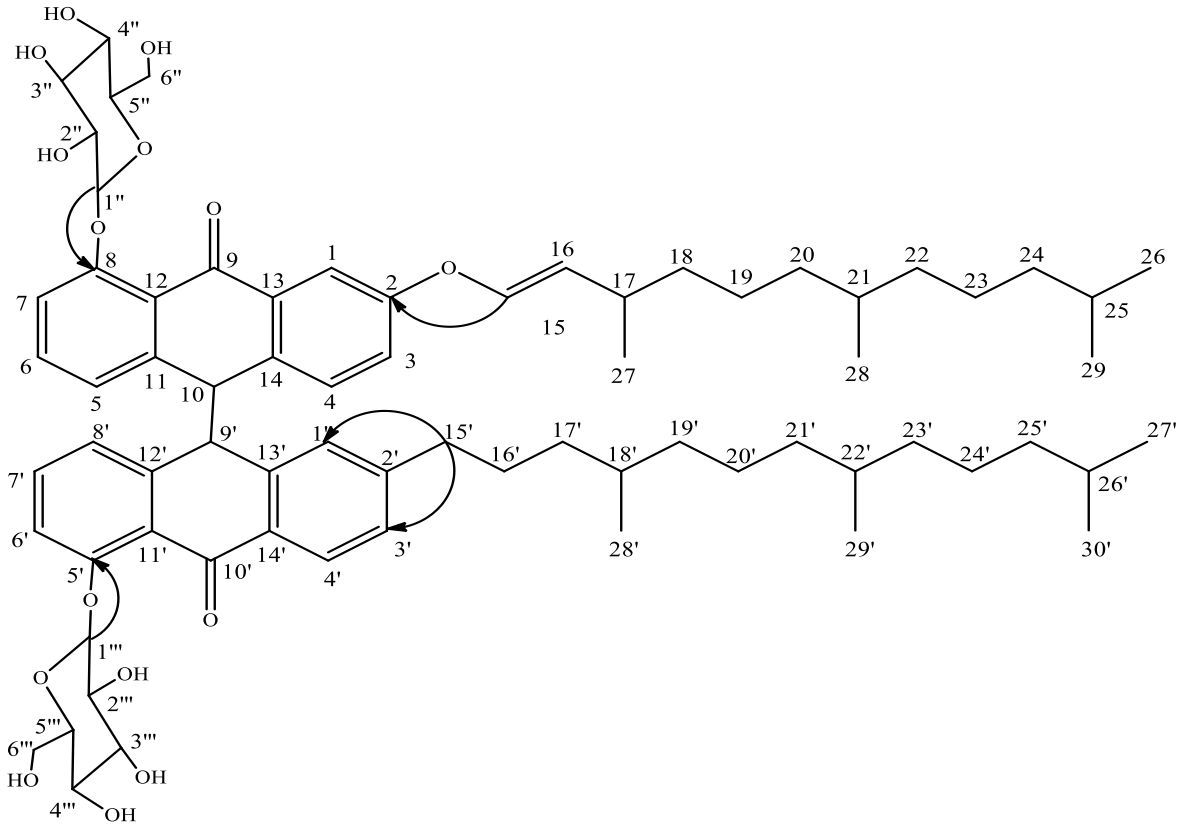


Fig 3.2.10 Mass spectrum of compound P2.

3.3 Characterization of Compound P3:

Compound **P3** (155 mg) was obtained as amorphous solid of white color and melted at 195-196 °C. Compound **P3** responded to positive color tests for an anthraquinone, appearance of red color with methanolic sodium hydroxide as well as with methanolic magnesium acetate (Kalidhar *et al.*, 1998). It also gave positive reaction for glycoside (Finer, 1975). The IR spectrum (Fig.3.3.2) of **P3** showed sharp absorption at $\bar{\upsilon}_{max}$ 16710 cm^{-1} for =CO functional group and broad absorption for O-H stretching at $\bar{\upsilon}_{max}$ 3497, 3409 cm^{-1}. ^{1}H NMR spectrum of compound **P3** (Fig. 3.3.3) revealed the presence of methyl, methine and methylene protons in **P3** which also supported by its ^{13}C NMR spectrum (Fig. 3.3.5). ^{13}C NMR of **P3** revealed that there were 8 methyl carbons, 17 methylene carbons and 18 methine carbons present in the compound. Form the literature review it was revealed that *Cassia* genus has a number of anthraquinone glycosides along with long alkyl chain (Kalidhar *et al.* 1998, Rao *et al.* 2003, Aminah *et al.* 2021). Several attempts were made to correlate **P3** with those of reported in the literature and this is in line with the observation that compound **P3** contains anthraquinone glycosides along with long alkyl chains in the molecule. ^{1}H NMR spectrum of compound **P3** revealed the presence of 12 aromatic protons attached to C2, at δ 6.29 (s, 1H, H-2), C3, at δ 6.82 (d,1H, *J* = 8.4, H-3), C4, at δ 7.19 (s, 1H, H-4), C5, at δ 6.78 (d, 1H, *J* = 8.4, H-5) C6, at δ 7.72 (m, 1H, H-6), C7, at δ 6.68 (d, 1H, *J* = 8.4, H-7), C1′, at δ 7.26 (s, 1H, H-1′), C3′, at δ 7.63(m, 1H, H-3′), C4′, at δ 7.33 (d, 1H, *J* = 8.4, H-4′), C6′, at δ 6.92 (d, 1H, *J* = 8.4, H-6′), C7′, at δ 7.63 (m, 1H, H-7′) and C8′, at δ 6.22 (d, 1H, *J* = 9.6, H-8′). Absorptions of two olefinic protons attached to C15 and C16 are shown at δ 7.84 (d, 1H, *J* = 9.2, H-15) and 5.36 (m, 1H, H-16) respectively. Presence of two anomeric protons at δ 5.90 (d, 1H, *J* = 3.2, H-1″) and 5.36 (d, 1H, *J* = 3.2, H-1‴) indicated compound **P3** contain two sugar moieties in the molecule. ^{13}C NMR spectrum of compound **P3** showed the presence of 71 carbons in the molecule. The mass spectrum (Fig. 3.3.8) of P3 exhibited highest molecular ion peak at *m/z* 1191.6936 [M+1] consistent with the molecular formula $C_{71}H_{96}O_{15}$. ^{13}C NMR (DEPT) spectrum of **P3** (Fig. 3.3.7) revealed the presence of 17 methylene carbons and 8 methyl carbons in the compound **P3**. Absorptions of 24 aromatic carbons appeared at δ 108.7(C1), 151.7(C2), 114.9(C3), 108.5(C4), 112.5(C5), 128.4(C6), 117.0(C7), 167.8(C8), 132.1(C11), 145.6(C12), 140.5(C13), 129.6(C14), 1 07.6(C1′), 132.1(C2′), 116.5(C3′), 127.5(C4′), 167.8(C5′), 128.5(C6′), 130.9(C7′), 111.1(C8′), 144.6(C11′), 132.1(C12′), 129.3(C13′) and 142.1(C14′) were obtained from ^{13}C NMR spectrum of **P3** (Table 3.3).

The important homo-nuclear H-H correlations and hetero-nuclear H-C correlations in the molecule **P3** observed from 2D COSY (Fig. 3.3.10) and HMBC (Fig. 3.3.9). The mass fragmentation pattern *m/z:* [M+1] 1191, 597, 373, 227, 226, 164 etc. supports the proposed structure of **P3**. Analysis of all spectral data structure **3** is suggested for compound P3 and was characterized as 5″[{(*2R,3S,5R,6S*)-3″,4″,5″-trihydroxy-6″(hydroxymethyl)tetrahydro-*2H*-pyran-2-yl}oxy]- 5‴[{(*2S, 3R,5S, 6R*) -3‴,4‴,5‴-trihydroxy-6‴(hydroxymethyl)tetrahydro-*2H*-pyran-2-yl}oxy]-17-[{(*E*)-17,21,25-trimethyldo dee-15-en-15-yl}oxy]-2′-(28′,29′,30′-trimethlytri decyl)--9, 10′-bianthracene]-9,10′(9′*H*,10*H*) -dione. It is a new natural product that is being reported for the first time through the study.



(3)

Compound P3

H → C HMBC correlation

Fig. 3.3 : Important HMBC correlations of compound P3.

Table 3.3: ^{13}C NMR and ^{1}H NMR data for Compound P3 (CD_3OD, δ, ppm, *J*/Hz)*.

