PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF PLANTS USED IN UNANI MEDICINE

A THESIS SUBMITTED TO

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SUBMITTED BY

Temesgen Hailu Hirigo

2K17/PhD/AC/16

UNDER THE GUIDANCE OF

Supervisor

PROF. ARCHNA RANI

Co-supervisor

PROF. RAJINDER K. GUPTA

DEPARTMENT OF APPLIED CHEMISTRY

DELHI TECHNOLOGICAL UNIVERSITY

SHAHBAD DAULATPUR, MAIN BAWANA ROAD,

DELHI – 110042, INDIA

July 2020

Dedicated

to

My Parents

DEPARTMENT OF APPLIED CHEMISTRY

DELHI TECHNOLOGICAL UNIVERSITY

SHAHBAD DAULATPUR, MAIN BAWANA ROAD,

DELHI – 110042, INDIA

DECLARATION

This is to certify that the work presented in this thesis entitled "**Phytochemical and Pharmacological Investigation of Plants used in the Unani Medicine**" is original and has been conducted by me for the degree of Doctor of Philosophy under the guidance of Prof. Archna Rani, and Prof. Rajinder K. Gupta, Department of Applied Chemistry. I declare that this work is original and has not been submitted in part or full to Delhi Technological University and any other institutions for the award of any other degree or diploma.

Temesgen Hailu Hirigo

(Candidate)

DEPARTMENT OF APPLIED CHEMISTRY

DELHI TECHNOLOGICAL UNIVERSITY

SHAHBAD DAULATPUR, MAIN BAWANA ROAD,

DELHI – 110042, INDIA

CERTICATE

This is to certify that the Ph. D thesis entitled "**Phytochemical and Pharmacological Investigation of Plants used in the Unani Medicine**" by Mr. Temesgen Hailu Hirigo (Enrol. No. 2K17/PhD/AC/16) for the award of the degree of Doctor of Philosophy at the Delhi Technological University (DTU), Shahbad Daulatpur, Bawana Road, Delhi-110042, India has been carried out at the Department of Applied Chemistry, under the guidance of Prof. Archna Rani, and Prof. Rajinder K. Gupta. This work is original, and has not been submitted in part or in full for any other degree or diploma of this or any other University.

Prof. Archna Rani
Supervisor

Prof. Rajinder K. Gupta
Co-supervisor

Prof. S.G. Warkar
Head of Department

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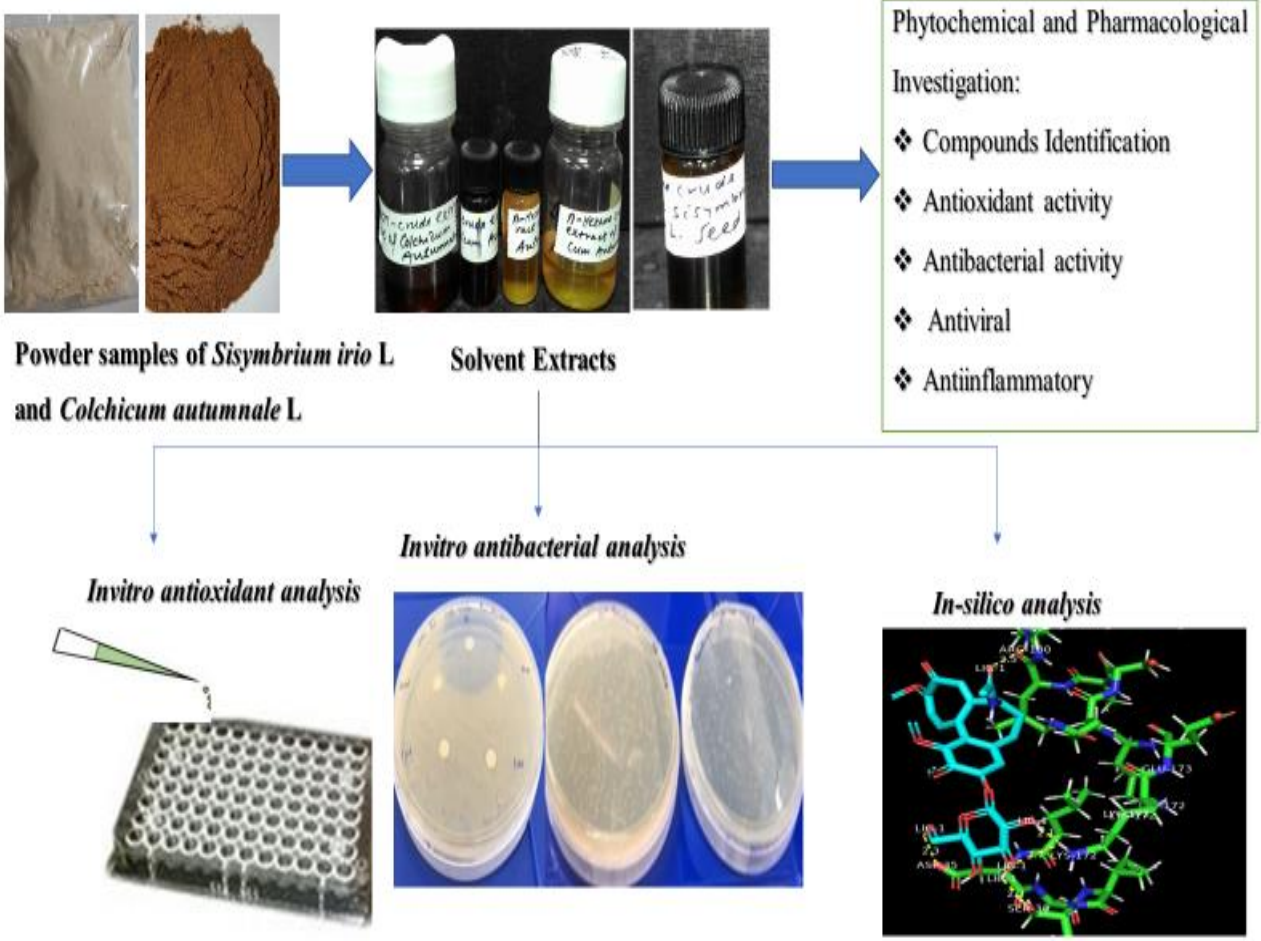
Overview of thesis



ABSTRACT

Unani system of medication is one of the oldest systems that prevails until date with its efficient drugs derived from plant, animal, and natural resources. In the Unani system of medicine, the drugs obtained from medicinal plants have a great interest due to their diverse applications. These plants comprise of various types of primary and secondary metabolites. Some of them have been playing a major role in the discovery of the new drugs for the treatment of various types of human and livestock diseases. *Sisymbrium irio* L is a medicinal plant and it belongs to the Family of *Cruciferae* and the various parts of this plant have been utilized as medicine and it is a world-wide distributed plant. Like *Sisymbrium irio* L, *Suranjan Shireen* (*Colchicum autumnale* L) which belongs to the family of the *Liliaceae* is primarily used for the treatment of arthritis in Unani medicine. The current investigation was carried out to analyze phytochemicals and pharmacological activities of *Sisymbrium irio* L and *Colchicum autumnale* L. The plant sample of *Sisymbrium irio* L and *Colchicum autumnale* L were bought from Khari Baoli Market, Delhi, Indian Drugs House, identified voucher ID 6238 & SC-0171/15 & extracted with n-hexane, dichloromethane, & methanol for 5-8 hrs in the Soxhlet apparatus.

Elemental analysis was done by using ICP-MS. Isolation, Identification, and Investigation of compounds were determined by a hyphenated spectroscopic and chromatographic techniques such as, GC-MS, FT-IR, NMR, MS, UV-Vis spectrophotometer and UHPLC-Q Exactive Orbitrap. The pharmacological properties for instance, the antibacterial, antioxidant and anti-dengue activity of *Sisymbrium irio* L and *Colchicum autumnale* L extracts were evaluated in vitro using paper disc diffusion, DPPH, ABTS, and MTT assay respectively. The qualitative phytochemical investigation demonstrated the presence of phenols, flavonoids, and terpenoids in every solvent extract of *Sisymbrium irio* L and *Colchicum autumnale* L, while steroids were recognized uniquely in n-hexane, dichloromethane and methanol extracts of the two plant extracts. However, saponins and tannins were not distinguished in any solvent extracts of these plants. Quantitative investigation of *Sisymbrium irio* L extracts affirmed that the methanol extract contained

the most notable number of phenolic substances and flavonoid substance, subsequent dichloromethane, and n-hexane, while the dichloromethane extract of *Colchicum autumnale* L contained the highest number of flavonoid and phenolic substances, following methanol and n-hexane extracts. The phytochemical constituents are responsible for its antioxidant, antimicrobial and anti dengue activity and other medicinal uses. Antioxidant analysis examination confirmed that the methanol extract of *Sisymbrium irio* L demonstrated the most notable antioxidant scavenging activity in comparison with n-hexane and dichloromethane extracts, while dichloromethane extract of *Colchicum autumnale* L exhibited the uppermost percentage of free radicals scavenging activity in comparison to n-hexane and methanol extracts. Antibacterial activity demonstrated dose dependent activity on the tested bacterial strains.

In vitro antiviral activity was performed against dengue virus-2 by the 3 (4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) strategy. Level of cell survival was likewise assessed by MTT. At this point of time maximum nontoxic dose (MNTD) of *Sisymbrium irio* L plant was examined through testing the dichloromethane, ethanolic, methanolic, and water extracts against Vero cells in vitro. Antiviral test dependent on cytopathic effects indicated via the level of inhibition upon treating DENV infected Vero cells with a maximum nontoxic dose (MNTD) of *Sisymbrium irio* L has the most antiviral inhibitory effects.

ICP-MS elemental analysis of *Sisymbrium irio* L and *Colchicum autumnale* L displayed the existence of Na, Mg, K, Ca, Sr, Mn, Zn, Al, P and Fe. GC-MS, and UHPLC-Q Exactive Orbitrap investigation of the extracts confirmed existence of:- Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, (E) - 9 - Octadecenoic acid, ethyl ester, 9, 12 - Octadecadienoic acid (Z, Z), Linoleic acid ethyl ester, β-sitosterol, Isorhamnetin, Isorhamnetin-3-neohesperidine, Isorhamnetin - 7 - O - beta - D - glucopyranoside, Isorhamnetin - 7 - glucoside, Isorhamnetin-3-Laminaribioside, Colchicine, (R/S)-Deacetyl Colchicine, 3-demethyl Colchicine, and Colchicoside (3-demethyl colchicine glucoside). The anti-inflammatory activity suggests that *Colchicum autumnale* L can be used for relieving the symptoms

of inflammation. Comparative docking studies revealed colchicoside as the most potent anti-inflammatory compound with minimum binding energy (affinity) as compared to other compounds and standard drug diclofenac.

Bioassay-guided effort yielded a compound which after characterization using UHPLC-QExactive Orbitrap, MS, ^{1}H-NMR, ^{13}C-NMR, and FT-IR identified as a class of apigenin, isorhamnetin derivative, and colchicine derivative. These spectroscopic techniques play an indispensable function in the isolation, identification & investigation of natural products for the discovery of new drugs. Hence, phytochemical analysis through the above spectroscopic techniques confirmed that *Sisymbrium irio* L and *Colchicum autumnale* L extracts contained the various types of bioactive compounds, and these bioactive compounds have an important role in the therapeutic system of medicine for treatment of disease. Further, *Sisymbrium irio* L and *Colchicum autumnale* L must be explored more on in vivo test in order to determine the mechanisms of action.

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List of Abbreviations and Symbols

&	And
°C	Degree Celsius
^{13}C-NMR	Thirteen Carbon Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
ABTS	2, 2'-Azino-bis (3-ethylbenzothiazoline-6-Sulfonic Acid)
CAT	Catalase
CC	Column Chromatography
CDCM	*Colchicum* Dichloromethane
CPE	Cytopathic effects
CDCM-8, CDCM-12	*Colchicum* dichloromethane extract of isolated compounds of 8 & 12
DCM	Dichloromethane
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	2, 2'-Diphenyl-1-Picrylhydrazyl radical
DSS	Dengue Shock Syndrome
FBS	Fetal Bovine Serum
FCPR	Folin-Ciocalteu phenol reagents
FDA	Food Developmental Agency
FT-IR	Fourier Transform-Infrared Spectroscopy
g	Gram
GAE	Gallic Acid Equivalents

GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GNTD	Greatest non-toxic dose
GPx	Glutathione peroxidase
GRx	Glutathione reductase
H	hour(s)
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IL-6	Interleukin
LC-MS/MS	Liquid Chromatography-Mass Spectrometry/Mass Spectrometry
LC-MS/TOF	Liquid Chromatography-Mass Spectrometry/Time of Flight
MeOH	Methanol
mg	Milligrams
min	Minutes
ml	Milliliter
mm	Millimeter
MNTD	Maximum non-toxic dose
MOI	Multiplicity of infection
MS	Mass Spectrometry
MTT	3-(4, 5-dimethylthaiazol-2-yl)- 2, 5-diphenyltetrazolium bromide
n-Hex	n-Hexane
ppm	Parts per million
PTLC	Preparative Thin Layer Chromatography
QE	Quercetin equivalents
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species

ROS	Reactive Oxygen Species
rpm	Revolutions per minute
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SM	*Sisymbrium* Methanol
SM-9, SM-17 & SM-19	*Sisymbrium* methanol extract of isolated compounds 9, 17 and 19
SOD	Superoxide Dismutase
TLC	Thin Layer Chromatography
Trolox	6-Hydroxy-2,5,7,8-tetramethyl- chroman-2-carboxylic acid
UHPLC	Ultra High-Performance Liquid Chromatography
UHPLC	Vanquish High-Performance Liquid Chromatography
UV	Ultraviolet
UV-VIS	Ultra violet-Visible Spectroscopy
w	Weight
WHO	World Health Organization
µg	Microgram
µl	Microliter

CHAPTER 1: INTRODUCTION

1.1. Historical Background

Unani Medicine likewise called as Yunani Medicine (in Arabic, Hindi-Urdu and Persian) signifies "Greek Medicine." Its origin is drawn back to the Greek writing, which has been an establishment of a considerable amount of scientific contributions and furthermore was set up through Persians, and Arabs into a detailed medical science. Meanwhile, it has been referred as Greco-Arab drugs. It is a great therapeutic art as well as science. It treats a person as a whole not as a group of individual parts. It's geared towards treating soul, mind, and body. Unani system of drugs was grounded in Hippocratic theory of four humors viz. blood, black bile, yellow bile and phlegm. According to World Health Organization (WHO), Unani system of medicine has been documented as an alternative system of medicine to provide the health care requirements for the world population. It is one of the famous traditional systems of medicine & draws on the old-style systems of medicine of China, India, Syria, Persia, Iraq and Egypt. It is vigorous & vibrant today and is being taught, practiced & researched under its local names in over twenty countries among China, India, Afghanistan, Denmark, Finland, Canada, Netherlands, Norway, Poland, Germany, Japan, Korea, Saudi Arabia, Sweden, Switzerland, UK, Turkey and USA (Sudhir, 2014). Unani system of medication is one of the oldest systems that prevails until date with its efficient drugs derived from plant, animal, and natural resources. According to this system of medicine, the methods of the treatment can be categorised into four various parts namely Regimenal therapy (Ilaj-Bil Tadbeer), Dietotherapy (Ilaj Bil-Ghidha), Surgery (Ilaj-Bil-Yada)& Pharmacotherapy (Ilaj-Bil-Dawa). In view of pharmacotherapy, both single and compound drugs are being used. Amongst the compound formulations, Dawa ul Misk Motadil Sada is a formulation that is used in Unani System of medicine and is being prescribed for the treatment of *Khafaqān* (Palpitation) from centuries with great reputation (Akhtar et al., 2019).

In Unani therapeutic, the medicines obtained from the medicinal plants have a great interest due to their diverse applications. The medicinal plants are the foundation of effective sources of natural products consumed as phytomedicines in Unani system of medicine and the chemical compounds derived from these medicinal plants have been playing a major part in the finding of novel drugs for the treatments of the various category of human, and animal diseases (Hailu et al., 2019).

They contain various types of phytochemicals and these phytochemicals have massive physiological activities in humans and animals. The therapeutic plant *Sisymbrium irio* L has a place with the family *Cruciferae*, and it is worldwide distributed plant (Khoshoo, 1966). In 1980, Vohora *et al*., investigated the seeds of *Sisymbrium irio* L Indian origin for antipyretic, analgesic and antimicrobial activity (Vohora et al., 1980). In 2011 and 2013, Haroon khan and Khan *et al*., isolated isorhamnetin, quercetin, β-sitosterol & β-sitosterol-3β-D-glucoside from aerial parts of the plants collected from the campus of Jamia Hamdard, New Delhi, India(Haroon Khan, 2011; U. A. Khan et al., 2013).

The phytochemical and biological studies (LD_{50}, antioxidant activity) of the aerial part of Saudi Arabia species (Najed Region) of *Sisymbrium irio* L contained flavonoids (N.A. Al-Jaber et al., 2011; N.A. A. Al-Jaber, 2011), β-sitosterol, stigmasterol & β-sitosterol glucoside (Al Massarani et al., 2017). Research study on the investigation of Baghdad-Iraq (Al-Jadrea) species of *Sisymbrium irio* L showed the presence of nicotine in the aerial part of the plant (Alsaffar et al., 201 7; Alsaffar, Abbas, & Dawood, 2016; Alsaffar, Abbas, Dawood, et al., 2016). The examination perf ormed on the aerial parts of *Sisymbrium irio* L gathered from the Irbid area confirmed the oil comp rised of acids, esters, sulfur, nitrogen - containing compounds, terpenoids, aromatic, and aliphatic compounds, and alcohols (Al-Qudah & Abu Zarga, 2010a, 2010b).

In 1998, Guil *et al*., from Spain investigated the leaves of wild edible *Sisymbrium irio* L (Hedge Mustard) for nutritional purpose as a result of the various amounts of nutrients (protein 3.43%, carbohydrates 1.43%, lipid 2.14%, oxalic acid/Calcium ratio 1.1), and fatty acids ω3 (46.57%) & ω6 (13.02%) together with erucic acid C22:1ω9 (2.52%) (Guil-Guerrero et al., 1999). Ethanolic solvent

extracts of a fresh plant of *Sisymbrium irio* L seeds were collected at the flowering stage from Peshawar University Campus, Pakistan reported the cytotoxic, phytotoxic and insecticidal activities (Shah et al., 2017)

In 2017, Gamal *et al*., from Egypt reported that the n-hexane extract of *Sisymbrium irio* L leaves collected from Bahariya Oasis, hindered the development of microbial strains; for example, *Klebsiella pneumonia* and *Staphylococcus epidermidis*, while the n-hexane extract of *Sisymbrium irio* L seed exhibited more noteworthy inhibitory impact against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The ethyl acetate fraction of the leaves of *Sisymbrium irio* L was dynamic against the gram-negative bacteria, for example, *Escherichia coli*, *Klebsiella pneumonia* & *Pseudomonas aeruginosa* & water extract of *Sisymbrium irio* L was dynamic against all tried pathogenic microbials, for example, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Enterococcus faecium, Acinetobacter baumanii, Enterobacter cloacae, and Candida albicans;* and the purified compound showed dose-dependent cytotoxic activity against Vero cell line (El Sherbiny et al., 2017). Solvents gradient based extracts of the seeds & leaves of *Sisymbrium irio* L gathered from Islamabad and Rawalpindi showed inhibition against the bacterial and fungal strains (Bibi et al., 2015). A study conducted at the aerial part of *Sisymbrium irio* L collected from Al-Jadriya area Baghdad-Iraq declared the ethyl acetate & methanol extract of the plant possesses hepato-protective activity against CCl_4 induced hepatotoxicity in rats (Alsaffar et al., 2017; Alsaffar, Abbas, & Dawood, 2016; Alsaffar, Abbas, Dawood, et al., 2016). Research conducted on the acute-toxicity, anti-inflammatory and bronchial smooth muscle investigation of an Indian variety of *Sisymbrium irio* L (seeds) is safe up to the doses of 1000mg/kg and causes no mortality and exhibits normal behaviour on the tested animal (R.K. Singh, 2015).

Our second medicinal research plant; *Colchicum autumnale* L belongs to the family *Liliaceae* that has an important part in the discovery of novel drugs in medicine. Physicians of Unani medicine make use of *Suranjan shireen* (*Colchicum autumnale* L) as the main line for the management of arthritis

and it's been referred to by nearly all renowned Unani authors in their books together with Unani Pharmacopoeia. The therapeutic values of *Colchicum autumnale* L have been well known to the Arabs (Siddiqui et al., 2020). The *Colchicum autumnale* L is so-called for the land-living of Colchis by the side of the eastern tip of the Black Sea. First completed descriptions & sketches of the plant known "Colchicon," were recognized in the first century AD by Dioscorides, father of botany in Unani medicine. *Colchicum autumnale* L is commonly a vital source of colchicines which was firstly extracted from bulbs and seeds. It is a class of alkaloids used for the remedy of gout and rheumatism, painful muscles, inflammation, and patients with familial Mediterranean fever (Jung et al., 2010; Spasevska et al., 2017), cirrhosis, sweet's syndrome, asthma, liver fibrosis, Behçet's disease and pericarditis with effusion (A. Poutaraud & Girardin, 2003; Anne Poutaraud & Girardin, 2002).

Colchicum autumnale L has antioxidant capacities owing to which it has been used to treat internal wounds for centuries and, its mixture is effectively used for curing piles and it has beneficial effects in gout such as small joints pain. The plant has minor toxic effects without any addictive element so it gives a revitalizing deep sleep to the users of its compositions (M. Akram, 2012). It is a well-known pain killer and has a great role in the removal of the pain of all types of muscular and burning muscular tissues, joint and gastric pains, periosteum and synovial membranes of joints and has a beneficial role for the treatment of foot palm burning (Anne Poutaraud & Girardin, 2002). The plant is chosen as a medication for the treatment of joint pain in the Unani arrangement of prescription (Ellington et al., 2003). It was concluded from the literature reviews *Suranjan Shirin* (*Colchicum autumnale* L) is mentioned in Unani classical literature for its various therapeutic efficacies but specifically in arthritis and join pain. Several preliminary studies reported its effectiveness in different forms of arthritis. In addition to effective uses for treatment of arthritis in Unani medicine, *Colchicum autumnale* L must be further explored to investigate the phytochemicals and dose-dependent pharmacological activity such as antibacterial, antioxidant and anti dengue activity of the plant extracts.

1.2. Statement of the Problem

In the course of the most recent decades, there has been an expansion in the number of individuals being determined to have maladies identified with oxidative harm such as malignant growth, joint pain, maturing, immune system issues, cardiovascular and neurodegenerative ailments. This uncontrolled expansion/proliferation of an ordinary/normal cell that produces hereditary instabilities and alterations accumulate within cells and tissues which transforms a normal cell into a malignant cell. Both external components/factors such as radiations, smoking, tobacco, toxins in drinking water, nourishment, air, synthetic compounds, certain metals, irresistible agents, internal variables/factors such as hereditary transformations, body invulnerable framework and the hormonal issue can cause malignant growth (Krishnamurthi, 2007). According to the research study conducted by Ferlay, around 10.9 million new malignancy cases, 24.6 million people living with the diseases, 6.7 million deaths reported the global over every year (Ferlay et al., 2015).

In view of World Health Organization information, above 14.1 million new disease cases & 8.2 million deaths were mentioned globally in the year of 2012 & over 70% new malignancy or cancer cases have been evaluated during the following twenty years (Ferlay et al., 2015; Faria et al., 2017). It is extraordinary that free radicals are the countless reason for one of a kind interminable chronic and degenerative diseases, for instance, coronary diseases, irritation, disturbance, stroke, diabetes mellitus, and cancer (Scalbert et al., 2007). Subsequently, it is fundamental to study the antioxidant activity of the plants used in the herbal medical prescription either to elucidate the mechanism of their pharmacological activity or to give data on antioxidant action of medicinal herbal plants (Molan et al., 2012). The sources of herbal antioxidant agents are principally phenolics that may appear in all parts of plants, for instance, herbal products, vegetables, nuts, seeds, leaves, roots, and barks (Mathew & Abraham, 2006). Antioxidant agent capacities are associated with decreased DNA damage, lessened lipid peroxidation, keeping up vulnerable capacity and inhibition of the malignant transformation of cells (Cao et al., 1996a, 1996b; Y. Li et al., 2019; Wan et al., 2011). The

growing incidence of cancer, the various limitations in the conventional therapy including high cost and the high toxicity of the present anticancer drugs has confronted an extreme test to the scientists to structure and build up other options which are eco-accommodating, biocompatible and practically feasible techniques in a greener way. Like in case of malignancy the event of dengue expanded 30-fold in the range of 1960 and 2010, because of a combination of urbanization, population development/growth, increased worldwide or international travel and global warming (Whitehorn & Farrar, 2010). It is endemic in more than 110 countries (Ranjit & Kissoon, 2011).

The World Health Organization (WHO) surveys that 50-100 million dengue diseases happen each year and that practically 50% of the total populace lives in nations where dengue is endemic; and as of now near 75% of the world populace presented to dengue are in the Asia-Pacific region (Abd Kadir et al., 2013). There's a promising future of medicinal plants as there are about half million plants round the world, and maximum of them haven't been investigated yet for their medical activities and their hidden ability of scientific activities will be decided within the remedy of present and destiny research (R. Singh, 2015). Within the development of human tradition, medicinal plants have performed an important function, for instance in religions and distinctive ceremonies (Hosseinzadeh et al., 2015). If we look at a few of the type of modern drug treatments, many of them are produced in a roundabout way from medicinal plants, for instance aspirin. A number of food crops have a medicinal activity for example, garlic is widely used as medicine. Investigation of therapeutic plants helps to realize their toxicity and protect human and animals from natural poisons and their medical consequences of plants are due to secondary metabolite compounds produced in the different parts of plants. Keeping this inattention, they have enhanced the interest of studies in the subject area of natural product chemistry. This is due to several factors, like along with therapeutic needs, the wonderful diversity of both chemical and biological activity of naturally occurring phytochemicals, the application of novel bioactive natural compounds as biochemical probes; the improvement of novel and sensitive strategies to locate biologically energetic herbal products, stepped forward

strategies to isolate, purify, and structurally represent those active constituents and also advances in solving the demand for supply of complex natural products (Clark, 1996).

1.3. Study Justification

In view of the inaccessibility of effective vaccines and proper or specific treatment of dengue fever in current or modern systems of medication, there is a need to search for a safe, effective and successful, adequate and acceptable treatment in medicine. The quest for alternative safe medicine in a current-day; most natural product researchers depend on natural remedies for various purposes. Hence, Isolation, Identification, and Investigation of biological activities (3Is) of compounds of natural origin with the help of spectroscopic and chromatographic techniques have several advantages in the discovery and development of novel drugs, for instance, it permits the production of semi-synthetic molecules, structural modification, and rationalization of mechanisms of action. The significant role played by trace compounds in the studied plants in overall biological activities of extracts needs further investigation and characterization of bioactive compounds for the use of Unani medicine. There is a sign of broadening the bioassay work on these plants to isolate bioactive compounds. There is the requirement for extra itemized examination concerning such practices to encourage their improvement and usage for the control of dengue fever, particularly patients worldwide. In addition to effective uses for treatment of diseases in Unani medicine, *Sisymbrium irio* L and *Colchicum autumnale* L must be further explored to investigate the phytochemicals and dose-dependent pharmacological activity such as antibacterial, antioxidant and the ant-dengue activity.

1.4. Objectives of the Study

I. Phytochemical Analysis

(a) Extraction of plant materials

(i) Cold pressed extraction of oil

(ii) Soxhlet extraction (hot extraction) using of solvent (n-hexane, dichloromethane and methanol)

(b) Bioassay guided isolation of phytochemicals and their characterization (CC, GC-MS, UHPLC-QExactive Orbitrap analysis, FT-IR, NMR (^{1}H-NMR, ^{13}C-NMR) & MS

II. Pharmacological investigation of plant materials

(a) Antibacterial

(b) Antioxidant

(c) Anti-dengue (anti-viral) activity

III. In-silico studies of anti-inflammatory activity of molecular docking

CHAPTER 2: LITERATURE REVIEW

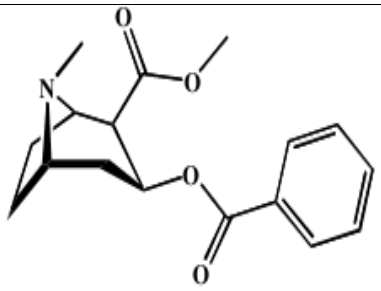
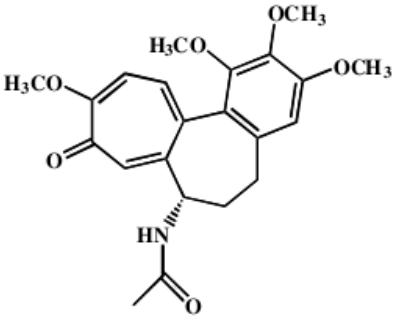
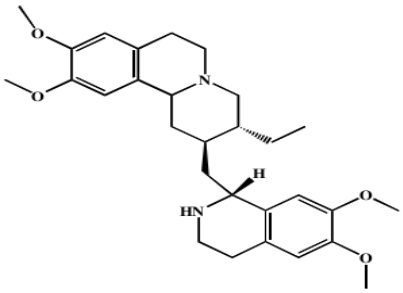
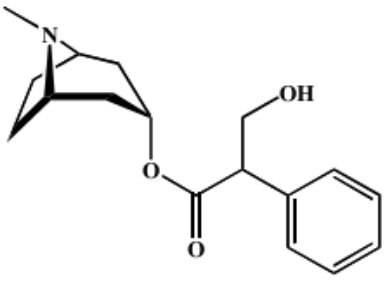
2.1. Medicinal Plants

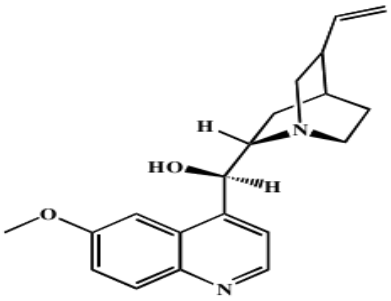
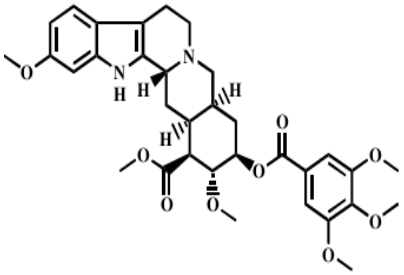
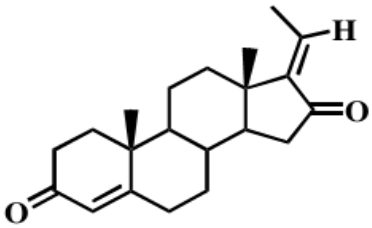
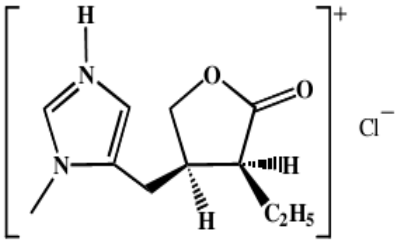
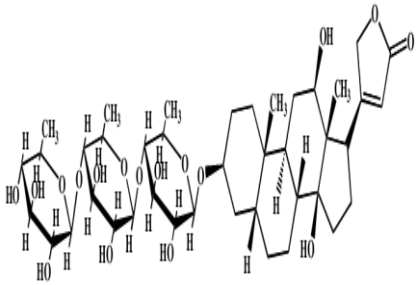
Medicinal plants have therapeutic and curative uses and they are considered as the backbone for folk medicine; in developing countries, more than 3.3 billion people utilize medicinal plants on a normal basis (Davidson-hunt, 2000). They have a great function in drug discovery and synthesis and additionally are considered as a prosperous source of aspects that are employed in drugs synthesis and improvement. They have a fundamental part in the growth of human cultures around the whole world, and livestock ailments are as historic as manhood itself. Still, most of the world population depends upon therapeutic plants for the medication of various diseases, and they are the richest resource for folkloric frame works of medication, present-day prescription, modern medicine, pharmaceutical intermediates, nourishment or food supplements & chemical entities for synthetic drugs (Hammer et al., 1999). In accordance with the World Health Organization (WHO) guide, about 80% of the world population depend upon the medicinal plants for their health care needs (Khan et al., 2013; A. et al., 2013). Moreover, an extended reliance on the usage of the therapeutic plants in the industrialized world has been followed to the extraction & advancement of numerous drugs and chemotherapeutics from therapeutic plants similarly as from traditionally utilized home grown remedies (Hoareau, 1999).

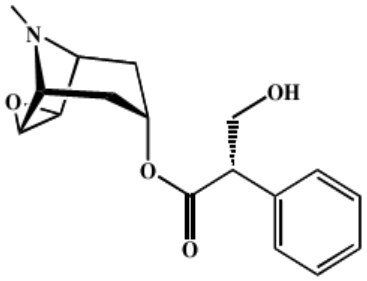
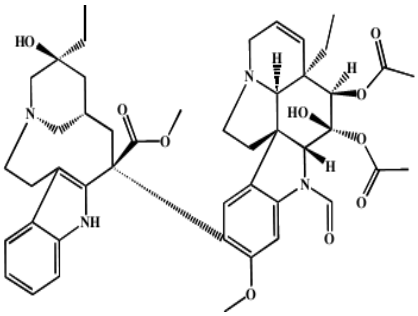
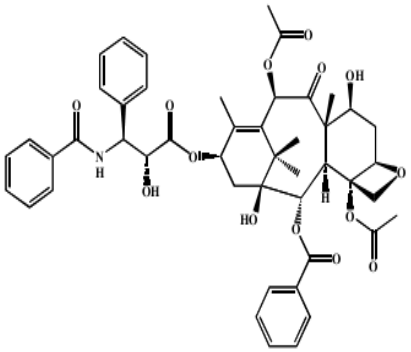
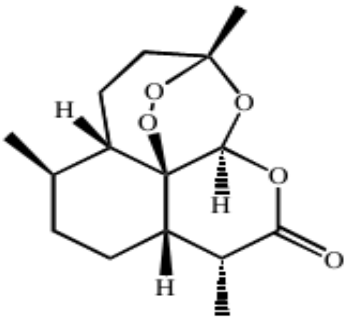
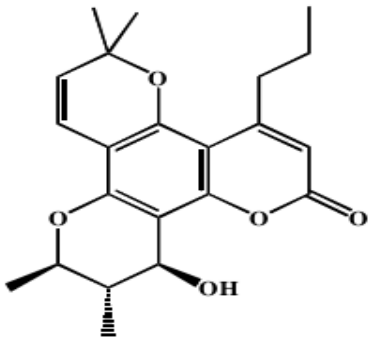
In various industries the role of medicinal plants is remarkable; for example, fine synthetic compounds, pharmaceuticals, cosmetics, drugs, and industrial raw materials and so forth. For the discovery of novel drug medicinal plants accomplish a dynamic part. In fact, even today, medicinal plants are fundamental in human care, and serve as the best hotspot for safe future medicines (Mahidol et al., 2002). Regardless of the way that now we have at our bearing different present-day drugs, it is still extremely critical to develop, and discover new therapeutic agents. It has been assessed the satisfactory treatment is accessible just for one-third of the human ailments. Along these

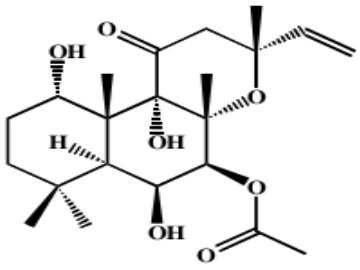
lines, battles against disorders have proceeded tirelessly. Folkloric plants medicines still acknowledge basic circumstances for the present medication or modern-day drug industries. A large part of the significant drugs of the last fifty years, which have been developed in the modern medicinal system are derived from plants. The World Health Organization expands, promotes and supports natural herbal drugs medications in national human care needs since they are easily available at a low price; have lesser side effects and are safer in comparison to modern synthetic drugs. Along these lines, the investigation of pharmacologically bioactive compounds is acquired by screening therapeutic plant sources or plants extracts for discovery of numerous therapeutically vital drugs for the treatment of human's ailments (Piccoli & Zatti, 1977). Present-day researches for bioactive molecules utilize modern bioassays and bioassay-guided fractionation of medicinal plants utilized by traditional healers. The bioassays guidelines prompted the separation of numerous novel therapeutically significant compounds by fractionation of various parts of the medicinal therapeutic plants. A decent number of ground-breaking or powerful medications and an enormous number of therapeutic leads and various new pharmacologically active constituents have been derived from herbal drugs as a result of the committed undertakings of researchers (Babu, 2019). Medicinally plants are often used as crude substances for the extraction of dynamic or active substances used in the synthesis of a number of drugs.