C atom	^{13}C, δ	^{1}H, δ	COSY	C atom	^{13}C, δ	^{1}H, δ	COSY
1	108.7	7.19, s		8′	111.1	6.22 (d, *J* = 9.6)	H-7′
2	151.7			9′	95.6	4.39, s	
3	114.9	6.82 (d, *J* = 8.4)	H-4	10′	192.3		
4	108.5	7.19, s		11′	144.6		
5	112.5	6.78 (d, *J* = 8.4)	H-6	12′	132.1		
6	128.4	7.72 (t, *J* = 4.0)		13′	129.3		
7	117.0	6.68 (d, *J* = 8.4)	H-6	14′	142.1		
8	167.8			15′	62.6	2.33, m	
9	196.3			16′	42.6	1.30, m	
10	94.7	4.70, s		17′	39.7	1.30, m	
11	132.1			18′	55.4	1.61, m	
12	145.6			19′	38.7	1.30, m	
13	140.5			20′	37.3	1.30, m	
14	129.6			21′	36.5	1.30, m	
15	144.6	7.84 (d, *J* = 9.2)	H-16	22′	47.9	1.44, m	
16	129.6	5.36, m		23′	33.7	1.30, m	
17	56.6	2.72, m		24′	33.5	1.30, m	
18	36.5	1.30, m		25′	31.8	1.30, m	
19	33.3	1.30, m		26′	45.8	2.06, m	
20	33.5	1.30, m		27′	13.0	.91 (d, *J* = 4.4)	H-26′
21	48.1	1.44, m		28′	22.6	.96 (d, *J* = 4.4)	H-18′
22	36.0	1.30, m		29′	18.4	97 (d, *J* = 4.4)	H-22′
23	32.2	1.30, m		30′	13.0	.90 (d, *J* = 4.4)	H-26′
24	31.8	1.44, m		1″	102.5	5.90 (d, *J* = 3.2)	H-2″
25	38.7	2.06, m		2″	72.3	3.92, m	
26	12.9	.86 (d, *J* = 4.4)	H-25	3″	69.3	3.32, m	
27	24.5	1.15 (d, *J* = 3.6)	H-17	4″	67.7	3.32, m	
28	18.0	.88 (d, *J* = 4.4)	H-21	5″	73.6	4.30 (t, *J* = 6.4)	H-4″, 6″
29	12.6	.99 (d, *J* = 4.4)	H-25	6″	65.2	4.23 (d, *J* = 3.6)	H-5″
1′	107.6	7.26, s		1‴	104.4	5.36 (d, *J* = 3.2)	H-2‴
2′	132.1			2‴	71.5	3.92, m	
3′	116.5	7.63 (d, *J* = 8.4)	H-4′	3‴	69.6	3.32, m	
4′	127.5	7.33 (d, *J* = 8.4)	H-3′	4‴	65.2	3.32, m	
5′	167.8			5‴	73.8	4.30 (t, *J* = 6.4)	H-4‴, 6‴
6′	128.5	6.92 (d, *J* = 8.4)	H-7′	6‴	67.7	4.23 (d, *J* = 3.6)	H-5‴
7′	130.9	7.63 (t, *J* = 4.0)					

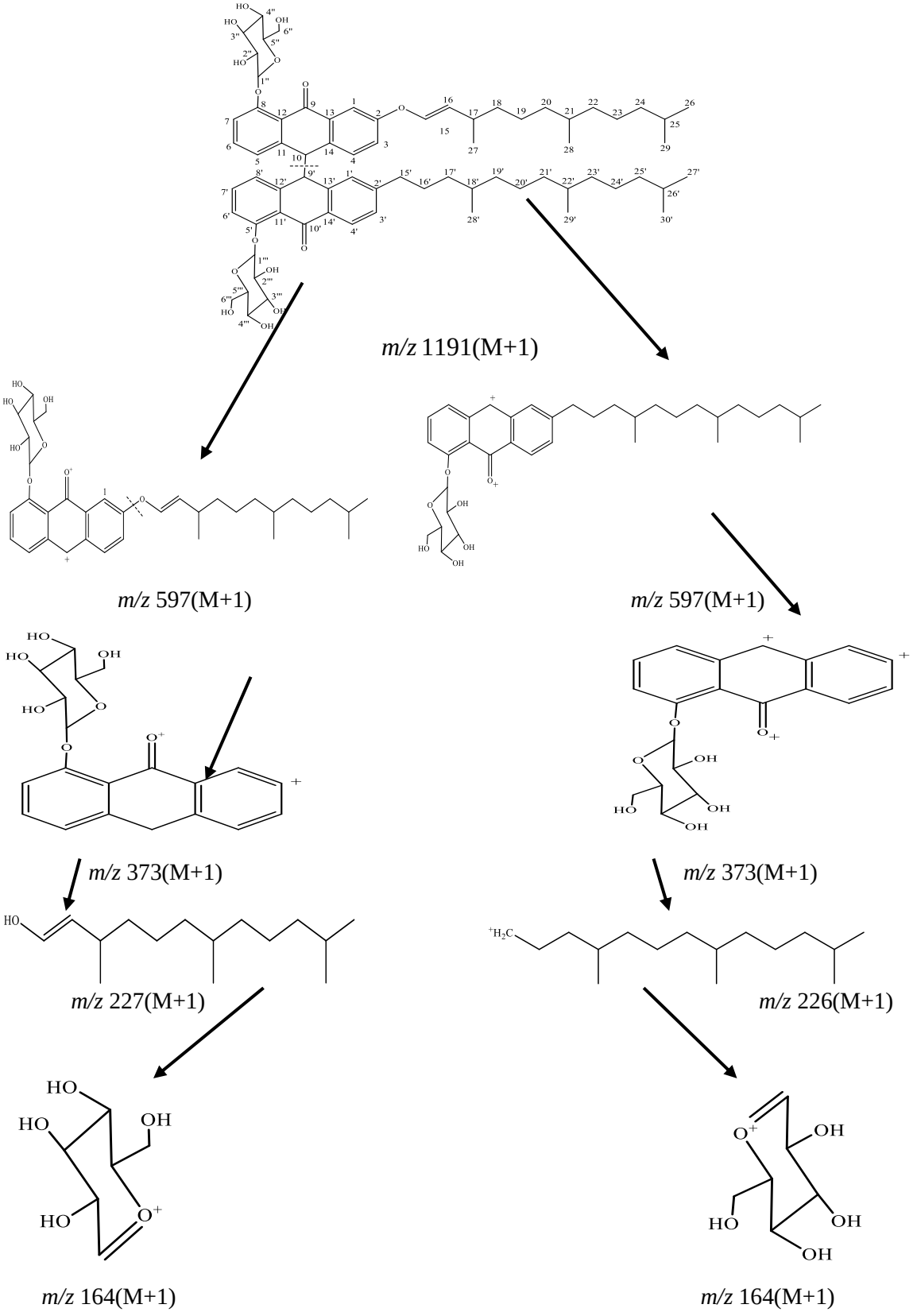


Fig 3.3.1 Mass fragmentation of compound P3.