Table 1: Name and chemical structures of some prominent plant-based drugs (Fabricant & Farnsworth, 2001; Cragg & Newman, 2005; Li et al., 2010; Pillay et al., 2008)

Name	Source	Uses	Structures
Cocaine	*Erythroxylon coca*	Anesthetic	
Colchicine	*Colchicum autumnale L*	Rheumatism treatment	
Emetine	*Psychotria ipecacuanha*	Used as anti-amoebic drugs	
Atropine	*Atropa belladonna*	Ophthalmic treatment	

Quinine	*Cinchona ledgeriana*	Antimalarial	
Reserpine	*Rauwolfia serpentine*	It is very important controls high blood pressure	
Guggulsterone	*Commiphora mukul*	Decrease the level of cholesterol in the human body	
Pilocarpine	*Pilocarpus jaborandi*	Cures glaucoma	
Digoxin	*Digitalis lanata*	To treat cardiac disorders	

Hyoscine	*Hyoscyamus niger*	Treats nausea	
Vincristine	*Catharanthus roseus*	It is important for cancer chemotherapy	
Paclitaxel and Taxol	*Taxus brevifolia*	Paclitaxel and taxol are used for cancer chemotherapy	
Artemisinin	*Artemesia annua*	Antimalarial	
Calanolide A	*Calophylum lanigerum*	Anti-HIV agent	

Forskolin	*Coleus forskohlii*	Vasodilator	

Nowadays in the pharmaceutical industry, therapeutic plants are the main component for research progressions & improvements. Such types of research focus on isolation, identification, investigation, examination, and utilization of bioactive medicinal constituents. The development and commercialization of medicinal plant-based bioindustries in the developing nations is needy upon the accessibility of facilities and data concerning downstream and upstream bioprocessing, extraction, filtration, purification, and marketing of the industrial potential of therapeutic plants (R. Singh, 2015). Rise of the present-day pharmaceutical industry is a result of various exercises including synthetic chemists, natural product chemists, pharmacologists, microbiologists, and natural chemists and so on, which has prompted the improvement of potent single molecules with very specific action for a wide assortment of diseases.

2.1.1. Medicinal Plants Used as Antimicrobial, Antioxidant, Antiviral, and Anticancer

2.1.1.1. Medicinal Plants Used as Antimicrobial

In a challenge to struggle with the various sorts of diseases that have proceeded to plaque people from time immemorial to the moment, numerous antimicrobials have been evolved to fight the pathogens responsible for the human diseases. Antimicrobials, which might be substances that repress or kill the development and improvement of microorganisms, can be as anti-microbials, which might be outcomes of microorganisms or incorporated or integrated derivatives, antimicrobial peptides produced by complex life forms or organisms just as some microbes and medicinal plants, which give off an impression of being the today pivotal point of standard medicine (Cowan, 1999). Medicinal plants are rich bio-assets of antimicrobial agents, they are utilized therapeutically in various countries

of the world and are the wellspring of potential and amazing drugs (Srivastava et al., 1996). Antimicrobial activity of some of the medicinal plants are represented here: -

***Allium sativum* (garlic):** Aqueous extracts of garlic have antibacterial influences against a wide sc ope of Gram-positive, Gram-negative bacteria, fungal growths (Hajimehdipoor et al., 2012) including multidrug-safe enterotoxic hereditary strains of *Escherichia coli* (Ankri & Mirelman, 1999) and thus utilized for the management of dental diseases like periodontitis (Bakri & Douglas, 2005). In 1999, Sasaki *et al* identified the antibacterial activity of garlic & indica ted the utilization of fresh garlic powder turned into extra effective than old garlic powder (Sasaki, 1999).

***Azadirachta indica* (Neem):** Pre-remedy of *Streptococcus sanguis* with the plant extract resulted in the noteworthy inhibition of bacterial bond or adhesion to saliva adapted hydroxyapatite, a composite of bone and teeth. Additionally, it inhibited insoluble glucan synthesis, recommending or suggesting plant can decrease the adherence of *streptococci* to enamel surface (Wolinsky et al., 1 996)

***Piper betle*:** Crude water extract of plant displayed reduced impact towards the development, follow ing potential, glucosyltransferase action to opposition *Streptococcus mutans* & further rosemary and cinnamon had antimicrobial activity towards *Streptococcus* mutans(Nalina & Rahim, 2007).

***Syzygium aromaticum*:** In 1996, Wu and Cai revealed the solvent methanolic crude extract was used for the treatment of periodontal ailments (Cai & Wu, 1996).

***Juglans regia*:** Adequacy of the plant extract (aqueous and acetone) has been evaluated via checking out on salivary samples of patients suffering dental carries(Deshpande & Salvekar, 2011).

***Myristica fragrans*:** The solvent ethanolic extract of the plant showed a great potential against the tested bacteria such as *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleactum, Periodontopathic, Porphyromonas gingivalis, Streptococcus salivarius, Streptococcus mutans*(Jaiswal et al., 2009).

***Mimusops elengi*:** The plant bark extract was screened to test antimicrobial properties of the plant,and the outcomes confirmed the antimicrobial functionality of the plant and proven that the ac etone extract can be used in the treatment of infectious diseases resulting from salivary microflora (Roqaiya et al., 2015).

***Punica granatum*:** Ethanolic, acetone, methanolic and water extract exhibited strong antimicrobial activity in various examinations done on both gram-positive and gram-negative non-oral microscopic organisms (Machado et al., 2002; Silva et al., 2008).

***Emblica officinalis*:** It comprises different chemical constituents, for example, ellagic acid, gallic acid, phenols, flavonoids, kaempferol polyphenols, and tannins (Nair & Chanda, 2007). They have the ability to prevent dental caries with the aid of hindering the virulence factors of *Streptococcus mutans*, and *Lactobacillus*(Hasan et al., 2012).

***Terminalia chebula*:** The essential constituents are hydrolysable tannins (thirteen%,) as an instance, chebulic acid, chebulagic acid, corilagin, & gallic acid (Han et al., 2006). These acids are discovered to have an antibacterial activity towards carcinogenic bacteria (Aneja & Joshi, 2009).

***Triphala*:** 0.1% chlorhexidine and 0.6% *Triphala* (*Terminalia chebula*, *Terminalia bellirica*, and *Emblica officinalis* blend) had been appeared to inhibitory plaque effect, gum disease and development of *Streptococcus mutans* and *Lactobacillus* (Bajaj & Tandon, 2011).

***Baccharis dracunculifolia*:** It had comparable effectiveness of the substances used to oral cleanliness or hygiene in the reduction of dental plaque and consequently, the anticipation of dentalcaries. In this way, it is able to be considered as a good candidate for new fabric to be implem ented in dental care (Pedrazzi, Leite, Tavares, Sato, Crivelaro, et al., 2015; Pedrazzi, Leite, Tavares, Sato, Nascimento, et al., 2015).

***Salvadora persica*:** It was shown in exceptional in vivo & in vitro examinations that alcoholic and aqueous extracts have potential to fight against extraordinary aerobic, and anaerobic organisms like *Streptococcus mutans* and *Emblica corrodens* had indicated robust antimicrobial activity (S.I. Khan, 2007; Poureslami et al., 2007; Darout et al., 2000).

***Quercus infectoria*:** The crude extracts demonstrating anti-dental caries interest may want to bring about the discovery of recent chemical classes of antibiotics (Vermani, 2009).

***Nidus vespae*:** The plant has been widely utilized in conventional Chinese medication, given their different pharmacological exercises, including antimicrobial, calming, against infection and sedative properties (Xiao et al., 2007).

***Cratoxylum formosum*:** An exploration indicated the plant gum has excessive antimicrobial action i n opposition to *Streptococcus mutans* and may turn into a promising natural varnish against caries (Suddhasthira et al., 2006).

Therapeutic plant-based antimicrobials represent an enormous unused source of pharmaceuticals and extra examination of plant antimicrobials needs to happen for treatment of various diseases both in plants and humans while at the same time for alleviating a considerable lot of the side impacts that are regularly connected with synthetic antimicrobials. Out of the few hundred thousand medicinal plant species around the world, just a little segment has been examined both phytochemically and pharmacologically (Hostettmann, 1999). In perspective on the huge number of the plant species conceivably accessible for the examination, it is fundamental to have effective frameworks of the techniques to assess the adequacy of therapeutic plants as antimicrobial agents. The pharmacological examination for an antimicrobial agent of plant cause starts with an intensive biological evaluation of plant extracts to guarantee viability & well being pursued by identification of active principles, dose details, adequacy and pharmacokinetic profile of the new drugs. Numerous plants have been utilized on account of their antimicrobial qualities and anti-microbial activities of plants have been explored using various researchers around the world-wide. Microbiologists, botanists, natural products scientists, and ethno-pharmacologists are looking at the world for phytocompounds which could be developed for the treatment of microbe's diseases (Tanaka et al., 2009)

2.1.1.2. Medicinal Plants Used as Antioxidant

Oxygen is one of the most critical components of the cells in human life; the cells use oxygen to produce energy and reactive free radicals are created due to ATP production by means of the mitochondria. The reactive free radicals are typically reactive nitrogen species (RNS) and reactive oxygen species (ROS) are created due to cellular redox process, and they play dual role in our body that means both as beneficial and toxic compounds, and their delicate balance between two opposing outcomes are manifestly a vast part of life. At low or slight stages, ROS and RNS play a big role within the cellular reactions and immune features. At high concentrations, they result oxidative stress, a malicious produce that can harm all cellular structures. Oxidative stress has a noteworthy effect in the development of chronic & degenerative diseases, for instance, malignant growth, joint aggravation, joint inflammation, joint pain, arthritis, aging, immune system issue, cardiovascular and neurodegenerative diseases (Barry Halliwell, 2006a, 2006b;Bahorun et al., 2006;Marian Valko et al., 2004;M Valko et al., 2006; Marian Valko et al., 2004;Willcox et al., 2010;Pacher et al., 2011;Genestra, 2007;B Halliwell, 2007).

The description of both free radicals' species is as follows: -

Reactive Oxygen Species: Incorporate lipid peroxyl ($LOO^{\bullet}$), peroxyl (ROO•), hydroxyl (OH•) & superoxide (O_2•-). The addition of one electron to a dioxygen molecule forms the superoxide anion radical ($O_2^{\bullet}$). Superoxide anion, creating both through metabolic systems or ensui ng oxygen "actuation "through physical light or illumination of light, is called the "essential" reactive oxygen species (ROS), and can moreover interface with quite a number of particles to make "secondary" reactive oxygen species, either immediate or by and large via compound, enzymatic or metal-catalysed processes (Marian Valko et al., 2004).

Reactive Nitrogen Species: These are nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), nitricoxide (NO), and peroxynitrite anion ($ONOO^-$).

These ROS, and RNS enclosed as the consequences of normal cell process are all-round saw for ass uming a double job as useful and deleterious species, because they can be both useful, and harmful to residing frameworks. Free radicals are unstable molecules that contain excess electron and they have ability to react with a variety of herbal substrates, for instance, lipids, proteins and DNA. The dangerous impact of free radicals causing conceivable normal harm named oxidative stress and nitrosative stress. By the day's end, oxidative stress consequences from the metabolic reactions that use of oxygen and speaks to an aggravation or disturbance in the harmony repute of antioxidant/pro-oxidant reactions in living organisms (M Valko et al., 2006).

The extra ROS can harm cell lipids, proteins or DNA, controlling their run of the mill limit. Along these lines oxidative stress has been associated with one of a kind human disease similarly as in the ageing approach and the sensitive concord amongst precious and hurtful impacts of free radicals is a noteworthy piece of residing creatures and is practiced by using frameworks called "redox regulation". The procedure of "redox guideline" protections of living beings from more than a few oxidative stresses and continues up "redox homeostasis" through monitoring the redox reputation in vivo. At the point when free radicals are made in vivo, quite a number of malignant growth counteractive action operators in the body show by means of guarding the living being from oxidative damage. The 1st line of protection, free radical scavenging antioxidant agents, such as, peroxidases and metallic chelating proteins suppress the era of free radicals. Next, nutrient, nutritional vitamins C and vitamins E scavenge radicals to block the oxidation chain initiation & chain growth or spread as a 2nd line of protection and this may additionally likewise incorporate the end of a chain by way of the reaction of two radicals. The restoration & once more chemical substances go about as the third line of the safety via fixing the damage and reconstituting membranes (Droge, 2002). The word antioxidant has gotten noticeable in current society as it got a presentation through wide correspondences consideration of its therapeutic points of interest. The antioxidant is "a substance

that restricts oxidation or represses responses advanced by oxygen or peroxides"(http://unabridged.merriam-webster.com).

The more organic significant meaning of antioxidants is "a synthetic/natural substance introduced to merchandise to stop or postpone their deterioration by using the action of oxygen in the air'. Inorganic chemistry and medicine states, "antioxidants are enzymes or substances, for example, Vitamin E or β-carotene that are outfitted for checking the harming effects of oxidation in animal tis sues"(http://cancerweb.ncl.uk/cgibin/omd?query=antioxidants). Antioxidants fight against the free radicals created in vivo, along these lines forestalling the organisms against oxidative damage. Therefore, the media thought about their medicinal preferences. Antioxidants can be classified as: non-enzymatic and enzymatic antioxidants. The principal enzymatic antioxidants immediately stressed in the balance of reactive oxygen species and reactive nitrogen species are catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GRx) and glutathione peroxidase (GPx) (Bahorun et al., 2006; Marian Valko et al., 2004; Marian Valko et al., 2007).

Antioxidants can be:

1. SOD, Catalase, GR_X and GP_X are known as enzymatic antioxidants
2. Carotenoids, vitamin E, vitamin C, thiol antioxidants such as glutathione, thioredoxin and lipoic acid, flavonoids and melatonin are known as non-enzymatic antioxidants

The non-enzymatic antioxidants can be classified as metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants retailers are endogenous antioxidants, conveyed with the aid of assimilation in the physique or digestion or metabolism, for instance, glutathione, lipoic acid, uric acid, coenzyme Q10, L-arginine, melatonin, bilirubin, metal-chelating proteins, so on. Nutrients antioxidants are exogenous antioxidants for instance, vitamins E, vitamins C, carotenoids, trace metals (manganese, selenium, zinc), flavonoids, omega-3 and omega 6 unsaturated fatty acid and so on.

Endogenous antioxidants are a substance which produced inside the human body for defense as an

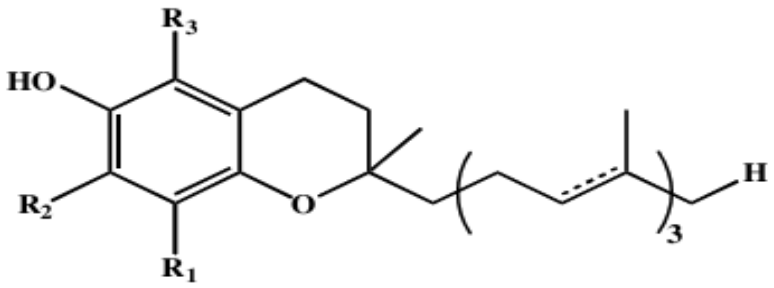
end result of ordinary metabolic strategies in the body. There is a splendid association of intracellular and extracellular antioxidants with special occupations interior in every area of the guard. Enzymatic antioxidant catalase converts hydrogen peroxide to oxygen molecule and water molecule whilst superoxide dismutase (SOD) modifications over the superoxide radical to hydrogen peroxide and oxygen molecule, a component of the antioxidants catalysts exist in one of a kind structures, for instance, cytosolic, layer and plasma kinds of glutathione peroxidase have been isolated. On the other hand, SOD has film, cytosolic and extracellular structures. Antioxidants catalysts for instance, super oxide dismutase, glutathione peroxidase & catalase work inside the cells to expel most superoxides and peroxides free radicals. Peroxidative chain responses started out via using free radicals that acquired away from the antioxidant's guards are ended via chain breaking water or lipid soluble antioxidants (Matés et al., 1999).

Exogenous antioxidants compounds come through diet and they play a simple exercise in the creation or generation of the antioxidants protect frameworks by way of giving the necessary job in the creation or manufacturing of antioxidant defence structures by imparting indispensable antioxidants supplement, for example, β-carotene, vitamin E, and vitamin C other antioxidant agent such as plant phenols which include flavonoids and critical natural resources that shape immense antioxidants phytochemicals. A medicinal plant is the super supply of naturally bioactive compounds known as phytochemicals or secondary metabolites. The predominant medicinal plant phytochemicals have been encouraged as naturally antioxidant attributes which may additionally contribute to the total antioxidant exercise of the plant materials together with polyphenols, aromatic, carotenoid and antioxidant vitamins, for example, vitamins C and E. These phytochemicals have been found to go about as antioxidants dealers via scavenging free radicals & might also have supportive or useful therapeutic plausible for free radical associated disorders (Dian a Victoria.T & Antony.V. Samrot, 2014). In fact, numerous authors have described a direct

relationship between total phenolic content material and antioxidant undertaking in a range of seeds, fruits and vegetables (Y. Li et al., 2019; Yang et al., 2017).

Vitamin E

It is a fat-soluble and a chiral compound with eight stereoisomers: α, β, γ, δ tocotrienol (with double bonds inside chain) and α, β, γ, δ tocopherol and it has high antioxidant capacity. α-Tocopherol is the most bioactive structure in humans (Nguyen et al., 2006). As it is fat dissolvable, α - tocopherol safeguards cell membranes from harm by way of free radicals. Its antioxidant work for the most part, dwells in the safety against lipid peroxidation. It plays an important role in the protection of prostate, colon, & breast malignant growths, cardiovascular infec tions, cataract, joint irritation & positive neurological issue. The main nutritional sources of vitamin E are: - wheat germ oil, vegetable oils, nuts, complete grains, oats, organic products, poultry, eggs, meat products.



α-tocopherol, $R_1 = R_2 = R_3 = CH_3$
α-tocotrienol, $R_1 = R_2 = R_3 = CH_3$

β-tocopherol, $R_1 = R_3 = CH_3$; $R_2 = H$
β-tocotrienol, $R_1 = R_3 = CH_3$; $R_2 = H$

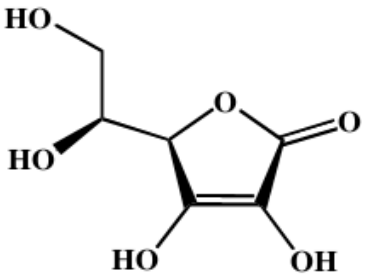
γ-tocopherol, $R_1 = R_2 = CH_3$ $R_3 = H$
γ-tocotrienol, $R_1 = R_2 = CH_3$ $R_3 = H$

δ-tocopherol, $R_1 = R_2 = R_3 = H$
δ-tocotrienol, $R_1 = R_2 = R_3 = H$

Vitamin C

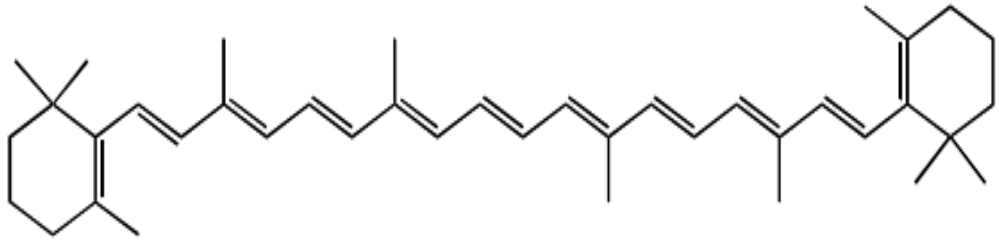
Water soluble vitamin and also known as ascorbic acid, and it is basic for collagen, carnitine, and neurotransmitters biosynthesis (Duh et al., 1999). Medical advantages of vitamin C are as antioxidant, in opposition to atherogenic as opposed to cancer causing, and as an immunomodulator. It has vital role in treatment of stomach cancer, lung, and colorectal cancer. It works in combination with vitamin

E to quench free radicals, and further recovers the reduced form of vitamin E. Medicinal plants such as lemon and orange, green vegetables, tomatoes so on are the sources of vitamin C.



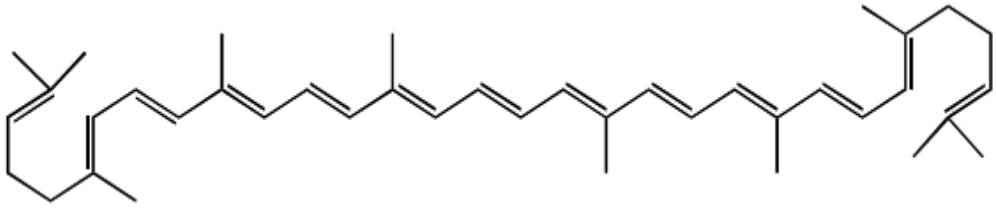
β -Carotene

It is a fat-soluble individual from the carotenoids which are considered as pro-vitamins given that they can be converted to active vitamin A. It is used to modify retinol, fundamental for vision. It is a robust antioxidant and is the excellent quencher of singlet oxygen. The herbal sources of β – Carotene are grains, oil, & veggies (carrots, green plants, squash, spinach and so forth.) (Nguyen et al., 2006)



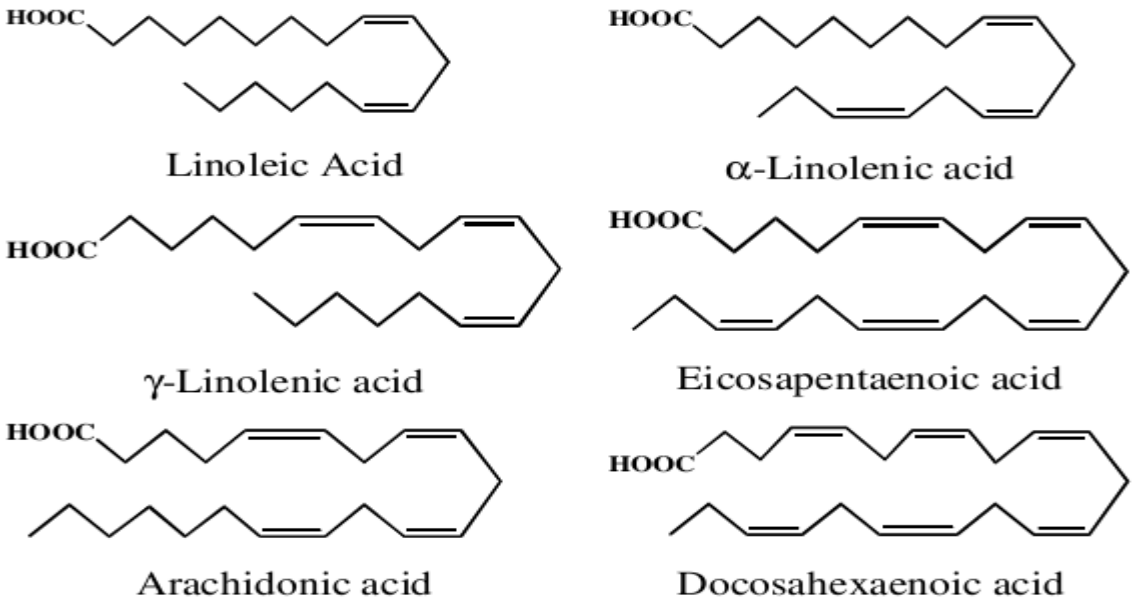
Lycopene

It is a carotenoid which possesses antiproliferative and antioxidant properties (Nguyen et al., 2006). It has been observed as defensive, specifically for prostate disease. Massive nutritional source of lycopene is tomatoes.



Omega-6- and Omega-3-fatty acids

They are basic long-chain polyunsaturated and unsaturated fatty acid in light of the fact that the human body can't synthesis them (Logan, 2004). They are simply gotten from nourishment. Omega-3 unsaturated fats can be found in oily fish (salmon, fish, halibut, sardines, pollock), nut oils, green growth, walnut, and flaxseed. Alpha-linolenic acid, Docosahexaenoic acid and Eicosatetraenoic acid are three sizeable dietary sorts of omega-3 unsaturated fats. Dietary sources of omega-6 unsaturated fats (linoleic acid) are vegetable oils, nuts, oats, eggs and poultry. It is fundamental to keep up a proper adjustment of omega-3s and omega-6s in the eating routine, as these two assets participate to help the welfare or wellness of people. Omega-3s reduce irritation and envision ceaseless ailments, for example, stroke, depression, coronary illness, memory misfortune, cataract, joint inflammation, and malignant growth. Omega-6s improve skin inflammation, diabetic neuropathy, psoriasis, osteoporosis and guide in many malignancies' action.



The capacity of the antioxidant compounds to inhibit the free radical reactions; thereby protecting the human body from ailments or diseases has prompted the expanding enthusiasm/interest in the discovery of a new novel antioxidant phytochemicals from medicinal plants. The utilization of the synthetic antioxidants, for example, butylated hydroxylanisole, and butylated hydroxytoluene are highly discouraged because of its cancer-causing properties (Thampi & Jeyadoss, 2015). Thus,

common natural antioxidants like phenolics and flavonoids from fruits, organic products, vegetables, flavours, spices, and herbs can be exploited as a substitute for these synthetic antioxidants.

2.1.1.3. Medicinal Plants Used as Antiviral

Dengue is the most broadly recognized viral (arboviral) disease in humans and it is transmitted via mosquitoes of the classification Aedes referred to as *Aedes aegypti* mosquito. Over the latest 50 years; frequency has elevated 30-fold. An expected 2.5 billion human beings are accounting to 40% of whole world population live in more than one hundred endemic nations and areas where dengue diseases can be transmitted. Up to 50 million diseases appear each yr with 500,000 instances/cases of dengue hemorrhagic fever and 22,000 deaths primary amongst children. Preceding 1970, simply nine countries had encountered cases of dengue hemorrhagic fever (DHF); from that factor, ahead the range has improved more than 4-fold and now largely allotted in subtropical and tropical areas of the world (Rahman, 2018).

Dengue ought to be suspected when a high fever (40°C/104°F) including the following symptoms. These symptoms are joint pains, ache behind the eyes, extreme headache, muscle pains, nausea, sickness, vomiting, swollen glands. These signs typically ultimate for 2-7 days, after an incubation duration of 4-10 days after the bite from a contaminated mosquito. Severe dengue is a possibly fatal complication because of fluid accumulation, extreme bleeding, plasma leaking, respiratory organ impairment. Warning signs and symptoms occur three-seven days after symptoms in conjunction with a limit in temperature (below 38°C/100°F) & contain extreme stomach pain, chronic vomiting, speedy breathing, bleeding gums, fatigue, restlessness & blood in vomit. The next 24-48 hrs of the necessary stage can be lethal; ideal scientific care is needed to avoid problems and risk of death. Despite the daunting & routine numbers of annual instances of Dengue fever (DF), most of the instances enhance inside days, solely 1-2% of cases progress to the greater severe prerequisites called dengue shock syndrome (DSS), and dengue hemorrhagic fever (DHF). DHF symptom is lowering platelet counts,

plasma leakage and severe bleeding may additionally occur ensuring in organ failure, and death. No unique therapy is handy other than supportive care and pain relief (Chaudhary et al., 2006; Kharya et al., 2011).

Request for therapeutic plant-based drug is increasing as they're typically being thought of easily available, safer, have lesser side effects, are non-toxic and have fewer harmful then synthetic drugs. Presently there is no antibody for the treatment of dengue viral disease since there are four diverse viral serotypes that cause the dengue diseases. Folkloric therapeutic plants have been reported to possess antiviral activity (Betancur-galvis et al., 1999; Kudi & Myint, 1999) & some are used to treat infections in humans & animals. Up to the present time, totally extraordinary healthful species are found to have the possibility to treat dengue; some of these haven't in any case been researched scientifically. Inside the Philippines, Euphorbia hirta, important provincially as (tawa-tawa), is utilized in folkloric medicine to fix break bone fever by people in rural areas (http://www. Stuartschange.org/GatesGates.html). Specialists of customary medications accept that decoction of tawa-tawa leaves will reverse virus diseases & prevent the fever from moving into urgent stages, however there are no logical examinations demonstrating its effectiveness (http://www. Curelibrary.com/blog/2007/04/). Tawa-tawa is prepared related to papaya leaves since papaya leaf extract incorporates and operate as associate degree to antibiotic to cure fever. Though papaya leaf extract kills the bacterial disease that caused the fever, tawa-tawa extract prevents haemorrhage or bleeding. Furthermore, unpublished examination has discovered that Psidium leaves are a decent way to increase platelets, along these lines serving to keep away from bleeding (http://abouthealth.com/dengue-fever-medicine). A water decoction of guava leaves contains quercetin, that demonstrations to hinder the development of protein layout RNA inside the virus (http://www.secondlifeblogs.info/gaava-leaf-prevent dengue heamorrhage.html).

The uses of some of therapeutic plants for the treatment of antiviral disease are as follows:

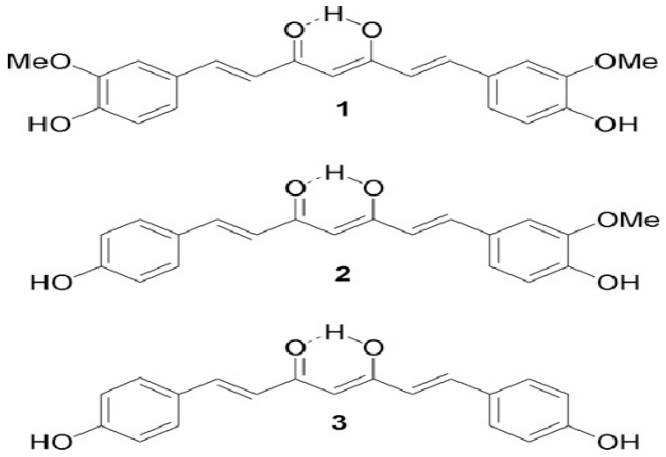
***Andropogon citratum*:** The citronella oil and essential oil are the bioactive constituent of this plant, and they are used to repulse mosquitoes. These mosquitoes repulsing activity can be checked through examination, together with effectiveness in repulsing of arthropod genus *aegypti* (Onanong et al., 2009).

***Andrographis paniculata*:** Methanolic extract of nontoxic dose of plant was checked to against Vero E6 cells in vitro, and documented the maximal dose that wasn't toxic to cells at 0.05-1. Solvent methanol extract exhibited the most noteworthy antiviral suppressive result on DENV-1 by antiviral check enthusiastic about cytopathic effects (Tang et al., 2012).

***Azidarachta indica*:** The leaves aqueous extract of the plant was assessed in vivo & in vitro inhibitory capability on the replication of DENV-2 (Parida et al., 2002).

***Carica papaya*:** The plant leaf was evaluated to check its capability to against dengue fever. The aqueous Carica papaya leaves extract shown latent activity to against dengue fever. This is by increasing the neutrophils, platelet count, and white platelets in blood tests of a 45-year-old patient bitten by infected mosquitoes (Ahmad et al., 2011). Another research examination confirmed the effectiveness of seed extract of carica (carica pubescens Lenna & K. Koch) as mosquito repellent to against dengue (*Aedes aegypti* Linn.) (Anggraeni & Laela, 2020).

***Curcuma longa*:** The ethyl acetate extract of the rhizomes of this plant gives three curcuminoids that are effective in inhibiting topoisomerase I & topoisomerase II, which play a critical role in DNA replication. Turmerone got from the volatile oil of curcuma longa gives 100% mosquitocidal activity against *Aedes aegypti* (Roth et al., 1998).



Chemical structure of curcumin I, II and III

***Euphorbia hirta*:** The tea obtained from this plant is used to treat dengue fever (http://www.stuartxchange.org/Gates-Gates.html, 2011). The water decoction of leaves of the plant, privately known as gates-gates, is used in the Philippines as customary drug to cure dengue fever (Abd Kadir et al., 2013).

***Momordica charantia*:** In vitro methanolic extract of the plant was examined to against Vero E6 cells at 0.20mg/mL dose. It has demonstrated inhibitory impact on DENV-1 by antiviral examine dependent on cytopathic effects (Tang et al., 2012).

***Murraya Koenigii*:** Ethyl acetate, dichloromethane, diethyl ether, and hexane extracts were prepared and the adult mosquitoes were fed on this normally. At the end of the experimentation, the observation was made, and the adult mosquito lose their consciousness and also are incapable to bite. Finally it was concluded that the plant extracts were used as mosquitocidal (Chandra et al., 2016).

***Piper longum*:** - It has a place with the family *Piperaceae* and they have been used to fight against *Aedes aegypti* (Chaithong et al., 2006).

***Psidium guajava*: -** Its ready organic product or juice has mending properties in instance of dengue fever by improving the declining levels of platelets (http://abouthealth.com/dengue-fever-medicine, 2011). The water boiled leaves of the plant was utilized to prevent bleeding in DHF, & enhanced

platelet counts 100.000/mm^3 a time of roughly within 16h (http://www.secondlifeblogs.info/guava-leaf-prevent dengue heamoorhage.html, 2010). Other investigation on the leaf extract of this plant demonstrated inhibitory effect on the growth of dengue virus (http://pinkroses.info/guave-leaf-extract-potential-cure-dengue-fever, 2011).

***Quercus lusitanica*:** The solvent methanol crude extract of the plant has the ability to inhibit the replication of dengue virus (Muliawan et al., 2006). The current treatment of severe dengue is steady or using supportive fluid therapy under medical supervision(Beesetti et al., 2016). Having no particular antiviral treatment or an antiviral specialist for dengue treatment, various techniques for counteractive action have been built up by controlling the mosquito reproduction or spread(Simmons et al., 2015; Lambrechts & Failloux, 2012). Because of the absence of compounds, some clinical researches have proposed the repurposing of understood medications, for example, prednisolone, chloroquine, celgosivir, lovastatin, and balapiravir; be that as it may, in spite of the fact that those medications are protected, they have not been productive at diminishing viral load, antigenemia, fever or inciting a beneficial impact to dengue patients (Low et al., 2017). Ethno pharmacology has contributed altogether to the discovery of new drugs (Mamedov, 2012; Petrovska, 2012). As of recent, the emphasis on therapeutic plants broadly utilized in customary frameworks has expanded worldwide (Taviad & Vekariya, 2018). In view of ethno botanical data, some studies have investigated some compounds with anti-dengue potential activity, for example, 7-O-methyl-glabranine & quercetin and fisetin (Zandi et al., 2011), catanospermine (Whitby et al., 2005), baicalein (Zandi et al., 2012). Bioactive compounds with a high capability against dengue movement ought to be additionally tried for lethality (in-vivo and in-vitro examines) and clinical tests for the application in the generation of the novel anti-dengue compounds from medicinal plants should be performed. Further, understanding of the life cycle, synthesis of viral RNA, functional genome of this virus and the mechanism of virus infection are also important to develop the appropriate drug. This approach could lead us to a new insight into the development of dengue antivirals from medicinal plants.

2.1.1.4. Medicinal Plants Used as Anti-cancer

Most cancers (malignant tumour) is an abnormal growth and proliferation of the cells and it is one of the fundamental life-threatening illnesses which creates a severe problem in each the growing and developed international locations and it's categorized as abnormal cellular proliferation. The maximum but not unusual motive in the back of the cancer is life-style adjustments and therefore a pressing want to find a better remedy for the disorder is needed. Most cancers development in human beings involves a complex process such as cellular and molecular modifications mediated by way of various exogenous and endogenous stimuli. It is prominent that oxidative DNA can hurt severely in malignant development (M Valko et al., 2006; Marian Valko et al., 2004, 2007).