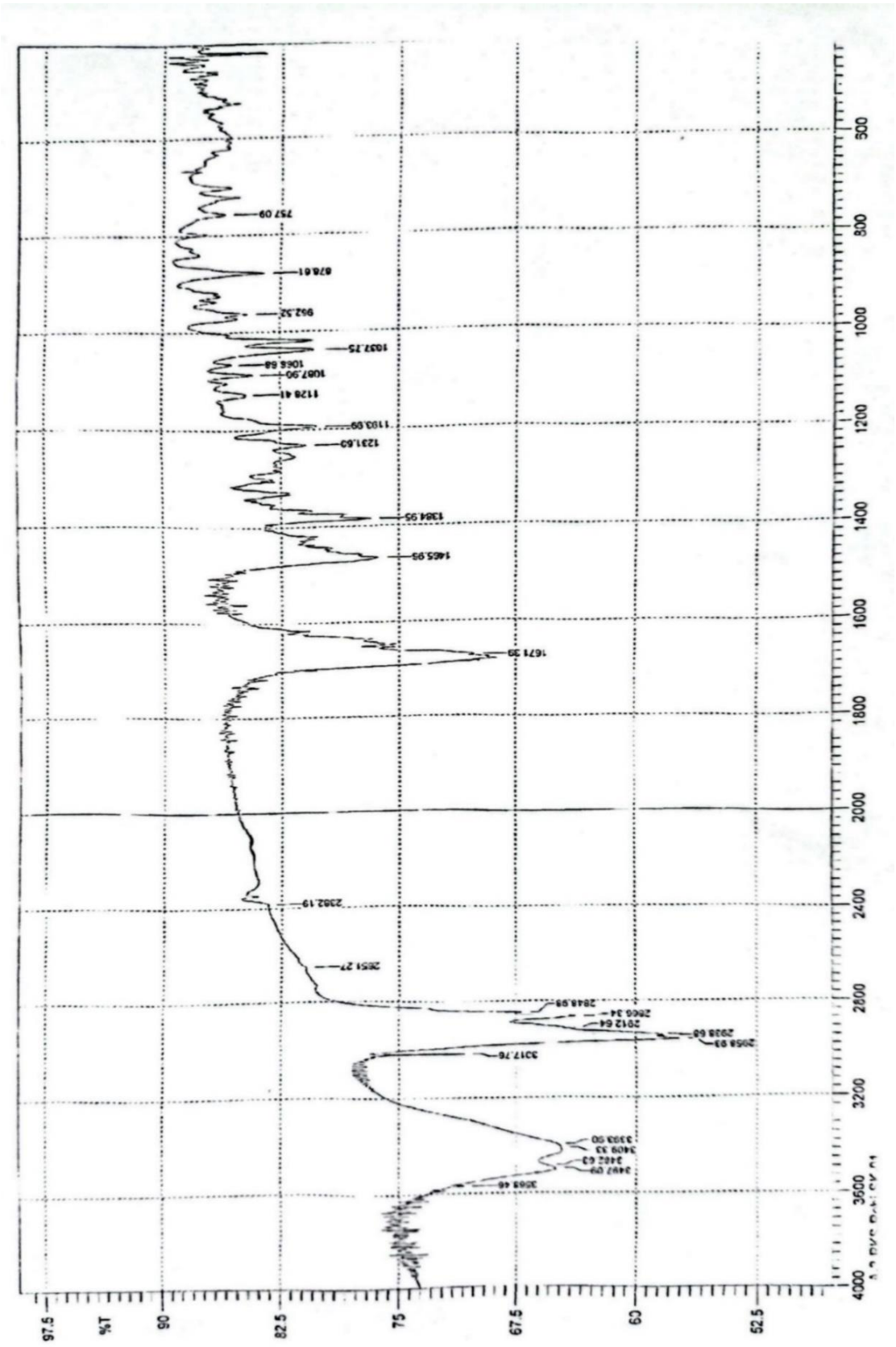
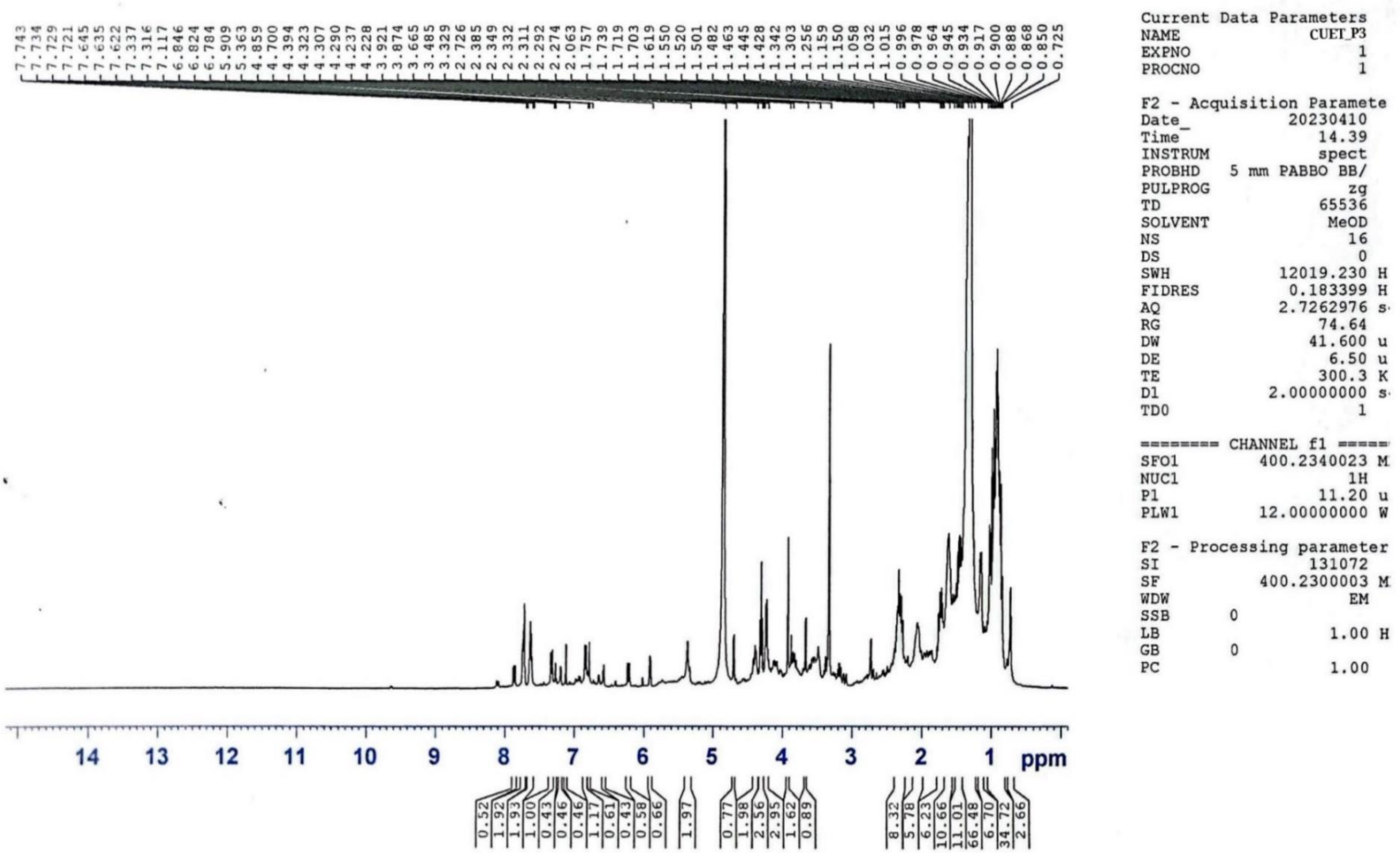
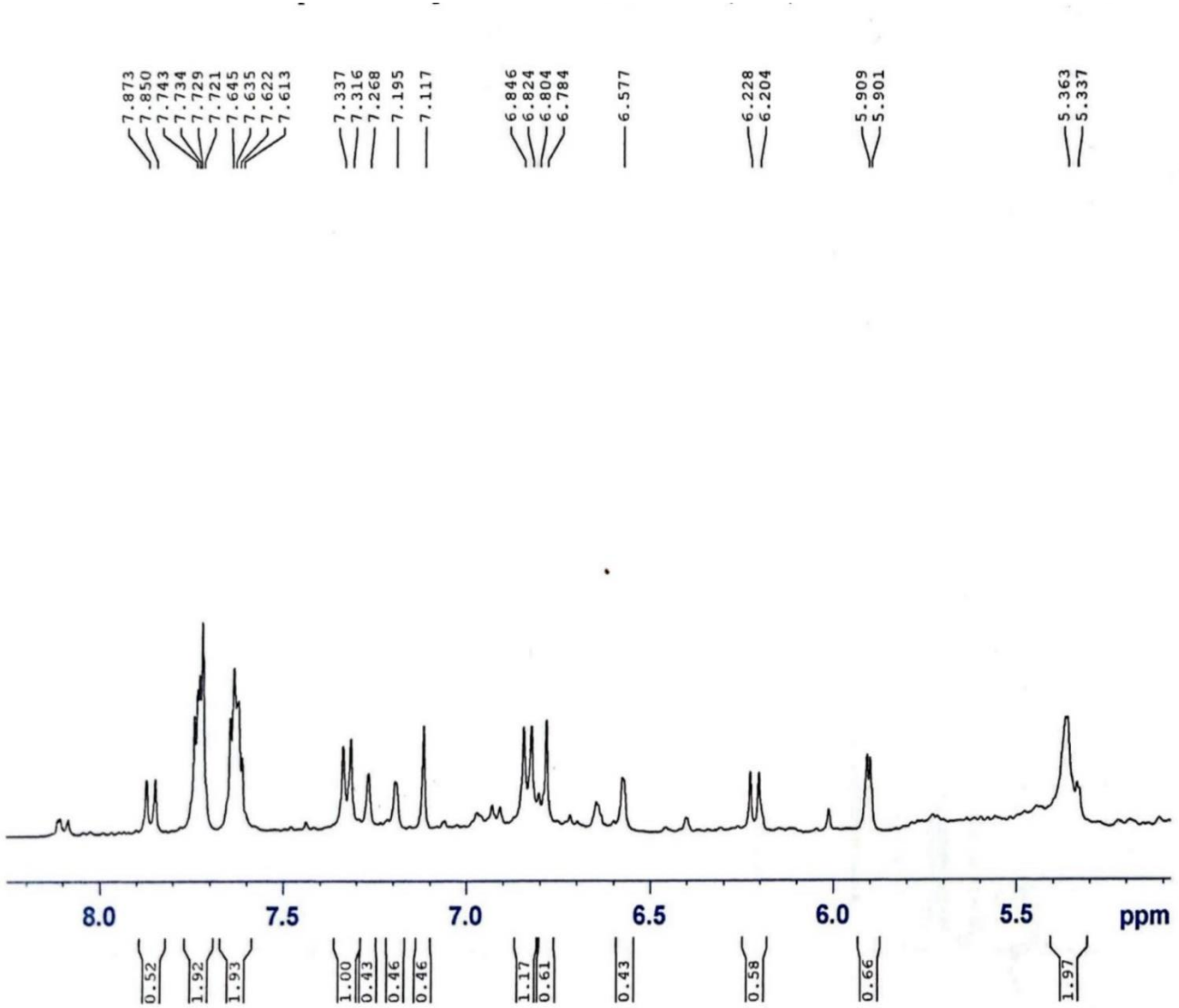