Most of the cancers initiation and promotion are associated with chromosomal defects and oncogene activation triggered through free radicals. Formation of hydroxyled bases of DNA is taken into consideration a critical event in chemical carcinogenesis(M Valko et al., 2006); (B Halliwell, 2007). This adduct development interferes with the conventional cell blast by methods for causing hereditary changes and altering normal gene transcription. Oxidative DNA harm further in delivering an assortment of alterations inside the DNA structure which incorporate base and sugar lesions, strand breaks, DNA-protein go-connections and without base sites. As an instance, tobacco smoking and continual infection because of noninfectious sicknesses like asbestos are resources of oxidative DNA damage and may make contributions to the development of most lung cancers and other tumors (M Valko et al., 2006; Willcox et al., 2010). Substances that provoke most cancers in the human body are termed as cancer agents. Chemical cancer agents, viruses, chromosomal rearrangements or spontaneous adjustments, inactive of tumour suppressor genes so on, have been implicated as causes of cancer. Hereditary predisposition to most cancers leads itself to similarly 20% of cancer instances and over-all public of cancers is being related to a number of environmental carcinogens (Doll & Peto, 2018).

The ninety percent of lung cancer is due to smoking, and it additionally causes belly, kidney, larynx, pancreas and bladder cancer. Tobacco is accountable for roughly one in five cancer-death instances globally (Kuper et al., 2002). Physical activity, weight problems and weight-reduction plans are associated with 30-35% of most cancer deaths. Physical inactivity is an idea to contribute to the most cancer risk. Overnutrition also causes cancer. Some precise foods are related to the unique type of cancers like excessive salt and weight loss plan cause gastric most cancers, aflatoxin B1 causes most liver cancers and chewing betel nut reasons oral cancer. The source of ionizing and non-ionizing ultraviolet radiations causes cancer (up to 10%). Source of ionizing radiations includes radon fuel and clinical imaging; this radiation is not always, in particular, a sturdy mutagen. Radiation combined with other most cancers-infecting retailers is far more potent like radon tobacco smoke (Droge, 2002).

The cancer-causing agents are categorized as procarcinogens, genotoxic cancer agents, epigenetic carcinogens and unclassified cancer-causing agents. Genotoxic carcinogens are the substances that react with nucleic acids. Those may be without delay appearing cancer agents as they could immediately have an effect on cellular constituents. Procarcinogens are substances that require metabolic activation to set off carcinogenesis. The exceedingly boundless relationship between utilization of fats and demise expenses from leukemia and breast, ovary, rectum malignant growths among older people is likely a reflected picture of more noteworthy lipid peroxidation (Droge, 2002). One of the most extreme fundamental systems adding to malignant growth is viewed as oxidative harm to the DNA. If a cell containing broken DNA divides earlier than it is repaired, the outcome is probably going to be a lasting hereditary change comprising the initial phase in carcinogenesis. The body cells that divide quickly are greater vulnerable to carcinogenesis because there is less opportunity for DNA restoration before cell division. The components which change in mutagenic signaling pathways also cause most cancers. Mutagenic changes in the components of signaling pathways also cause many types of cancers. Hence, to deal with these issues medicinal plants have their very own roles and those therapeutic plants have numerous advantages over chemical products

because plant-derived compounds are greater tolerant and non-poisonous to the normal human cells. Existed conventional therapies for the treatment of most cancers are radiotherapy and chemotherapy that have various side outcomes like cardiac, neurological, renal and pulmonary toxicity, thus significantly affecting the fitness of the person. Hence, an alternative technique is required to broaden that is much less toxic than stronger anticancer drugs available inside the market. Research has been made on formation of compounds recognized to own cytotoxicity outcomes, as they display the ability to damage cancer cells. Because of these advantageous of medicinal plants, they may be in excessive demand and numerous species of medicinal plants were investigated and selected for the preparation of many cancers drugs.

These days, there has been an elevated medical interest inside to have a look at materials from plant supply as anticancer compounds. Those studies have determined the role of medicinal plants in the prevention and treatment of cancer (Greenwell & Rahman, 2015a, 2015b). Therapeutic plants such as *Podophyllum hexeandrum, Withania somnifera, Rubia cordifolia, Linum usitatissimum, Catharanthus roseus, Berberies aristata, Allium sativum, Annona muricata* and so on display capacity function inside the inhibition of most cancers cell proliferation (Tiezzi, 2017). Medicinal plant-derived anticancer drugs are powerful inhibitors of most cancers cell lines. Because of which there is an excessive request for therapeutic plants for the manufacturing of medicinal crucial bioactive compounds. There are numerous different medicinal plants everywhere globally which are being used historically for most cancers' prevention and treatment. Some phytochemicals that are crucial bioactive lively components of plants, along with catechins, ursolic acid, silymarin, hecogenin glycyrrhizin, berberine, Camptothecin, gallic acid and numerous types of flavonoids which have proven promise in future most cancers management. The list of some useful anticancer medicinal plants is discussed in the table 2: (Tiezzi, 2017).

Table 2: The list of some of the medicinal plants with anticancer activity

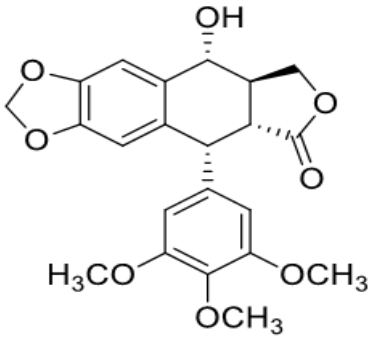
Medicinal plants	Family	Anti-cancer compounds	Sources
Echinops setifer	Asteraceae	Echinopsine	(G. Pandey & Madhuri, 2014)
Junchus effuses	Juncaceae	Phenylpropanoid, effusol Tridecanone, juncanol	(G. Pandey & Madhuri, 2014)
Newbouldia laevis	Bignoniaceae	2-acetylfuro-1, 4-naphthoquinone	(Kuete et al., 2011)
Alpinia galanga	Zingiberaceae	Pinocembrin	(Madhuri et al., 2011))
Aegle marmelos	Rutaceae	Lupeol	(Madhuri et al., 2011)
Berberis vulgaris	Berberidaceae	Berberine	(Madhuri et al., 2011)
Rubia cordifolia	Rubiaceae	Rubiadin, rubidianin, purpurin, xanthopurpurin	(Madhuri et al., 2011)
Prunella vulgaris	Labiatae	Ursolic acid, oleanolic acid	(Madhuri et al., 2011)
Ailanthus altissima	Simaraubaceae	Ailantenol, ailnthanone	(Das et al., 2019)
Andrographis paniculate	Acanthaceae	Andrographolide	(R. A. Kumar et al., 2004)
Apium graveolens	Umbelliferae	Apigenin	(Sultana et al., 2005)

Aloe ferox	Liliaceae	Emodin, Aloe-emodin	(Wasserman et al., 2002); (Tumors et al., 2000)
Ananas comosus	Bromeliaceae	Ananas bromelain	(Sakarkar & Deshmukh, 2011)
Astragalus membranaceus	Papilionaceae	Swainsonine	(Sakarkar & Deshmukh, 2011)
Brucea antidysenterica	Simaraubaceae	Bruceantin	(Cragg & Newman, 2005)
Campotheca acuminate	Nyssaceae	Campothecin	(Cragg & Newman, 2005)
Cephalotaxus harringtonia	Cephalotaxaceae	Homoharringtonine	(Cragg & Newman, 2005)
Dysoxylum binectariferum	Meliaceae	Rohitukine	(Cragg & Newman, 2005)
Diphylleia grayi	Berberidaceae	Diphyllin	(Cragg & Newman, 2005)
Indigofera tinctoria	Leguminosae	Indirubins	(Cragg & Newman, 2005)
Croton lechleri	Euphorbiaceae	Taspine	(Ayele, 2018)
Euphorbia semiperfoliata	Euphorbiaceae	Jatrophane	(Ayele, 2018)
Lantana camara	Verben	Lantadene, isocamerine, camerine, lantanine, micranine	(Cavallito et al., 1944)

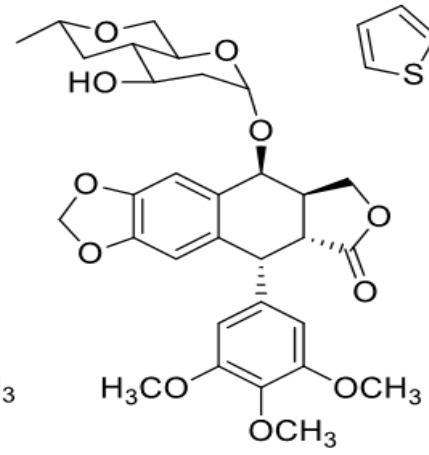
Pteris multifida	Pteridaceae	Pterokaurane	(Cavallito et al., 1944)
Glycyrrhiza glabra	Leguminosae	Glycyrrhizin	(Roy & Bharadvaja, 2017)
Gossypium barbadense	Malvaceae	Gossypol	(Roy & Bharadvaja, 2017)
Larrea tridentate	Zygophyllaceae	Terameprocol	(P. Kumar & Kumari, 2015)
Lonicera japonica	Caprifoliaceae	Luteolin	(G. Pandey & Madhuri, 2014)
Mappia foetida	Icacinaceae	Camptothecin	(Desai et al., 2008)
Podophyllum hexandrum	Berberidaceae	Podophyllin	(Roy & Bharadvaja, 2017)
Ocimum sanctum	Lamiaceae	Eugenol, orientin, vicenin	(Roy & Bharadvaja, 2017)
Oldenlandia diffusa	Rubiaceae	Ursolic acid	(Roy & Bharadvaja, 2017)
Plumbago zeylanica	Plumbaginaceae	Plumbagin	(Roy & Bharadvaja, 2017)
Scrophularia nodosa	Scrophulariaceae	Iridoid	(Ardeshiry et al., 2010)
Zingiber officinale	Zingiberaceae	Curcumin, gingerenone A, gingeols, zingerone	(Katiyar et al., 1996)
Ziziphus nummularia	Rhamnaceae	Betulinic acid, betulin	(Sarek et al., 2005)

In addition to the above review on the anticancer activity of some medicinal plants; the properties, and chemistry of some of the major plant-derived anticancer drugs are discussed as follows. One of

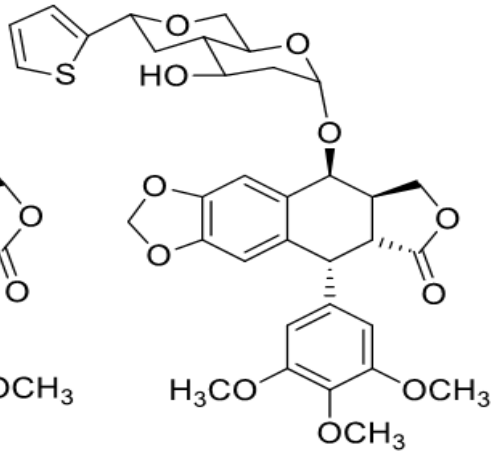
the first known anticancer drugs is podophyllotoxin and this was used for the treatment of warts and as a purgative and was later found to act as an anti-cancer agent by irreversibly binding to tubulin (K. Lee, 2004). The development of etoposide and teniposide were due to the synthetic modification of podophyllotoxin molecule and they are powerful effect for small cell cancers of the lungs and testes (Cragg & Newman, 2005). These modified drugs act by inhibiting topoisomerase II, thus disrupting the enzyme-DNA complex and causing cell death (M.R. Lee, 1999).



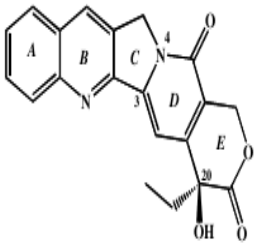
Podophyllotoxin



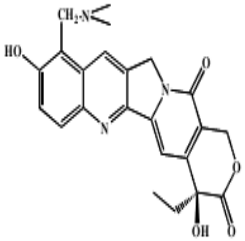
Etoposide



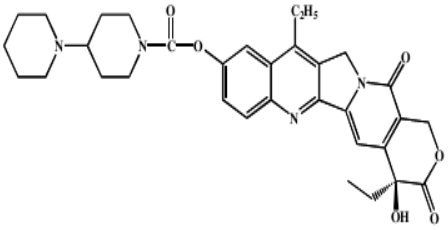
Teniposide



Camptothecin



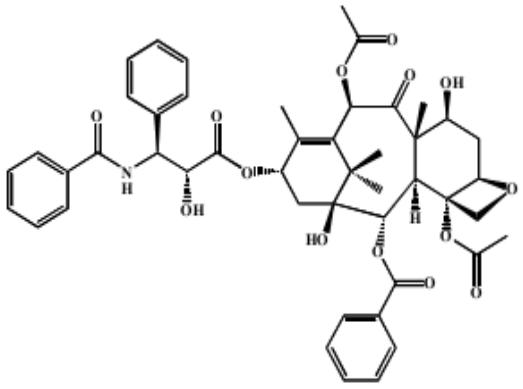
Topotecan



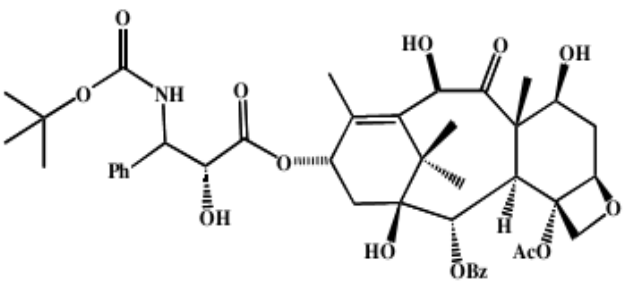
Irinotecan

Wani and Wall's discovery Camptothecin as an anticancer drug in the mid 1960 brought an entirely new dimension to the field of chemotherapy. It is a broadly studied natural alkaloid, and it is isolated from the tree of Chinese Camptotheca acuminate (Mahidol et al., 2002; Zhang et al., 2013). The molecule Camptothecin, camptosar (CPT-II, irinotecan) and hycamtin (topotecan) are utilized for curing of colon and ovarian cancers. It is a member of the quinolinoalkaloid group and it contains a pentacyclic ring structure that consists of a pyrrole (three, 4β) quinoline moiety and one asymmetric center inside the α-hydroxy lactone ring structure that consists of a pyrrole (three, 4β) quinoline

moiety and one asymmetric center inside the α-hydroxy lactone ring with 20(S) configurations. The stereochemistry at C-20 of CPT could be very important for its interest, as 20(S) hydroxyls are lively whilst the corresponding 20(R) hydroxyl compound is inactive (Bodley & Shapiro, 1995). One of the basic disadvantages found in the utilization of CPT similarity in medical research became a marked loss of healing interest because of their intrinsic instabilities resulting from the quick hydrolysis of the lactone ring in the frame. Apart from the above disadvantage, it's far from being a powerful cytotoxic agent. It indicates anticancer interest in particular for strong tumors. It shows anticancer activity in particular in opposition to ovarian, colon and pancreatic cancers cells. Nevertheless, its analogues confirmed anticancer interest in breast, liver, prostate cancers and so forth. Camptothecin inhibits DNA topoisomerase I (Laco, 2011) thereby preventing DNA replication. The development of artificial and semisynthetic techniques has facilitated the take a look at of the CPT mechanism, further to the identification of analogues with better properties. The most successful derivatives of CPT have been received because of modifications in ring A and B. So far, the handiest CPT analogues regularly occurring for clinical use (Laco, 2011) are topotecan and irinotecan. All of the analogues of CPT have proved as potent cytotoxic agents with the useful resource of inhibiting cell DNA topoisomerase I by means of a mechanism much like CPT with similar or higher interest. Non-stop studies on the Camptothecin-DNA topoisomerase I interplay similarly to its unique mechanism of motion might also suggest new instructions within the synthesis of latest camptothecins.



Taxol



Docetaxel

Taxol is isolated from the taxus brevifolia pacific yew. It is one of the most outstanding agents and has been determined beneficial in the treatment of refractory ovarian cancers, metastatic breast, lung cancer and Kaposi's sarcoma. Docetaxel is considered as one of semisynthetic derivatives of taxol and it has better anticancer drug in comparison to taxol. Taxol has a basic pentadecane, tetracyclic ring system. It has a N-benzoyl-β phenylisoserine side chain attached on the C-13 hydroxyl as an ester linkage. This aspect chain is largely required in taxol for anticancer activity and so is the C-2'-hydroxyl. Taxol has a completely unique mode of action (Weaver & Bement, 2014).

The discovery of novel drugs compounds from medicinal plants and their mechanism of action might offer an alternative and powerful remedy closer to cancer prevention and those medicinal plant species are already being used to treat or prevent the development of cancer. More than one researcher has recognized species of plant life which have proven anticancer properties with numerous cognizance on those which have been utilized in natural remedy in developing countries. The medicinal plant incorporates numerous secondary metabolites that show their potential pastime in opposition to diverse sicknesses. These medicinal plant merchandise demonstrating anticancer activity remain the concern of huge-ranging research pointed on the improvement of drugs for the remedy of various human tumors. Plant-derived phytochemicals possessing anticancer activities have received considerable interest in current years due to the unfavourable results produced via chemotherapy and radiation remedy. Phytochemicals obtained from traditional therapeutic plants have been found to possess anticancer and chemo shielding effects. They may be more secure for lengthy-term use in most cancers' patients. They provide nutrients and decrease the facet outcomes of traditional most cancers therapy due to powerful antioxidant agents. Anti-carcinogens derived from the plant source have largely contributed to the improvement of the latest drugs (Pradesh, 2016).

Anticancer drugs got from the plant sources have generally added to the advancement of new medications. Along these lines, it tends to be presumed that homegrown therapeutic plants and their subordinates are dynamic against various kinds of malignant growths. Herbal drug medication or

treatment might be prescribed to the provincial and poor people to treat adequately the malignant growths as it is less expensive. Investigation of therapeutic plants for anticancer treatments gives a marvelous space to the advancement & development of strong pharmacological agents. On the basis of the present literature review on anticancer activity of medicinal plant species, further investigation should be continuous in order to test pharmaceutical applications & for the accomplishment of novel antioxidant, antibacterial, anti-dengue & anticancer compounds.

2.1.2. Medicinal Plant *Sisymbrium irio* L

Sisymbrium irio L is one of the therapeutic plants and it belongs to the family *Cruciferae*, called Asalio, Khubkalan, khubkhala (Hindi); Khubah (Arabic); Khakasi, Khubakalan (Urdu); Khakshi (Persian), and London Rocket/Rocket Mustard (common names) and is found in different parts of the world(Khoshoo, 1966). Scientific description of *Sisymbrium irio* L follow as: -

Kingdom:	Plantae
Order:	Brassicales
Family:	*Cruciferae*
Genus:	*Sisymbrium*
Botanical name:	*Sisymbrium irio* L
Arabic:	Khubah
English:	London Rocket
Hindi:	Asalio, khubkaln, Khubkala
Urdu:	Khubakalan
Used in:	Ayurveda, Unani
Common names:	London Rocket, Rocket Mustard

A. Whole plant B. Seeds C. Leaves D. Flowers

Figure 1: Pictorial representation of different parts of *Sisymbrium irio* L.

2.1.3. Phytochemistry of the *Sisymbrium irio* L

Numerous phytochemicals are identified from various parts of *Sisymbrium irio* these are derivative of terpenes (Al-Qudah & Abu Zarga, 2010a, 2010b) triterpenes, tannins, saponins, ste rols (Khalil et al., 2017), flavonoids (N.A. Al-Jaber et al., 2011; N.A. A. Al-Jaber, 2011, 2011) alkaloids (Alsaffar *et al*., 2016), sitosterols, stigmasterol's, glycosides (Al-Massarani et al., 2017) & Glucosinolates (Nengroo & Rauf, 2019). The research study conducted on the aerial part of the plant showed the oils obtained from *Sisymbrium irio* L comprised of seven acids and two esters (38.80%), eleven sulfur & eleven nitrogen-containing compounds (36.41%), fifteen terpenes derivatives (terpenoids) (8.19%), five aromatic compounds (3.53%), six aliphatic hydrocarbons (6.29%), four fatty alcohols (2.49%) & three additional chemical compounds (1.17%) (Al-Qudah & Abu Zarga, 2010a, 2010b). Other research examination conducted on the phytochemical investigation of the various parts such as leaves, stem, flowers & roots of *Sisymbrium irio* L exhibited the existence of tannins, saponins, triterpenoids/steroids, carbohydrates, alkaloids & flavonoids at different levels in different extracts of plant organs and the absence of cardiac glycosides and anthraquinones (Khalil et al., 2017). The diverse kinds of bioactive secondary metabolites that were found in various parts of *Sisymbrium irio* L shown in Table 3.

Table 3: Compounds identified in the *Sisymbrium irio* L

Phytochemicals	Part of the plant
Flavonoids (apigenin, apigenin-7-galactoside, apigenin-7-*O*-β-D-glucoside, luteolin-7-*O*-glucoside, apigenin-7-di-glucoside, apigenin-7-*O*-(6″ acetyl) glucoside, apigenin-7-*O*-gluco(6″,1‴)rhamnoside, apigenin-7-*O*-gluco(6″,1‴) rhamnoside, apigenin-7-*O*-gluco(6″, 1‴) rhamnoside-5-methoxide, Kaempferol, kaempferol-3-*O*-xylosoid-7-galactoside	Aerial part (N.A. Al-Jaber et al., 2011)
Alkaloid (nicotine)	Aerial parts (Al-Qudah & Abu Zarga, 2010)
β-sitosterol-glucoside, β-sitosterol, and stigmasterol	Aerial parts (Al-Massarani et al., 2017)
Glucosinolates	Aerial parts ((Nengroo & Rauf, 2019))
Isobutyl isothiocyanate, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, cis-8,11,14-eicosatrienoic acid, heptacosane, palmitic acid, n-butyl isothiocyanate, di-methoxyacetophenone, aliphatic hydrocarbons, aromatic compounds, N-(n-propyl) acetamide, dioctyl adipate, isopropyl isothiocyanate &Terpenoids	Aerial parts (Al-Qudah & Abu Zarga, 2010)
Indole-3-carboxaldehyde, indole-3-carboxylic acid, apigenin, naringenin-4′-O-glucopyranoside, -adenosine and apigenin-7-O-glucoside, sitosteryl-6′-*O*-undecanoate-*β*-D-glucoside, 1,2-dipalmitoyl-3-*O*-α-6‴-sulfoquinovosyl glycerol, (Z)-8, 11, 12-trihydroxyoctadec-9-enoic acid, -sitosterol-D-glucoside, crotanoyl cosmosiin, *β*-sitosterol, ursolic acid, tetracosanoic acid,	Aerial parts (Al-Qudah & Abu Zarga, 2010)

2.1.4. Pharmacological Applications of *Sisymbrium irio* L

The plant *Sisymbrium irio* L is used for the remedy of different sorts of diseases consisting of inflammatory conditions and rheumatism, as an expectorant, febrifuge, for the remedy of voice disorders (Marzouk et al., 2010), chest congestion, rheumatism, detoxifying liver and spleen, reduces swelling and smooth wounds, antipyretic, analgesic, antimicrobial and antioxidant potential (Alsaffar et al., 2017). *Sisymbrium irio* L has a wide pharmaceutical use inside the remedies of irritation and rheumatoid (Alsaffar, Abbas, Dawood, et al., 2016). The seeds of London rocket are used for the remedy of inflammatory situations, boils, pimples, cough, cholera and non-precise fever. The seeds crude extracts of the *Sisymbrium irio* L have been tested for analgesic, antipyretic & antimicrobial effects. Solvent ethanolic extract shown tremendous antipyretic & analgesic consequences as well (Al-Mujalli et al., 2013). It additionally showed marked antibacterial action against each gram-negative and gram-positive organism & turned into determined to be safe in acute studies (Vohora et al., 1980). The polarity-based extract of the *Sisymbrium irio* L was energetic to inhibit the growth of foremost ailment-causing bacterial strains (Bibi et al., 2015).

The n-hexane extract of plant leaves inhibited the growth of microbial strains inclusive of *Klebsiella pneumonia* and *Staphylococcus epidermidis*. The ethyl acetate extract of the leaves turned into potent against the gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, while the plant seed confirmed extra inhibitory effect towards *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (El Sherbiny et al., 2017). *Sisymbrium irio* L aqueous extract was active against all examined pathogenic microbes such as *Acinetobacter baumanii, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus aureus ATCC 29213, Enterococcus faecium, Escherichia coli ATCC 25922, Enterobacter cloace and Klebsiella pneumoniae* (El Sherbiny et al., 2017). The plant has numerous therapeutic effects and are used for the treatment of acute diseases, persistent, ribs bottleneck, to decrease ache in the joints and muscle

mass ache, inflammation, deal with the liver and the infection, accidents, painkilling, to reduce fever & for antibiotic uses (Shah et al., 2013).

The ethanolic extracts of *Sisymbrium irio* L seeds suggest cytotoxic, phytotoxic, and insecticidal activities and also the ethanolic extracts of the seeds of this plant confirmed anti-inflammatory activity, antidepressant, swim pressure immobility and broncho protective role (R.K. Singh, 2015). Secondary metabolites along with isothiocyanates and nitriles which are discovered in *Sisymbrium irio* L have huge programs in opposition to unique varieties of microbial diseases (Conrad *et al*., 2013). The research examination indicates the uses of *Sisymbrium irio* L for the treatment of cancers and the improvement of more secure and greater effective therapeutic agents (Yukes & Balick, 2010). The activity may be attributed to the presence of β-sitosterol, as in vivo observation showed β-sitosterol had a huge function within the diets of mice & rats fed with colon carcinogens that decreased the proliferative variations of the most cancers growth (Ramprasath & Awad, 2015). β-sitosterol had distracted the shape of most cancers cell membranes & altered the signalling pathways that alter cancer growth and apoptosis (Shahdaat et al., 2015). The *Sisymbrium irio* L extracts confirmed hepatoprotective effects in opposition to CCl_4 prompted hepatotoxicity in albino rats (Alsaffar et al., 2017). The hepatotoxic houses of the CCl_4 became mainly because of the presence of intermediate reactive metabolite, trichloromethyl radical. Trichloromethyl radical bound covalently to the macromolecules & encouraged peroxidative deprivation of membrane phospholipids of endoplasmic reticulum wealthy in polyunsaturated fatty acids that leads to pointless construct-up of phospholipids in tissues along with liver (Parkash & Patel, 2015).

A histopathological study found out that post-treatment with *Sisymbrium irio* L absolutely exhibited the significant protection of liver cells. The effectiveness of the antihepatotoxicity of the drug may additionally depend on its capacity in decreasing the damaging impact the usual hepatic functioning impaired with the aid of a hepatotoxin (Alsaffar et al., 2017). *Sisymbrium irio* L extracts have the effective reducing potential of carbon tetrachloride action to elevate the levels of the enzyme in tested

groups, this suggests the defence of structural integrity of hepatocyte membrane or renewal of injured liver cells, that can be due to the existence of bioactive phytochemical flavonoids (Alsaffar, Abbas, Dawood, et al., 2016). In Mediterranean location, *Sisymbrium irio* L leaves are nourished as food and used as folk medicine for infections of the throat and chest (N.A. A. Al-Jaber, 2011). This plant is utilized in Unani medicine for various healing processes makes and is endorsed for the prevention of dengue fever because of the presence of bioactive metabolites. From this literature review, it is certain that numerous phytochemicals are found in various parts of *Sisymbrium irio* L, which are needed in addition of phytochemicals and pharmacological investigation to become beneficial healing properties and is a novel drug molecule.

2.1.5. Medicinal Plant *Colchicum autumnale* L

Colchicum autumnale L belongs to the family *Liliaceae* and is called Falheeqan, Aqeemaroon, Balboose, Falheeq, Asmaroon, Qabaroon (Unani); Ukba, Laeba bararbaria (Arabic), Barbari, Jangli Singara, (Hindi), Hageer, Surangan, *Suranjan Shireen* (Persian) and Meadow Saffron, *Colchicum* (English) (Suhail & Ansari, 2017).

Table 4: Scientific classifications of *Colchicum autumnale* L

Rank	Scientific and Common name
Kingdom	Plants- plantae
Subkingdom	Vascular Plants-Tracheobionta
Super-division	Seed plants- Spermatophyta
Division	Flowering plants- Magnoliophyta
Class	Monocotyledons-Liliopsida
Subclass	Liliidae
Order	*Liliales*
Family	Lily family-*Liliaceae*

Genus	*Colchicum- Colchicum* L.
Species	*Autumn crocus-Colchicum autumnale* L (Suhail & Ansari, 2017).

Figure 2: The picture representation of *Colchicum autumnale* L.

The plant grows in woodland clearings, moist meadows, and shady rocky habitats on non-calcareous substrates. It should be resolved up to an altitude of 2,000 meters and are developed or cultivated for their long, ornamental flora. This perpetual herb develops to about 0.3m in top and has light pink vegetation and a fleshy conical root (corm). The corm has a sour, acrid taste, bitter and radish-like odour. Low-lying leaves are arranged across the base of the plant, creating from bulb. Springtime, the plant has leaves alternatively no blossoms (M. Akram, 2012).

2.1.6. Chemical Constituents in the *Colchicum autumnale* L

Medicinal plant metabolites have a significant role in our lives since nowadays most of the world population relies on herbal sources of drugs for the curing of various ailments. These medicinal plants constitute diverse types of bioactive metabolites/phytochemicals. It is one of medicinal plants used to cure certain ailments and whose medicinal benefit is attributed to the phytocompound colchicine present in it. Colchicine is the major active component which was found

in different parts of *Suranjan Shireen* in various composition; such as, in amount of nearly 0.6% of corm; amounts may exceed 1% in the seeds, flowers, and dried leaves contain the same amount of colchicine as fresh parts; nevertheless, dried flowers and seeds contain fifteen times as much colchicine as leaves. Different types of other related alkaloids have been isolated from the *Colchicum autumnale* L together with colchicoside, demethyl-3-colchicine, colchicerine, colchicine, 2-demethylcolchifoline, cornigerine (M. Akram, 2012), 2-desmethylcolchicine, 3-demethylthiocolchicine are chemical constituents while bioactive compound is 'Colchicine'. It is the main alkaloid of *Suranjan Shirin* (*Liliaceae*) and is beneficial for treatment of acute attacks of gout (Ellington et al., 2003). It is a phenethylisoquinoline alkaloid (amino alkaloid) which occurs in seeds of *Colchicum autumnale*. In 2003, Ellington *et al*., reported the extraction of alkaloids three-dimethyl colchicine, colchicoside, and colchicine from seeds of *Colchicum autumnale* L (*Suranjan shireen*) by using supercritical carbon dioxide method. The quantitative determination of the alkaloids was done via HPLC and the percentage of recovery was higher than 98% for the three alkaloids. This extraction method was in comparison with a conventional method involving sonication and maceration, and the equal levels of alkaloids had been acquired or obtained in each case. The supercritical carbon dioxide method is but very efficient, extra rapid and greater environmentally friendly than conventional techniques (Ellington et al., 2003). Phytochemical investigation of hydroalcoholic extract of *Colchicum autumnale* L flower and root exhibited the occurrence of tannins, terpenoids, flavonoids, polyphenols whereas carbohydrate & protein were absent (Suica-Bunghez et al., 2017).

The research examination demonstrated the presence of six chemical constituents in the flowers of *Colchicum autumnale* L which was introduced from Europe to China which are- 2-demethyl colchicine, colchicine, 2-demethyl colchicine 2-demethyl β-lumicolchicine, 2-demethyl demecolcine and β-lumicolchicine. It is also reported in the study that the content of colchicine was low while the content of 2-demethyl colchicine was high in the flowers of *Colchicum autumnale* L (Yue-Ling et al., 2014). Another research investigation on the HPLC profiling of marker compounds of *Colchicum*

autumnale L (*Suranjan Shirin*) showed the presence of 0.33 ± 0.06% of alkaloid and 0.15 ± 0.08% of colchicines (Siddiqui et al., 2020).

Table 5: Phytochemicals present in *Colchicum autumnale* L

Phytochemicals	Part of the plant
Phenethylisoquinoline alkaloid (amino alkaloid)	Seeds (Evans, 2006)
Colchicine	Seeds, corms, flowers & leaves (M. Akram, 2012).
Colchicine, colchicoside, 3-demethylcolchicine	Seeds (Ellington et al., 2003)
Flavonoids, Tannins, Terpenoids, Polyphenols	Flowers and roots (Suica-Bunghez et al., 2017)
2-demethyl colchicine, 2-demethyl colchicine 2-demethyl β-lumicolchicine, 2-demethyl demecolcine and β-lumicolchicine	Flowers (Siddiqui et al., 2020)

2.1.7. Pharmacological Applications of *Colchicum autumnale* L

It has been utilized in India in anti-inflammatory, and against gout therapeutics for roughly 4000 years and it's far broadly perceived for its toxicity, the plant is described by methods for around 30 tropolone alkaloids. Fundamentally colchicine, used to against gout, and colchicoside, which, changed over into thiocolchicoside, has myorelaxant properties (Suhail & Ansari, 2017). Other plenteous employments of these alkaloids: liver fibrosis, cirrhosis, and familial Mediterranean fever are inside the way of development (Ben-chetrit, 2014). The active principles mentioned above are in a large part extracted from seeds of meadow saffron is called alkaloid, and their structure is widely known, and it provides dramatic relief from acute attacks of gouty arthritis. A number of other pharmacological actions of colchicine including depressing the respiratory center, improving the action of central depressants, and decreasing body temperature. Its role has been shown to set off T lymphocytes (M. Akram, 2012). All T-lymphocytes are activated and explicit high levels of the

activation markers CD44, and CD69 (Siddiqui & Akhtar, 2019). Acute poisoning of *Colchicum autumnale* L was reported in cattle through experimental histopathological study. *Colchicum autumnale* L or autumn crocus crude or dehydrated bulbs have been fed to 11 calves, and all of the calves evolved extreme diarrhoea, & euthanized within 63 hours. At necropsy, the gastrointestinal mucosa became oedematous & hemorrhagic. Necrosis, histologically & deterioration with karyopyknotic & karyorrhexis had been proven within the basal moveable coating of the tongue, forestomach, urinary bladder, renal pelvis, neck cellular layer of the stomach gastric glands, and intestinal crypts. Those findings had been also seen in renal tubular epithelial cells, lymphocytes and Kupffer cells Kupffer cells inside hemopoietic system & the lymphoid. The lesion of the prevailing acute crocus poisoning of livestock intently resembled those said in people with colchicine intoxication. Purified acetone extract of organs poisoned cattle confirmed to encompass demecolcine and colchicine via HPLC (Yamada et al., 1998)

Another research examination confirmed the suicidal poisoning with *Suranjan Shirin* (*Colchicum autumnale* L). It is commonly mentioned to autumn crocus & 'gowri gedde' within the southern region of Karnataka nation in South India. Over-dosage of colchicine leads closer to intense vomiting, diarrhoea, and epigastric ache, and even to die because of multiorgan failure. Chemical examination of blood & viscera gained from post mortem affirmed the existence of colchicine. Over-dose of it is high harmfulness & inaccessibility of one of a kind remedy treatment. It traditionally provides with gastroenterocolitis and might achieve multiorgan failure in deadly cases (Nagesh et al., 2011). *Colchicum autumnale* L also absolutely modified the clinical pathology of subclinical hyperthyroidism and thyroidal quantity in sufferers with euthyroid goiter via using normalization of the regulation of thyroidal hormones. Thyroid stimulating hormone, and free triiodothyronine linear regression indicated a regulative therapeutic effect of *Colchicum autumnale* L (Scheffer et al., 2016). The bulb-like corms of *Colchicum autumnale* L incorporate colchicine, a treasured remedy with a close-becoming beneficial document. Colchicine is endorsed by means of the US FDA used for the treatment of familial Mediterranean fever & gout. According to Najmul Ghani, corms of colchicum

is mainly used for medicinal purpose and the efficacy of the drug remains for 3 years, and It is also used to treat sciatica and to increase Aphrodisiac activity (Siddiqui & Akhtar, 2019).

Suranjan Shirin (*Colchicum autumnale* L) was found effective in all three important arthritis that means osteoarthritis, gouty arthritis and rheumatoid arthritis. It comprises good anti-arthritic and anti-inflammatory activity in all three major types of arthritis, which is equivalent to the effect of the potent standard inflammatory agent Diclofenac sodium (Siddiqui & Akhtar, 2019). It becomes clinically evaluated that *Colchicum autumnale* L is powerful within the remedy of gouty arthritis as a component of Gouticin tablet and in the remedy for rheumatoid arthritis as an aspect of Arthritin tablet (M. Akram, 2012). At decrease doses (0.0001 g) it's miles used in the remedy of gout, and it was confirmed to have an antitumor effect. In the world literature, it's been declared that the medicinal drugs received from the plant species are used in veterinary medications for arthritis and as a diuretic (Spasevska et al., 2017). In Anthroposophic remedy, a shape of integrative medicine (Kienle et al., 2013), the plant used the medication of diverse thyroid issues along with goiter. Primer data from nonrandomized preliminaries has also bolstered the use of colchicine for the cure and avoidance of intense pericarditis.