Fig 3.3.2 IR spectrum of compound P3.




```
Current Data Parameters
NAME                  CUET_P3
EXPNO                       1
PROCNO                      1

F2 - Acquisition Paramete
Date_                20230410
Time                    14.39
INSTRUM                 spect
PROBHD    5 mm PABBO BB/
PULPROG                    zg
TD                      65536
SOLVENT                  MeOD
NS                         16
DS                          0
SWH                12019.230 H
FIDRES              0.183399 H
AQ                 2.7262976 s
RG                     74.64
DW                    41.600 u
DE                     6.50 u
TE                    300.3 K
D1                 2.00000000 s
TD0                         1

======== CHANNEL f1 =====
SFO1             400.2340023 M
NUC1                      1H
P1                     11.20 u
PLW1            12.00000000 W

F2 - Processing parameter
SI                     131072
SF               400.2300003 M
WDW                       EM
SSB          0
LB                     1.00 H
GB           0
PC                     1.00
```

Fig 3.3.3 **^{1}H spectrum of compound P3.**




```
Current Data Parameters
NAME                 CUET_P3
EXPNO                      1
PROCNO                     1

F2 - Acquisition Paramete
Date_               20230410
Time                   14.39
INSTRUM                spect
PROBHD   5 mm PABBO BB/
PULPROG                   zg
TD                     65536
SOLVENT                 MeOD
NS                        16
DS                         0
SWH               12019.230 H
FIDRES             0.183399 H
AQ                 2.7262976 s
RG                     74.64
DW                    41.600 u
DE                     6.50 u
TE                    300.3 K
D1                2.00000000 s
TD0                        1

======== CHANNEL f1 =====
SFO1              400.2340023 M
NUC1                       1H
P1                     11.20 u
PLW1             12.00000000 W

F2 - Processing parameter
SI                    131072
SF                400.2300003 M
WDW                       EM
SSB          0
LB                     1.00 H
GB           0
PC                     1.00
```

Fig 3.3.4 **^{1}H (Expand) spectrum of compound P3.**

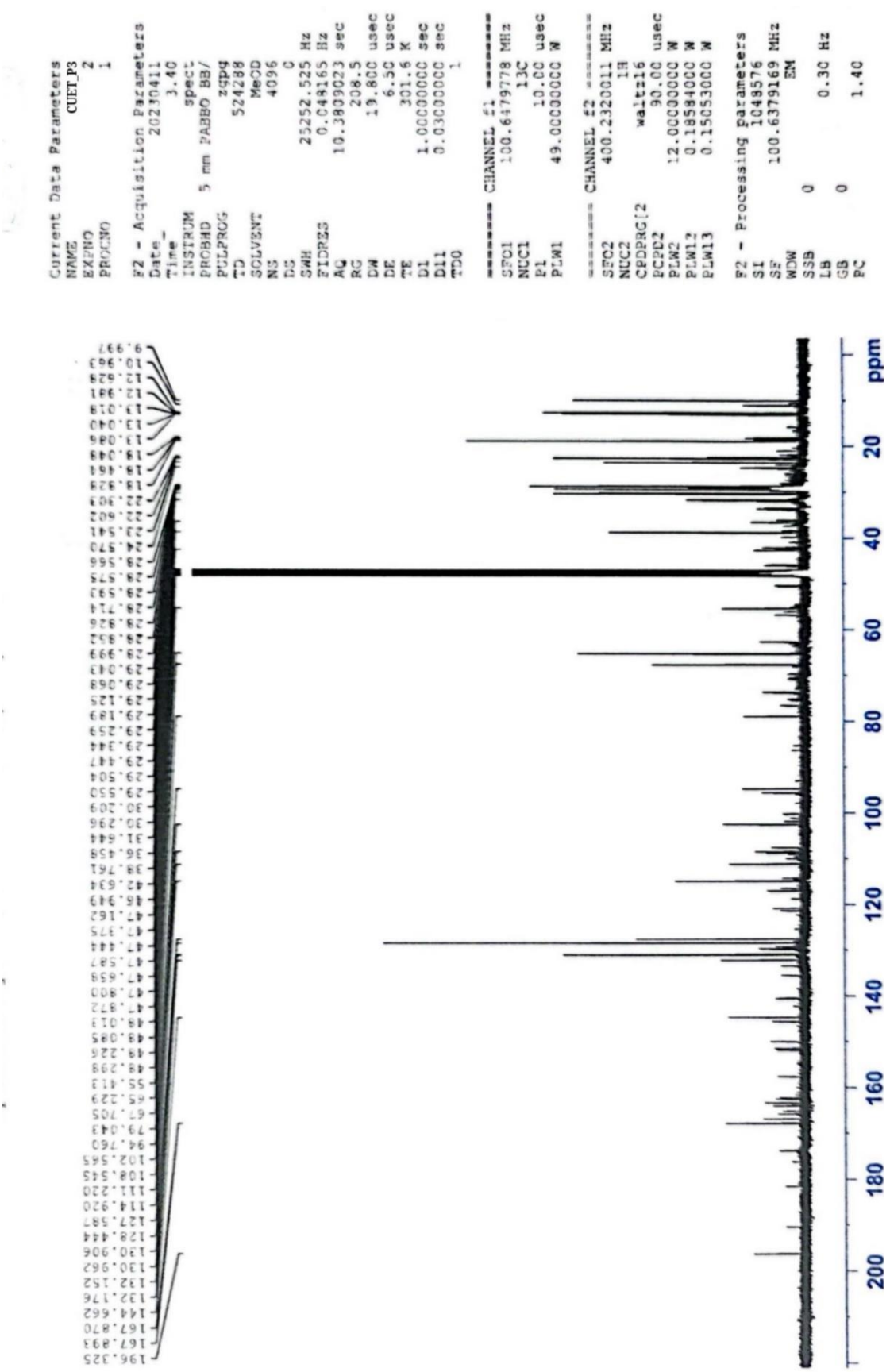
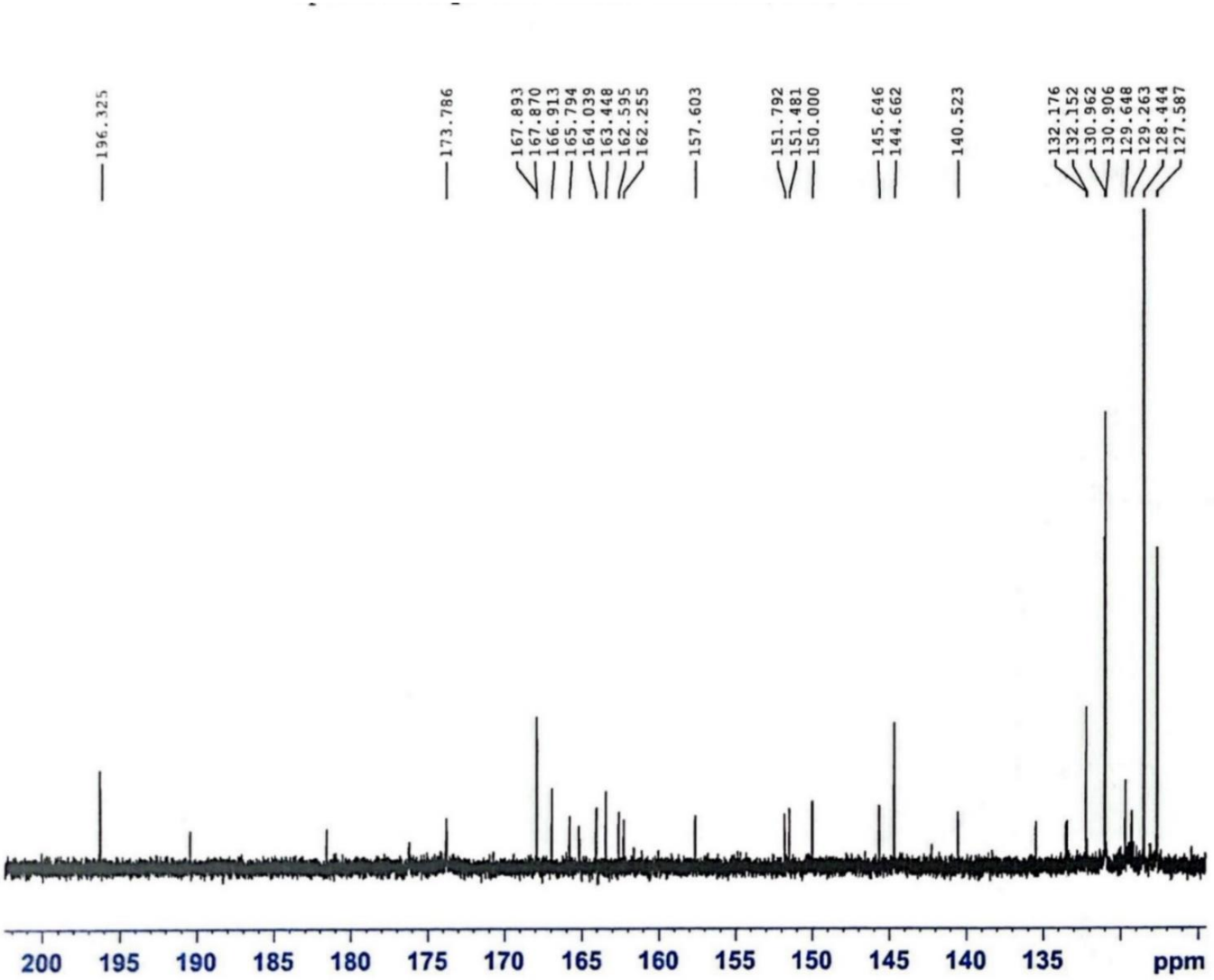
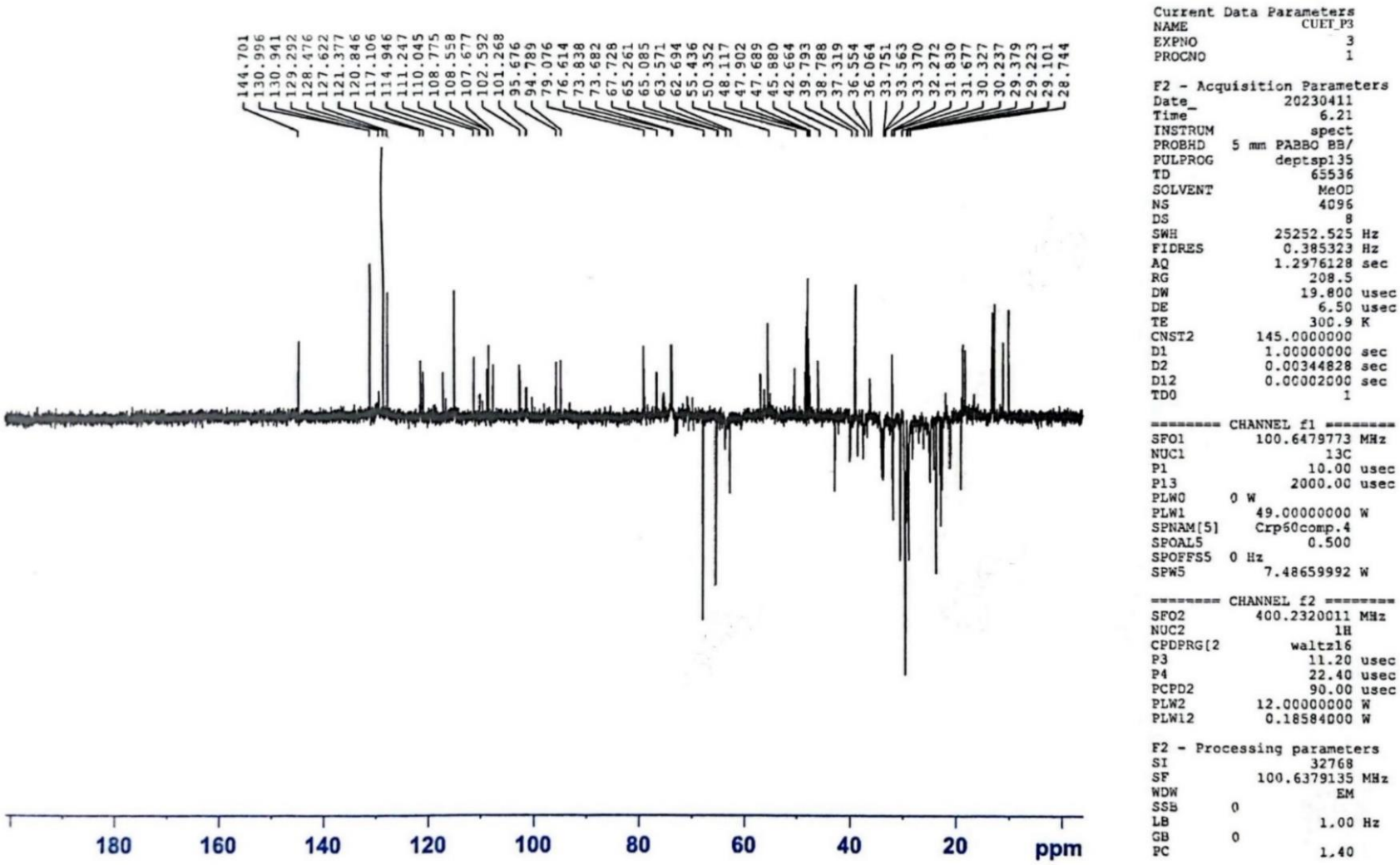


Fig 3.3.5 ^{13}C NMR spectrum of compound P3.




```
Current Data Parameters
NAME                  CUET_P3
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_                20230411
Time                     3.40
INSTRUM                 spect
PROBHD   5 mm PABBO BB/
PULPROG                 zgpg
TD                    524288
SOLVENT                 MeOD
NS                      4096
DS                         0
SWH                25252.525 Hz
FIDRES             0.048165 Hz
AQ                10.3809023 sec
RG                     208.5
DW                    19.800 usec
DE                     6.50 usec
TE                    301.6 K
D1                1.00000000 sec
D11               0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1           100.6479778 MHz
NUC1                    13C
P1                    10.00 usec
PLW1           49.00000000 W

======== CHANNEL f2 ========
SFO2           400.2320011 MHz
NUC2                    1H
CPDPRG[2             waltz16
PCPD2                 90.00 usec
PLW2           12.00000000 W
PLW12           0.18584000 W
PLW13           0.15053000 W

F2 - Processing parameters
SI                    1048576
SF             100.6379169 MHz
WDW                        EM
SSB          0
LB                     0.30 Hz
GB           0
PC                     1.40
```