In a single-center, open-label, randomized trial, called the Colchicine for Acute Pericarditis have a look at, the addition of colchicine to standard remedy with either aspirin or glucocorticoids halved the recurring charge after a preliminary attack of acute pericarditis (Imazio et al., 2016). In atherosclerotic vascular issue, an artery wall thickens because of the collection of calcium and fatty substances alongside low density lipoprotein cholesterol. It's far a disorder influencing blood vessel, vein, a constant fiery response inside the dividers of corridors especially because of atheromatous plaques. Disturbance of the plaques may likewise prompt intense coronary disorder (alongside ischemic chest throb, intense myocardial localized necrosis, risky angina); heart failure; or potentially stroke together with non-high-impact embolic ischemic stroke. Coronary sickness, and coronary heart issue are a type of atherosclerotic vascular diseases on account of plaque developing along the internal

dividers of the corridors of the coronary heart, which limits the veins and decreases blood stream to the coronary heart. Stable coronary ailments are those that happen typically in power, character or recurrence at known degrees of effort or other upgrades. Unstable coronary infections are those other in force, individual or recurrence. The research report shown the use of colchicine in treating major repetitive aphthous stomatitis & preventing further repeats of ulcers (Katz et al., 1994).

2.1.8. Different Extraction Techniques of Plants Extracts

Extraction is fundamental, basic, and essential footstep in the assessment of medicinal plants. As a result of the reality it is integral to extract the favoured chemical compounds from the plant materials for further isolation, identification, investigation & characterization. The steps involved in the preparation of plants materials for extraction are: prewashing, drying or freeze-drying, grinding to acquire a homogenous pattern and often improving the kinetics of analytic extraction and moreover increasing the contact of the sample surface with the solvent system. Appropriate actions ought to be made to guarantee that potential bioactive ingredients are not lost or destroyed during the preparation of the extract from plant samples. If the plant became selected on the evidence of traditional uses (Fabricant & Farnsworth, 2001), at that point it's much desirable to make the extract as defined with aid of the standard healer on the way to mimicking as intently as viable to the common herbal drug. The choice of appropriate solvent system mainly depends upon the unique nature of both primary and secondary bioactive metabolites, or phytochemicals being targeted. For instance, the extraction of hydrophilic compounds makes use of polar solvents inclusive of ethanol, ethyl acetate, or methanol, and for extraction of greater lipophilic compounds dichloromethane or a mixture of dichloromethane and methanol in the ratio of 1: 1 is used in some occurrences. Chlorophyll was extracted using hexane (Deshmukh Krishi Vidyapeeth et al., 2017).

The products thus obtained from medicinal plants are particularly powders, semisolids or impure liquids meant best for outside/oral use. Therefore, extraction is still of sizeable interest in

order to obtain improved yields of drugs derived from plant and animal resources. Various extraction techniques are used for hundreds of years for extracting bio-active compounds.

The aims of all extraction techniques are (Smith, 2003): -

1. Extraction of target bioactive compounds from plant parts

2. To increase selectivity of analytical methods

3. To increase sensitivity of bioassay by means of increasing the amount of targeted compounds

4. For transformation of bioactive compounds right into a more appropriate forum used for separation & detection

5. To afford a robust and reproducible approach definitely impartial of variations inside the sample matrix

2.1.8.1. Conventional Extraction Techniques

Different types of the above extraction techniques are used to extract numerous types of phyto chemicals or secondary bioactive compounds from plants sources. They need various types of solvents & application of heat. For extracting bioactive compounds from medicinal plants, the existing classical techniques are: (1) Soxhlet extraction, (2) Maceration, (3) Hydro distill ation, (4) Decoction, and (5) Infusion. The description of these conventional extraction techniques is: -

2.1.8.1.1. Soxhlet Extraction: It is one of existing classical extraction technique that is used to extract bioactive compounds from natural sources. In this technique the thimble is used to hold plant material and situated in a refining jar which contains the solvent of particular interest. After reaching an overflow level, the solution of the thimble-holder is suctioned with the help of a siphon & the siphon unloads the solution back into the distillation flask. This solution includes extracted solutes into the bulk liquid. The solvent is passed to the solid bed of plant & the solute remains in the distillation flask. The procedure runs over and over until the extraction is finished. This method is not suitable

for thermo labile compounds as extended heating may lead to degradation of compounds (Kasiramar & Gopalasathees kumar, 2018).

2.1.8.1.2. Maceration: It is a broadly utilized technique in therapeutic plants research and useful for making wine. It involved soaking of plant materials (coarse or powdered) in a stoppered holder with a dissolvable solvent & permitted to remain at room temperature for a time of at least three days with frequent agitation. The process is intended to soften & break the plant's cell wall to release the soluble phytochemicals. Following 3 days, the mixture is pressed or strained by filtration. In this conventional technique heat is moved through conduction & convection and the choice of solvents will decide the sort of compound removed from the samples (Azwanida, 2015).

2.1.8.1.3. Hydro distillation: It is a regular system for the extraction of essential oils and bioactive compounds from plants parts. Natural organic solvents are not included during the extraction of plant extracts & it could be performed before drying out of plant materials. In this traditional extraction procedure, samples of the plant materials are packed in a still compartment & water is included in sufficient adequate amount, & subsequently brought to boil or by-passing steam through it. The heat & steam cause the cell structure of the plant material to blast & separate, accordingly freeing the essential oils. The essential oil molecules & steam are brought along a funnel & diverted through a cooling tank, where they come back to the fluid structure & are gathered in a tank. The developing fluid is a mixture of water and oil. Since essential oils are not water dissolvable, they can be effectively isolated from the water & redirected. Essential oils which are lighter than water will float on the surface. Hydro distillation can be:

A. Water and steam distillation

B. Water distillation

C. Direct steam distillation (Vanker, 2004).

2.1.8.1.4. Decoction: This is suitable system for the extraction of constituents soluble in water and that can't be destroyed by means of the effect of heat. In the decoction of the plant's materials,

distilled water is introduced to the dried sample and the mixtures are subjected to warming constantly for a while at a temperature of 100°C. At that point it is permitted to cool to at 25℃ and filtration performed to acquire the filtrate. Then, the extract is obtained by concentrating the filtrate (Bimakr et al., 2010).

2.1.8.1.5. Infusion: In this technique, extraction consists of soaking the solids plants powder both in boiling or cold water for a period of time (A. Pandey & Tripathi, 2014).

2.1.8.2. Advanced Extraction Techniques

The main challenges of the above conventional extraction techniques are the requirement of highly-priced and excessive pure solvent, longer extraction time, evaporation of the large amount of solvent, selectivity of extraction is low & decomposition of thermal labile compounds (Luque de Castro & Gatcia-Ayuso, 1998). In order to conquer the above-mentioned obstacles of classical extraction strategies, new & promising extraction techniques are brought. Those methods are called non-traditional extraction techniques. Some of those approaches are viewed as ‘‘green techniques’’ as they fulfil with requirements established through the manner of environmental protection agency, USA. These encompass designing safer chemicals, secure solvents auxiliaries, using much less hazardous chemical synthesis, design for electricity efficiency, atom economic system, usage of renewable feedstock, design to save degradation, reduce derivatives, catalysis, time analysis for pollution prevention & inherently safer chemistry for the prevention of accident (http://www.epa.gov/geenchemistry/pubs/about-gc.html). The description of some of the advanced extraction techniques are:

2.1.8.2.1. Ultra-Assisted Extraction Technique: This technique involves the use of high-frequency sound waves, excessive-intensity & their interaction with materials. It is a probably beneficial technology since it does not require complex instruments & is fairly low-cost. It could be used both on large & small scale (J. Dai & Mumper, 2010).

2.1.8.2.2. Microwave Assisted Extraction (MAE) Technique: It is a simple, economical & environmental friendly technique for the extraction of phytocomponents from various sample materials of the plant (Hemwimon et al., 2007). The use of this technique for extraction of plant materials becomes 1st stated via Ganzler & co-people in 1986 (Kasiramar & Gopalasatheeskumar, 2018). It possesses magnetic and electric fields which can be perpendicular to each other. The electric field causes heating by means of two synchronous components in particular- dipolar rotation and ionic conduction. Sample components absorb microwave energy in accordance to their dielectric constants (Gupta & Kothari, 2014). Once the sample of plant materials are submerged inside a microwave with noticeable solvent, the heat of microwave radiation straightforward reaches the solid without being absorbed with the aid of the solvent, following in promptly heating of the residual moisture within the solid. Heating causes the moisture to evaporate & creates a high vapour pressure that breaks the cell wall of the substrate and releases the content material into the solvent. Solvents utilized for these extraction procedures are individuals with an excessive dielectric consistency & potentially assimilate microwave energy; be that as it may, the extraction selectivity & the capability of the medium to engage with microwaves may be modulated through utilizing combinations of solvents. It can be practiced in two exceptional modes- one is closed vessel operation- that is under controlled (expanded) pressure & temperature, another is open vessel operation accomplished at atmospheric pressure. Those technologies are called as focused microwave-assisted extraction & pressurized microwave-assisted extraction technique, respectively (Gupta & Kothari, 2014).

MAE is tremendously powerful for acquiring extracts below moderate situations. According to the research study conducted by Gupta & Kothari, the microwave assisted extraction requires shorter extraction time, lesser solvent requirement, low price & higher extraction yield in assessment to Soxhlet extraction. Consequently it's been taken into consideration as a capacity opportunity to standard methods (Gupta & Kothari, 2014). Both sonication assisted extraction & microwave assisted

extraction demonstrated to be successful in increasing the yield of tannins & phenolics and in rising the potential of antioxidant activity (Thomas et al. , 2012).

2.1.8.2.3. Supercritical Fluid Extraction: - It is predominantly supercritical carbon dioxide for the reason that carbon dioxide is close to room temperature & it has low critical pressure that offers the opportunity to perform at moderate pressures, usually between 100 hundred and 450 bar. This method was introduced as an alternative to the extraction methods using solvent (Yepez et al., 2002). Numerous solvents may be used for supercritical fluid extraction, together with nitrous oxide, butane, pentane, hexane, sulphur hexafluoride, and fluorinated hydrocarbons. Carbon dioxide is frequently used as an extraction solvent. It alone is non-selective, however its potential and selectivity of extraction can be advanced through the utilization of a co-solvent or modifier. After that the extraction co-solvent can be removed without any difficult (Gupta & Kothari, 2014). A simple supercritical fluid extraction system consists of the following elements: a tank of the mobile phase, commonly carbon dioxide, co-solvent vessel and pump, a pump to pressurize the gas, an oven that comprises of the extraction vessel, a controller to preserve the excessive pressure inside the device and a trapping vessel. Regularly exceptional sorts of meters like float meter, dry/moist fuel meter can be connected to the framework (Azmir et al., 2013).

2.1.9. Isolation, Identification, and Characterization of Bioactive Compounds

After the extraction of plants materials; isolation, identification, investigation and characterization of bio-active secondary metabolites or compounds become a big task. This is because of most plant extracts occur as a mixture of diverse varieties of bioactive compounds with distinct polarities. Phytochemical screening assay is an easy, brief, and cheaper manner that offers the researcher a short method to the diverse types of phytochemicals or secondary metabolites determination in herbal plants. In separation or isolation of these bioactive compounds, wonderful chromatographic

separation techniques, together with TLC, HPLC, CC, flash chromatography and sephadex chromatography could be used to attain natural bioactive phytochemicals.

Thin layer chromatography- It is a favourite approach of most researchers because it offers a brief answer as to how many components are there in aggregate. It is likewise used to help to know the identity of a compound in a combination while the retention factor of unknown compound is in comparison with the retention factor of a known compound (Sasidharan et al., 2011). After TLC analysis, the structure elucidation of pure compounds is determined by using several spectroscopic and chromatographic techniques. These are high performance liquid chromatography (HPLC), liquid chromatography (LC)/electrospray ionization tandem mass spectrometry (MS/MS), capillary electrophoresis, ion spray mass spectrometry (MS), fuel chromatography/MS, nuclear magnetic resonance spectroscopy (NMR), and gas chromatography-mass spectrometry (GC-MS)(Jeong et al., 2012). Some of the analytical techniques and their uses are: -

2.1.9.1. Thin Layer Chromatography (TLC): It is a quick, simple & cheaper system that researcher can rapidly apply as to how many components are in a mixture. It consists of both mobile and stationary phase. For instance, a thin glass plate coated with either silica gel or aluminium oxide is used as the stationary phase, and the mobile phase solvent is chosen according to the properties of the components in the mixture. The principle of this technique is the distribution of a compound between a solid fixed phase (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of the TLC plate. The plate is then evolved inside the developing chamber that has a shallow pool of solvent just under the extent at which the pattern became implemented. The solvent is drawn up via the particles at the plate through the capillary motion and because the solvent moves over the combination each compound will both continue to be with the strong section or dissolve in the solvent and flow up the plate. Whether the compound moves up the

plate or stays back depends upon the physical properties of that individual compound and for that reason we rely on its molecular shape, especially functional groups.

The solubility rule “Like Dissolves Like” is accompanied. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind (S. Kumar et al., 2013; Bele, 2011). It is additionally used to assist the identity of a compound in a combination when the retention factor of unknown compound is compared with the retention factor of a known compound. Further assessments involve the spraying of phytochemical screening reagents, which cause colour modifications in accordance with the phytochemicals existing in a plant extract; or with the aid of viewing the plate under the UV light. This is very important for the confirmation of the purity and identity of isolated compounds. The short coming of TLC analysis is: low sensitivity, low resolution and the difficulty of detection of trace components, etc. (Yongyu et al., 2014).

2.1.9.2. Column Chromatography (CC): It is a common technique for the separation and purification of both liquids and solids. It contains both stationary bound and mobile phases and in a solid-liquid approach - the mobile phase is a liquid and stationary phase is a solid. The principle of this technique is based on the differential adsorption of substance through the adsorbent. The common adsorbents are alumina, silica, calcium phosphate, calcium carbonate, starch, magnesia, etc. The choice of solvent is largely dependent upon nature of both the solvent and the adsorbent. The cost at which the additives of an aggregate are isolated depends upon at the interest of the adsorbent and polarity of the solvent. If the interest of the adsorbent may be very high and polarity of the solvent could be very low, then the separation is very slow but gives an excellent separation. However, if the interest of adsorbent is low and polarity of the solvent is high the separation is speedy however it gives a bad separation and that means the components separated aren't hundred percent pure. The

adsorbent is made into a slurry with an appropriate liquid and located in a cylindrical tube that is plugged at the lowest through a bit of glass wool or porous disc. The combination to be separated is dissolved in the perfect solvent and introduced at the top of the column and is authorized to skip through the column. Because the aggregate moves down the column, the components are adsorbed at unique areas relying on their capability for adsorption. The component with more adsorption power might be adsorbed on the top and the other can be adsorbed at the lowest. The one-of-a-kind components can be desorbed and collected one after the other through greater solvent on the top and this technique is known as elution. So, the system of dissolving out of the components from the adsorbent is known as elution and the solvent is known as eluent. The weakly adsorbed component is probably eluted more hastily than the other. The one of a kind fractions are gathered one at a time. Distillation or evaporation of the solvent from the specific fractions gives the pure components (Vlab.arita.edu, 2011).

2.1.9.3. High-Performance Liquid Chromatography (HPLC): It's by far a versatile, long lasting, safest, fastest, dependable and broadly used technique for the isolation, identification, quantification and purification of herbal products. This technique involves two phases (1) mobile phase, and (2) stationary phase and the separation of constituents is on the basis of the difference between partition coefficients of the two phases. It may be utilized efficiently to isolate or separate individual compounds from a combination of compounds and it is utilized in phytochemical and analytical chemistry to perceive, identify, quantify and purify the character components of the mixture (Boligon & Athayde, 2014).

2.1.9.4. Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS), and Liquid Chromatography-Mass Spectrometry/Time of Flight (LC-MS/TOF):

With the extension of natural remedy marketplace, the difficulty to exercise techniques on qualitative analysis of herbal medicine have been updated to guarantee their great efficacy, and safety has been arousing wonderful attention (Chen et al., 2011). LC-is an extensively utilized technique to analyse

sample and it is regularly coupled with mass spectrometry. In LC-MS, soluble compounds (the mobile phase) are handed through a column filled with a stationary (solid) phase. This efficaciously isolates the compounds dependent on their weight and affinity for the cellular and stationary phases of the column. Liquid chromatography-mass spectrometry can examine non-volatile metabolites without derivatization, hence gives amazing insurance of the metabolome. In the current time, this technique has become the maximum selective approach for surprisingly screening and characterization of regarded and unknown parts from the extracts. Interfaces at the side of atmospheric pressure chemical ionization and electrospray ionization have been efficaciously utilized in liquid chromatography mass spectrometry configuration and is appropriate for natural medication analysis. There are 3 common places in single mass analysers: ion trap (IT), quadrupole (Q), and time-of-flight (TOF). The characteristic features of TOF device is its correct mass measurement which gives the basic elemental composition of parent and even of fragment ions and may be used for the identification of unknown compounds and the differentiation of isobaric compounds. The measurement of accurate masses with the concentration of five ppm is usually accepted for the confirmation of the elemental compositions (Chen et al., 2011).

In order to achieve such accurate mass measurement, TOF instruments require frequent tuning and calibration of the spectrometer. Time of flight-mass spectrometry is a powerful device which is capable of ten thousand or greater resolving strength expressed in phrases of complete height width at one-half maximum, and it has a high acquisition speed and gives accurate mass measurement along with complete scan spectral sensitivity. Precise mass estimation gives the basic arrangement of parent and fragment ions utilized for the identification of unknown species and more prominent separation of isobaric species (two distinct mixes with the indistinguishable nominal mass yet the diverse basic structure, also with various accurate masses) (Fernandez-alba et al., 2006; J. Zhou et al., 2009).