Fig 3.3.6 ^{13}C NMR (Expand.) spectrum of compound P3.




```
Current Data Parameters
NAME                   CUET_P3
EXPNO                        3
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20230411
Time                      6.21
INSTRUM                  spect
PROBHD     5 mm PABBO BB/
PULPROG              deptsp135
TD                       65536
SOLVENT                  MeOD
NS                       4096
DS                          8
SWH                25252.525 Hz
FIDRES             0.385323 Hz
AQ                 1.2976128 sec
RG                       208.5
DW                    19.800 usec
DE                     6.50 usec
TE                     300.9 K
CNST2              145.0000000
D1                 1.00000000 sec
D2                 0.00344828 sec
D12                0.00002000 sec
TD0                          1

======== CHANNEL f1 ========
SFO1               100.6479773 MHz
NUC1                       13C
P1                      10.00 usec
P13                   2000.00 usec
PLW0          0 W
PLW1             49.00000000 W
SPNAM[5]         Crp60comp.4
SPOAL5                 0.500
SPOFFS5       0 Hz
SPW5              7.48659992 W

======== CHANNEL f2 ========
SFO2               400.2320011 MHz
NUC2                       1H
CPDPRG[2                waltz16
P3                      11.20 usec
P4                      22.40 usec
PCPD2                   90.00 usec
PLW2              12.00000000 W
PLW12              0.18584000 W

F2 - Processing parameters
SI                         32768
SF                 100.6379135 MHz
WDW                          EM
SSB           0
LB                      1.00 Hz
GB            0
PC                      1.40
```

Fig 3.3.7 **^{13}C NMR (DEPT) spectrum of compound P3.**

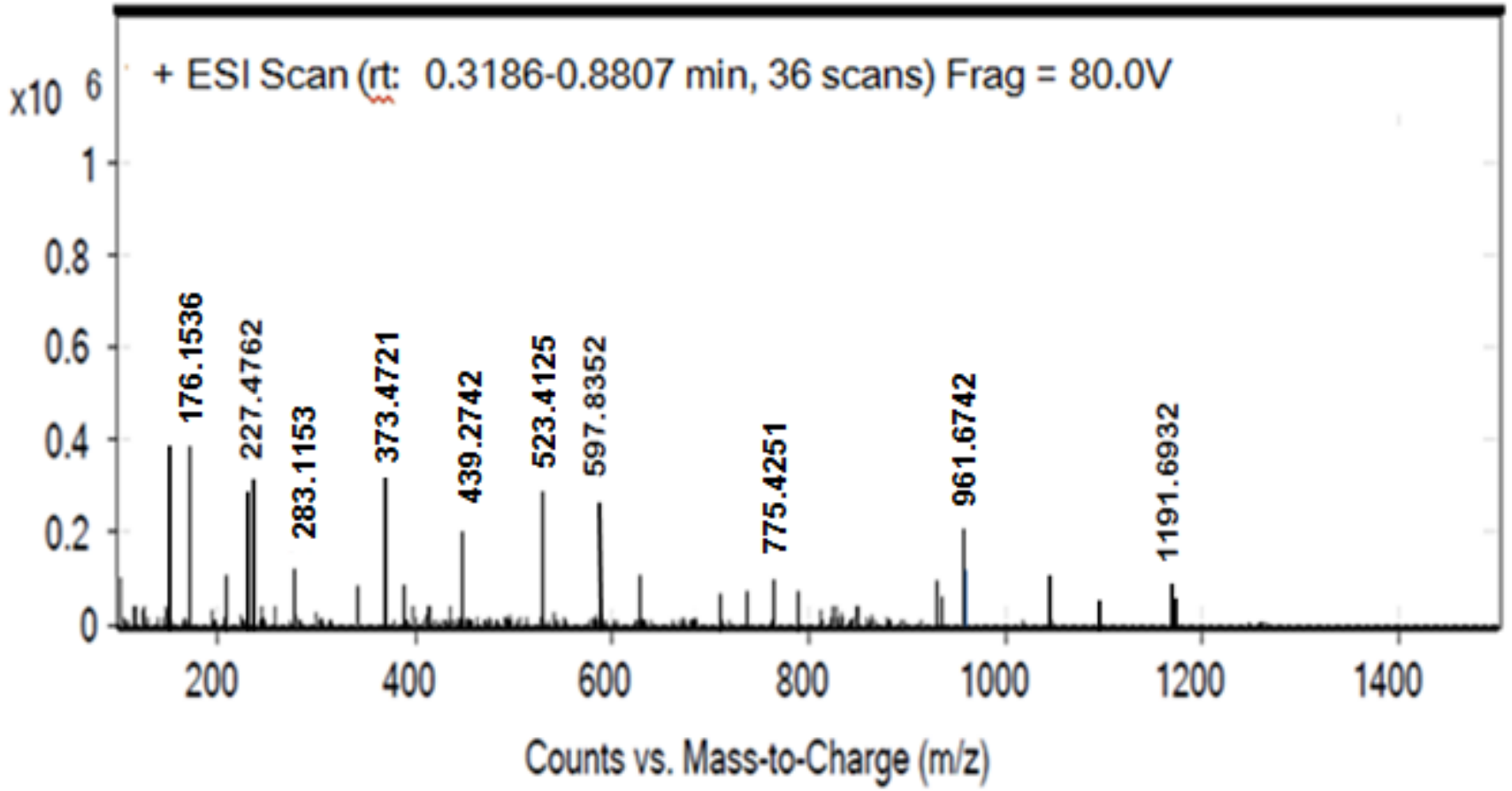


Fig 3.3.8 Mass spectrum of compound P3.

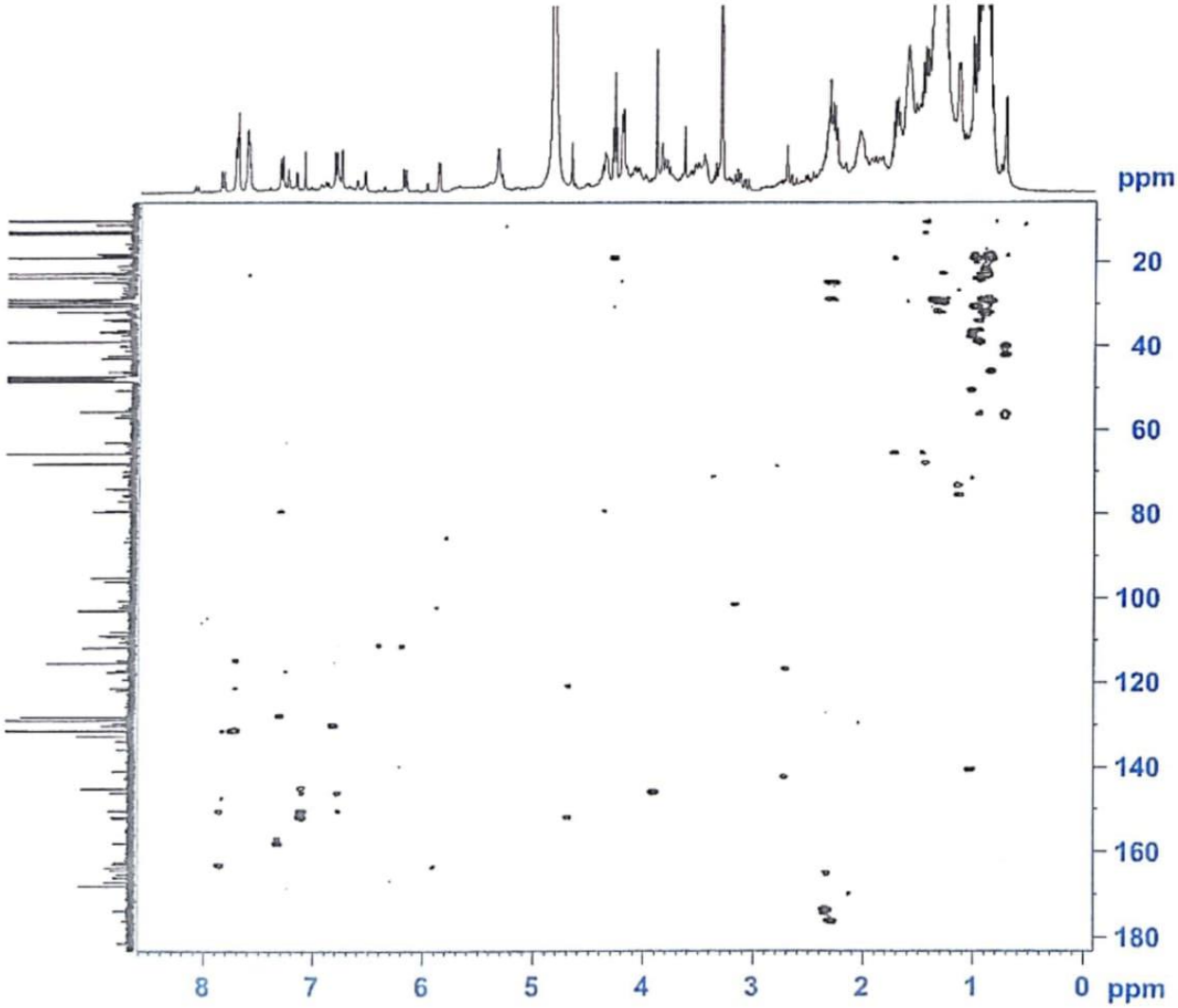
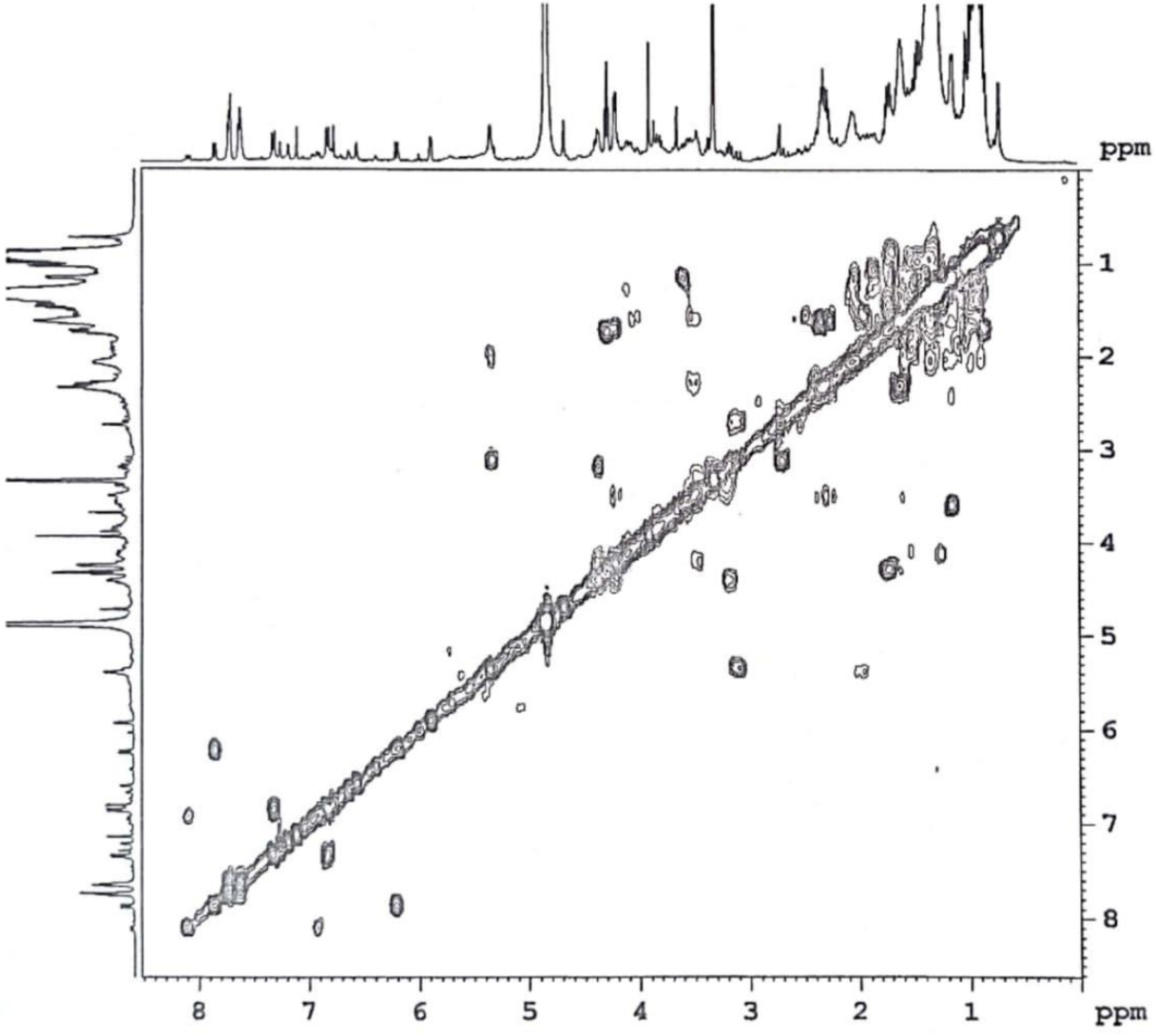


Fig 3.3.9 HMBC spectrum of compound P3.

```
Current Data Parameters
NAME                  CUET_P3
EXPNO                       6
PROCNO                      1

F2 - Acquisition Parameters
Date_               20230411
Time                    7.00
INSTRUM                 spect
PROBHD   5 mm PABBO BB/
PULPROG    hmbcgplpndqf
TD                      2048
SOLVENT                 MeOD
NS                         4
DS                        16
SWH                12019.230 Hz
FIDRES              5.868765 Hz
AQ                  0.0851968 sec
RG                      208.5
DW                     41.600 usec
DE                      6.50 usec
TE                     300.5 K
CNST2              145.0000000
CNST13              10.0000000
D0                  0.00000400 sec
D1                  1.00000000 sec
D2                  0.00314828 sec
D6                  0.05000000 sec
D16                 0.00020000 sec
IN0                 0.00001960 sec

======== CHANNEL f1 ========
SFO1               400.2340023 MHz
NUC1                       1H
P1                       11.20 usec
P2                       22.40 usec
PLW1               12.00000000 W

======== CHANNEL f2 ========
SFO2               100.6479773 MHz
NUC2                       13C
P3                       10.00 usec
PLW2               49.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]          SMSQ10.100
GPNAM[2]          SMSQ20.100
GPNAM[3]          SMSQ10.100
GPZ1                    50.00 %
GPZ2                    30.00 %
GPZ3                    40.10 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                         512
SFO1                100.648 MHz
FIDRES             49.321439 Hz
SW                   250.899 ppm
FnMODE                     QF

F2 - Processing parameters
SI                        2048
SF                400.2300000 MHz
WDW                       SINE
SSB          0
LB           0 Hz
GB           0
PC                        1.40

F1 - Processing parameters
SI                        1024
MC2                         QF
SF                100.6379135 MHz
WDW                       SINE
SSB          0
LA           0 Hz
GB           0
```




```
Current Data Parameters
NAME                  CUET_P3
EXPNO                       4
PROCNO                      1

F2 - Acquisition Parameters
Date_              20230411
Time                   6.21
INSTRUM                spect
PROBHD   5 mm PABBO BB/
PULPROG             cosygpqf
TD                      2048
SOLVENT                 MeOD
NS                         4
DS                        16
SWH               12019.230 Hz
FIDRES             5.868765 Hz
AQ                 0.0851968 sec
RG                     208.5
DW                    41.600 usec
DE                     6.50 usec
TE                    300.6 K
D0                 0.00000300 sec
D1                 1.00000000 sec
D13                0.00000400 sec
D16                0.00020000 sec
IN0                0.00008320 sec

======== CHANNEL f1 ========
SFO1              400.2340023 MHz
NUC1                       1H
P0                      11.20 usec
P1                      11.20 usec
PLW1              12.00000000 W

====== GRADIENT CHANNEL =====
GPNAM[1]          SMSQ10.100
GPZ1                   10.00 %
P16                  1000.00 usec

F1 - Acquisition parameters
TD                        256
SFO1                  400.234 MHz
FIDRES             46.950119 Hz
SW                     30.031 ppm
FnMODE                    QF

F2 - Processing parameters
SI                       2048
SF                400.2299975 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
PC                       1.40

F1 - Processing parameters
SI                       1024
MC2                       QF
SF                400.2300029 MHz
WDW                     QSINE
SSB          0
LB           0 Hz
GB           0
```

Fig 3.3.10 COSY spectrum of compound P3.

CHAPTER – IV

BIOLOGICAL STUDIES

PAGE NO. 59

4. Biological Studies

4.1 Antimicrobial potency assay:

In vitro antimicrobial studies of the isolated compounds **(P1, P2** and **P3**) were assayed against two gram-positive and two gram-negative bacteria along with two fungi by using agar disc diffusion method. The development of diameter of inhibition zones (mm ± SD) are presented in **Table 3.4 -3.5**. Ceftriaxone and Amphotericin B were used as standard. All the tested compounds showed activity against bacterial and fungal strains. Compound P2 and P3 were biologically inactive towards gram-positive bacteria. P1 displayed activities against all bacterial and fungal strains. P2 exhibited inhibition value 19.0 ± 1.0 mm against *S. typhimurium*. P3 exhibited inhibition value 21.0±1.0 mm against *S. typhimurium*. Compounds P1, P2 and P3 showed promising ani-fungal activities value 27.7±0.6 mm, 19.3±0.6mm and 41.3±0.6 mm, 45.0±1.0 mm and 33.3±0.6 mm, 36.3±0.6 mm against respected fungal strains respectively. Compounds P1, P2 and P3 showed remarkable antibacterial activities and compound P3 exhibited significant antifungal activities against fungal strains.

Table 3.4: Anti-bacterial activity of the compounds **P1, P2** and **P3.**

Test samples	**Gram positive bacteria**		**Gram negative bacteria**	
	S. aureus	*B. subtilis*	*S. typhimurium*	*E.Coli*
P1	12.3±0.6	13.3±0.6	18.3±0.6	17.3±1.2
P2	-	-	19.0±1.0	-
P3	-	-	21.0±1.0	-
Ceftriaxone	38.0±1.0	34.0±1.0	44.3±0.6	40.0±1.0
DMSO	-	-	-	-

The individual data expressed as mean ± SD (Standard deviation) of three experiments

Table 3.5: Ani-fungal activity of the compounds P1, P2 and P3.

Test samples	***T. harzianum***	***A. niger***
P1	27.7±0.6	19.3±0.6
P2	41.3±0.6	45.0±1.0
P3	33.3±0.6	36.3±0.6
Amphotericin B	17.7±0.6	8.3±0.6
DMSO	-	-

The individual data expressed as mean ± SD (Standard deviation) of three experiments

CHAPTER – V
CONCLUSION
PAGE NO. 60

5. Conclusion:

The main objective of the research work was to isolate some compounds from the fruit of *Cassia fistula* along with other secondary metabolites as well as to determine the molecular structure of the isolated compounds. In the study, it was succeeded in finding some of them those were isolated and established the structures by ^{1}H NMR, ^{13}C NMR, Mass spectroscopy of three natural products **P1**, **P2** and **P3**. They are new natural products and reported for the first time from the *Cassia fistula* fruits. The isolated products **P1**, **P2** and **P3** were tested antimicrobial activities where **P1** showed potential antibacterial activities against all experimental bacterial strains, **P2** and **P3** showed against *S. typhimurium.* In the antifungal screening, compounds **P1**, **P2** and **P3** showed remarkable antibacterial activities.

References

Bharatiya banaushadhi, Biswas K, Ghosh A B, (Calcutta University Advancement of learning, Calcutta), 1973, 336-338.

Ethnophramacol J, Iggnacimuthu S, Perumal R, Samy S, Sen A, Screening of 34 medicinal plants antibacterial properties, 62 (1998) 173-182.

Duthie C, Duthie SJ, Kyle JAM (2000). Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nut. Res. Rev.13: 79-106.

Gupta M, Mazumder UK, Mukhopadhyay DK (2000) Rath N, Antitumour activity of methanolic extract of *Cassia fistula L*. seed against Ehrlich ascites carcinoma. J. Ethnopharmacol. 72:151-156.

Indian Herbal Pharmacopoeia. Indian Drug Manufacturer's Association. Mumbai. 2002; 106.

Ramachandra Rao S, Ravishankar GA (2002). Plant cell cultures: chemical factories of secondary metabolites. Biotechnol. Adv. 20: 101-153.

Ganapaty S, Thomas P. S, *et al*. "A review of phytochemical studies of *Cassia* species" Journal of Natural Remedies, 2002, 2(2): 102 – 120.

Bahorun T (2002), Neergheen V. Optimisation of growth and polyphenolic production in *Cassia fistula* callus cultures. Asian J. Microbiol. Biotechnol. Environ. Sci. 4: 181-185.

Becker K, Mohan P S, Siddhuraju P, Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula L.):* a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp, Food Chem, 79 (2002) 61-67.

Anonymous, WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants, (World Health Organization, Geneva), 2003, 19-32.

Akanmu MA and Elujoba AA, Iwalewa EO. Toxicity potentials of *Cassia fistula* fruits as laxative with reference to senna. Afr. J. Biomed. Research. 2004; 7: 23

Apparanantham T, Bhavapriya V, Govindasamy S, Kalpana S, Manonmani G and. Anti-oxidant activity of *Cassia fistula (Linn.)* flowers in alloxan induced diabetic rats. J. Ethanopharmacol. 2005; 97: 39.

Aruoma OI, Bahoran T, Neergheen VS, Phytochemical constituents of *Cassia fistula.* Afr. J. Biotech. 2005; 4: 1530

Gokhale SB, Koakte CK, Purohit AP and. 'Pharmacognosy', CBS Publishers and distributors. 2005, 169

Chauhan NS, Kumar VP, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. J. Ethnopharmacol. 2006; 107: 182

Prakash D, Suri S, Singh BN. Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. Int. J. Food Sci. Ntr. 2007; 58: 18.

Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of *Cassia fistula*. J. Ethanopharmacol. 2007; 112: 590

Govindarajan M, Jebanesan A, Pushpanathan T, Bioefficacy of *Cassia fistula Linn*. (Leguminosae) leaf extract against chikungunya vector, Aedes aegypti (Diptera: Culicidae). Parasitol Res. 2008; 102: 289.

Deshmukh T Pradeep, Jarald E Edwin, Jain Neetesh, Silawat Narendra, Yadav Akash. The mechanism of hypogl ycemic and antidiabetic action of hydro alcoholic extract of *Cassia fistula Linn* in rats. Journal of the Pharma Research, 2009; 01: 82-9

Chowdhury JU, Hoque M. N, Jaripa B, Yousuf M, 2009. Medicinal plants of Bangladesh, BCSIR, LAB, Ctg. 624.

Mahobia N. K, Patel R. D, Panchal U. R, Sheikh N.W, Seth M. V, Upwar N.I, Analgesic study of methyl alcohol extract of *Cassia fistula* Pod, J. Pharmacy Res., 2010; 3(9), 2218-2219

Malpani S.N. *et al*. Antidiabetic Activity of *Cassia fistula Linn* Bark in Alloxan Induced Diabetic Rats. Int. J. Phar. Sci. and Res. Jan-April, 2010; 2(1): 382-385

Agrawal R C, Gupta H, Kumar K, Evaluation of antibacterial and antioxidant activity from fruit extract of *Cassia fistula L*., Biomed Pharmacol J, 3 (2010) 207-210.

Anonymous, Quality control methods for herbal materials, (World Health Organization, Geneva), 2011, 45-57.

Charaka Samhita, Dash B, Sharma R K (Chowkhamba Sanskrit Series, Varanasi), 2012, 81-83.

Acharya R N, Bhalodia N R, Nariya P B, Shukla V J,*In vitro* antibacterial and antifungal activities of *Cassia fistula* Linn. fruit pulp extracts, AYU, 33 (2012) 123–28.

Ali A, Al-Fatlawi A A, Md. Irshadab, Mehdi S J, Md. Zafaryab & *et al*, Phytochemical composition of *Cassia fistula* fruit extracts and its anticancer activity against human cancer cell lines, J Biol Active Prod Nat, 4 (2014) 158–170.

Acharya R N, Jasani M D, Kolhe R, Pandya T N, Shukla V J, Experimental study on collection methods of *Cassia fistula* Fruit pulp, J Herbal Sci, 3 (2014) 15-19.

Lam K S, New aspects of natural products in drug discovery, Trends Microbiol, 15 (2017) 279–289

Hada D, Sharma K, Isolation and characterization of chemical compounds from fruit pulp of *Cassia fistula* and their antimicrobial activity, J Drug Deliv Ther, 8 (2018) 15-20

Rani L. *et al.* "Ethno-medicinal Species of *Cassia (C. fistula, C. siamea, C. tora):* Documentation and Metabolites Estimation" Int.J.Appl.Sci.Biotechnol. 2019, 7(3): 387-394.

Hussain S, Iqbal M, Naseer S, Saeed M, Phytochemical composition and pharmacological effects of Cassia fistula, Sci. Inq. Rev., 4 (1) (2020), pp. 59-69

Conference Presentation

The Scientific sub-committee of BCSIR Congress-2023 invited me to oral presentation of my work on the abstract titled - Bioactive phytoconstituents from the fruits of *Cassis fistula*. 8 – 10 March 2024.

Proshanta Shil
M. Phil. Student
ID No. – 17MCHEM003P
Chittagong University of Engineering & Technology (CUET)

Dr. Ranajit Kumar Sutradhar (Corresponding Author)
Professor
Department of Chemistry
Chittagong University of Engineering & Technology.