Similarly, ion trap analysers are particularly suitable for more than one fragmentation step. In direct ion traps, ions are separated and collected because of an exceptional course of action of hyperbolic

and ring-framed electrodes in addition to electric powered fields. At this point the ions can be divided along these lines as portrayed above with the aid of collision-induced decomposition. This procedure can be rehashed in a consecutive manner all together and significant basic certainties are obtained (Jonscher et al., 1997), which might be utilized for the separation of isomers (two extraordinary compounds with the identical exact mass and elemental composition). In this sense, the combination of LC/TOF-MS accurate mass measurements to generate empirical formulae and LC/IT-MS providing extra fragmentation data for structure confirmation represents an effective technique for the analysis of complex structures. Coordinating LC-MS, LC-MS/MS, LC-MS/TOF, and LC/IT-MS information, the proposed structure utilizes more than one computational procedures to derive and organize putative distinguishing pieces of proof for the choice of metabolites which includes ion annotation, mass-based totally search, isotopic sample analysis, spectral interpretation and spectral matching. Directly, this methodology has been effectively developed and applied for the assessment of natural contaminants (Martı et al., 2008; Martı et al., 2008), home grown drug(Liu et al., 2010; W. Dai et al., 2011; Chen et al., 2011), metabolites(Huang et al., 2011); (Liu et al., 2010); (Kosina et al., 2011); (Chen et al., 2011); W. Dai et al., 2011; Liu et al., 2010) and numerous different fields.

2.1.9.5. Gas chromatography-Mass Spectrometry (GC-MS): This technique consists of a gas chromatogram coupled to a mass spectrometer. It is used to separate, recognize, and quantify a complex mixture of chemical compound. It is the best technique for the investigation of the many remarkably low molecular weight compounds in plants materials. In order to analyze a compound via GC-MS, it has to be sufficiently volatile and thermally stable. Further, functionalized compounds may require chemical amendment prior to analysis to do away with unwanted adsorption outcomes that might in any other case affect the quality of data obtained. Samples are generally analyzed as organic solutions and consequently materials of interest as an example, soils, sediments, tissues so on if are to be solvent extracted then the extract is subjected to numerous 'wet chemical' techniques earlier, and then GC/MS analysis is possible. Gas chromatography-mass spectrometry is frequently

used for direct analysis of complex chemical components existing in traditional medicines, and medicinal plants. Nowadays, Gas chromatography-mass spectrometry research has been increasingly more applied for the evaluation of medicinal plants as this method has proved to be a precious technique for the evaluation of non-polar components and volatile essential oil, fatty acids, lipids terpenoids, steroids, and alkaloids; and only grams of medicinal plants materials are required(Roman, 2008; Sermakkani & Thangapandian, 2012).

2.1.9.6. Ultraviolet-Visible Spectroscopy: This spectroscopic strategy is utilized for subjective examination and for the recognizable proof of specific classes of phytocompounds in both pure and organic biological mixtures. Specially, it may be utilized very well for quantitative investigation in light of the fact that aromatic molecules are power chromophores in the UV range. Phytocompounds are analysed through by means of this spectroscopic technique (Kemp, 1991). Natural phenolic phytocompounds such as phenolic compounds complexed with iron, polymer dyes, tannins, and anthocyanins have been distinguished by means of UV-Vis spectroscopy(Kemp, 1991). Additionally, the methods were seen as less selective and give data on the composition of the total polyphenol content. This spectroscopic technique is used to determine the total anthocyanins (520 nm), phenolic acids (360 nm), flavones (320 nm), and the total phenolic extract (280 nm). In comparison to other techniques, this spectroscopic technique is not time-consuming and presents reduced costs(Luque et al., 2006).

2.1.10.7. Fourier Transform - Infrared Spectroscopy (FT-IR): It has been a long-established method for the identification and characterization of compounds, and the functional groups present in an unknown combination of extracts(Eberhardt et al., 2007; Hazra et al., 2007). A number of the frequencies may be assimilated when infrared light goes through a natural compound; nevertheless, some frequencies could be transmitted through the sample with no retention occurring. The absorption infrared radiation is related with the vibrational changes that occur inside a molecule while its miles exposed to infrared radiation. Consequently, this technique is also known as vibrational

spectroscopy, and it is used to identify different function groups such as carbon-carbon single bonds, carbon-carbon double bonds, carbon-carbon triple bonds, carbon-oxygen single bonds, carbonyl-oxygen-hydrogen bonds, and nitrogen-hydrogen bonds which have numerous vibrational frequencies. The above types of the chemical bonds can be identified in organic compounds within the aid of detecting the characteristic feature of the frequency of the absorption band in the infrared spectrum(Luque et al., 2006). FT-IR gives fast & non-destructive examination to fingerprint herbal extracts or powders(Cherkaoui et al., 2010). Similarly, FT-IR spectra of pure compounds are usually so unique that they're like a molecular 'fingerprint'. For most common plant compounds, the spectrum of an unknown compound may be recognized with the aid of assessment to a library of recognized compounds. Sample preparation for FT-IR are for liquid samples and the best way is to place one drop of pattern between two plates of NaCl. The drop forms a thin film among the plates. Solid samples can be milled with potassium bromide (KBr), after which they are compressed into thin pellets which may be analyzed. In any other case, solid samples may be dissolved in a solvent which includes methylene chloride, and the solution then positioned onto a single salt plate. The solvent is then evaporated off, leaving a skinny film of the original fabric at the plate.

2.1.9.8. Nuclear Magnetic Resonance Spectroscopy (NMR): It is associated with the magnetic properties of certain atomic nuclei; extensively used to know the nuclei of atom such as, carbon, hydrogen atom, and isotopes. It has empowered numerous analysts to watch atoms by means of recording the varieties among the various magnetic nuclei, and in this way giving a clear picture of what the positions of these nuclei are in the compound. Additionally, it proves as to which atoms are found in neighbouring groups. In the long run, it also concludes how many atoms are found in each of these environments (Kemp, 1991). Numerous efforts were made within the past by way of the use of PTLC, CC, and LC to isolate individual phenols and the structural elucidation of compound which can be determined ultimately through nuclear magnetic resonance spectroscopy off-line (Agu et al., 2007; Luque et al., 2006). It miles an effective method for the structural determination of organic

molecules & widely used tools in chemical profiling and plant metabolomics; in light of its exemplary advantages over the other techniques, and alongside with non-destructive nature, relative quantification is possible without using internal standard, and recovery of a sample. Low sensitivity is one of the most important weaknesses and creates a problem for successful usage in a few cases (Simpson et al., 2011) but this problem can be solved by the use of a better magnetic field at low temperature (Cryogenic nation). Nuclear magnetic resonance spectroscopy offers the macroscopic view of metabolome & needless attempt for sample preparation(Ward et al., 2007). In particular, plant metabolomics needs a successful extraction of metabolites from plant parts on account of chemical compound variety and diversified variety in plants(Cuyckens & Claeys, 2004). Metabolomic labs around the world introduce a wide scope of spectroscopic procedures yet the utilization of NMR method as a first pass screen has numerous points of interest over various analytical strategies directly being used (Ward et al., 2007).

2.1.9.9. Mass Spectrometry (MS): Highly energetic electrons are used, and this technique is used to determine the molecular weight and fragmentation patterns in the given organic molecule. In this case the organic molecule is bombarded with either lasers or electrons in mass spectrometry, and thereby converted to charged ions, which might be highly energetic. The chromatogram of mass spectrum is plotted as the percentage of relative abundance of each fragment ion and molecular ion with respect to retention time. In the use of MS, relative molecular mass (molecular weight) may be decided with excessive accuracy and a specific molecular technique may be determined with an information of locations wherein the molecule has been fragmented (Praul, 2005).

Mass spectrometry presents plentiful facts for the structural determination of the organic molecules. In this method molecular masses of huge biomolecules may be decided with an accuracy of 0.01% of the whole molecular mass of the pattern(Devanshu et al., 2010). In MS, there are three steps: (1) ionization, (2) mass analysis, and (3) detection. The sample receives ions even as delivered into the mass spectrometer, and the molecular mass of the compound of interest is calculated based totally on

the mass/charge ratio. The important components required for the right selection of mass analyser are detection limit, the resolution, mass variety, scan rate, and test charge. Numerous varieties of mass analysers are mechanically used together with magnetic/electric field mass analyser, quadrupole analysers and time of flight (TOF). In mass spectrometer, detectors are the critical component that produces a signal from incident ions via secondary electrons or by the induced current. Depending up on the compounds to be characterized, detectors can be used in step wise mode. The mass spectrometry detectors are recorded according to a current or charge produced thru the ions (Devanshu et al., 2010). Mass spectrometry scans compounds inner a particular mass range. The electron spray ionization, and atmospheric pressure chemical ionization are commonly used to determine the fragmentation patterns of the ions. Every ionization mode gives a rapid and entire fragmentation pattern with a whole perception into their metabolite composition.

Electron spray ionization is the approach of desire in herbal product assessment. Its mode is ideal for liquid chromatography-mass spectrometry analysis of secondary metabolites from plant life. It's far an easy ionization method, capable of producing small fragmentation patterns through electrical strength, which lets in the ions to switch from liquid to gaseous phase before being analyzed in mass spectrometer (Steinmann & Ganzera, 2011).

CHAPTER 3: METHODOLOGY

3.1. Experimental Sites

Solvent extraction, chromatographic separation, isolation & purification of compounds, UV-Vis & FT-IR analysis were done at Delhi Technological University (DTU), Department of Applied chemistry research laboratory. The ^{1}H-NMR,^{13}C-NMR, Mass spectroscopy (MS), GC-MS, UHPLC-QExactive Orbitrap Analysis, antibacterial, antioxidant, anti dengue, and cytotoxicity activity were done in (JNU, GGSIPU, and ICGEB, New Delhi, India).

3.2. Apparatus and Instruments

The different apparatus and instruments which have been used are as follows: Column chromatography, separatory funnel, oven, TLC plate & jars, soxhlet apparatus, filter paper, analytical mills, pipettes, water bath, UV lamp, beakers, UV light cabinet, Tubes, culture, media, round bottom, UV-Vis Spectrophotometer, electronic balance, conical flasks (different size), measuring cylinder, rotary evaporator, chromatographic chamber, TLC plate, FT-IR, NMR, MS, GC-MS, LC-MS, and UHPLC.

3.3. Chemicals, Reagents, and Media

Silica gel S 32-63 μm (60-120 mesh) ASTM (Germany), solvents (99.8% methanol ACS grade, 99.0 % n-hexane ACS grade, and 99.5% dichloromethane ACS grade New Delhi, India), anhydrous sodium sulfate (Techno pharm chem Delhi, India), 99% sulphuric acid, distilled water, sodium hydroxide pellets AR 98%, 99% acetic acid glacial, acetic anhydride, ferric chloride, Hydrochloric acid, bacteria growth media, Trolox, Ascorbic acid ($C_6H_8O_6$), ABTS, and DPPH. All reagents are of analytical grade.

3.4. Experimental Methods

3.4.1. Assortment and Documentation of the Plant Material

The seeds of *Sisymbrium irio* L & *Colchicum autumnale* L were collected from Indian Drugs House, Khari Baoli, Delhi. The Botanical specimens of the plant were identified by Dr. Mokhtar Alam, Central Council for Research in Ayurvedic Sciences, Ministry of Ayush, Government of India and National Institute of Science Communication and Information Resources, New Delhi and the voucher specimen/file number 6238 and SC-0171/15 were deposited to the ministry of Ayush, Government of India; and Raw Material Herbarium and Museum, Delhi (RHMD).

3.4.2. Preparation of Extracts

500 g of a powdered seeds of *Sisymbrium irio* L, and *Colchicum autumnale* L were extracted with 5 L of n-hexane, dichloromethane, and methanol for 5-8 hrs in Soxhlet apparatus consecutively. At that point, the extracts were dried with 5 g of anhydrous sodium sulfate and separated or filtered and concentrated with a rotary evaporator under reduced pressure at 40^{o}C. Then all concentrates (crude extracts) were kept at 4℃ until investigation.

3.4.3. Phytochemical Screening Tests

Both qualitative and quantitative analysis of the crude extracts of n-hexane, dichloromethane, and methanol were done using standard procedures for identification of bioactive phytochemicals constituents that were present in the different solvent extracts.

3.4.3.1. Qualitative Phytochemical Tests

The qualitative phytoconstituents analyses of solvents extracts were done using standard procedures (Majgaine & Verma, 2017; Verma et al., 2016).

Tannins

About 0.5 mg of the two plants extracts were placed in separate test tubes, and 20 mL of distilled H_2O was added and boiled. It was then filtered and one percent of $FeCl_3$ was added to the filtrate and perceptions were made. The absence brownish green colour showed the absence of tannins.

Saponins

About 1 mg extracts were mixed with 5 ml of water and vigorously shaken. The absence of stable foam confirmed the absence of saponins.

Flavonoids

Alkaline reagent test: *Sisymbrium irio* L and *Colchicum autumnale* L extracts were treated with few drops of NaOH solution. The formation of powerful or sturdy yellow colour that turns into colourless on the addition of dilute acid indicated existence of flavonoids.

Terpenoids

About 0.25 mg extracts of the *Sisymbrium irio* L and *Colchicum autumnale* L were taken in a test tube; 1 ml of the $CHCl_3$ was added, vigorously shaken and dried completely. To this 1 mL of conc. H_2SO_4 was added and heated for about 2 min. The formation of a grey color confirmed the existence of terpenoids.

Glycosides

Salkowsks' test: The *Sisymbrium irio* L and *Colchicum autumnale* L extracts were mixed with 1 mL of conc. H_2SO_4 and 1 mL of $CHCl_3$ was carefully added and shaken gently, at that time the observations were made and the formation red-brown colour showed the presence of glycone portion of a glycoside.

Steroids

Liebermann Bur chard reaction: About 1 mg of the extracts was put in a different test tube and 5 mL of $CHCl_3$ was added and filtered, 2 mL of the filtrate was mixed with 2 mL of the mixture of CH_3COOH and conc. H_2SO_4 was added along the side of the test tube. The formation of a blue-green ring revealed the existence of steroids.

Phenols

Sisymbrium irio L and *Colchicum autumnale* L extracts were put in the various test tubes, and treated

with a few drops of 2% of $FeCl_3$; the formation of black or bluish-green coloration revealed the existence of phenols.

3.4.3.2. Estimation of the Total Phenolic Contents

Estimation of the total phenolic contents was estimated by means of the Folin - Ciocalteu index protocol (Khalil et al., 2017). The procedure for the determination of the standard gallic acid curve is as follows:

1. 1 mg Gallic Acid was dissolved in 1 millilitre of DMSO (1 mg/mL as stock)
2. Seven different concentrations were taken from stock in separate test tubes
3. 2 mL of FCPR (1: 10 v/v stock with water) was added to each test tube
4. 1 mL of 1M sodium carbonate was added to each test tube
5. Dist. H_2O was added till to the volume of the solution became 4 mL
6. It was allowed to stand for 15 min. in dark at room temperature
7. Absorbance was taken at 765 nm in a spectrophotometer
8. Control: 1 ml DMSO + 2 mL of FCPR + 1 mL of 1M sodium carbonate

Procedure for determination of total phenolic content in extracts: 10 µg of crude extracts from stock (10 mg/mL) was taken in triplicates and measurements were made up to one mL using dist. H_2O, then 2 mL FCPR (1:10v/v stock with water) was added to each test tube followed by the addition of 1 mL of 1 M Na_2CO_3 to each test tube. It was permitted to stand for fifteen minutes at room temperature in the dark and at that point, absorbance was taken at 765 nm in a spectrophotometer. Calibration was performed by serial dilution of Gallic acid as a standard (5, 10, 15, 20, 25, 30 and 35 µg/mL) in distilled water $y = 0.0251x - 0.00501$, $R^2 = 0.987$). The total phenolic amount was established as the equivalence of micrograms of Gallic acid per milligram of the dried plant extracts (µg GAE/mg dry plant extracts).

3.4.3.3. Estimation of the Total Flavonoid Contents

This was calculated according to some modifications of (Khalil et al., 2017). Procedure for the standard curve of Quercetin is as follows:

1. 1 mg Quercetin was added in 1 mL dimethyl sulfoxide (DMSO) (1 mg/mL as stock)
2. Seven different concentrations were taken from the stock in separate test tubes
3. 100 microlitre (µL) of ten percent of aluminium trichloride was added to each test tube
4. 100 microlitre of 1 molar potassium acetate was added to each test tube & mixed well 2.8 mL distilled water was further added to individually test tube
5. Overall volume of the solution was made up to 4 mL using DMSO
6. Above reaction was incubated for thirty minutes at room temperature in the dark
7. Absorptance was taken at 415 nanometres

Procedure for calculation/estimation of the total phenolic content in different solvent extracts: ten µg of crude extracts from stock was taken in triplicates and volume made up to 1 mL using DMSO and 100µL of 10% $AlCl_3$ and 100µL of 1 M potassium acetate were added and mixed well, and 2.8 ml of dist. water was further added and the reaction mixture was incubated at room temperature for 30 min. in dark and at that point absorbance was taken at 415 nm in a spectrophotometer. Calibration was determined by means of quercetin as a reference substance and from that a standard calibration curve got with solutions of 5, 10, 15, 20, 25, 30 and 35 µg/mL ($y = 0.0184x + 0.0154$, $R^2 = 0.9956$). The determination of total flavonoid contents was revealed as the equivalence of micrograms of quercetin per milligram of dried plant extract (µg QE/mg).

3.4.3.4. DPPH Antioxidant Assay:

Free radical trapping activity of extracts was carried out on the basis of a method with some modification (Cuendet et al., 1997). Antioxidant activity of various solvent extracts of *Sisymbrium irio* L, and *Colchicum autumnale* L and L-ascorbic acid (Vitamin C as standard) were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The standard

solutions were prepared by adding ten milligrams of ascorbic acid and crude extracts in one millilitre of dimethyl sulfoxide in separate reagent bottles (10 mg/mL as stock). Different concentrations of ascorbic acid and crude extracts were taken in separate test tubes from stock solutions & 900 µL of DPPH reagent (0.1 mM in methanol) were added in each test tubes and 1 mL reaction volume was made up using DMSO, then mixture was permitted to stand at room temperature for thirty to forty five minutes next, the absorbance was taken at 517 nm in spectrophotometer. Control was prepared by using a 900 µL DPPH solution with 1 mL of DMSO. The percentage of free radical scavenging activity was calculated as (Mothana *et al*., 2009):

$$\% \text{ FRSA} = [(A_{DPPH} - A_{EXTR})/A_{DPPH}] \times 100$$

Whereas, A_{DPPH} (absorbance control sample), A_{EXTR} (absorbance of extracts)

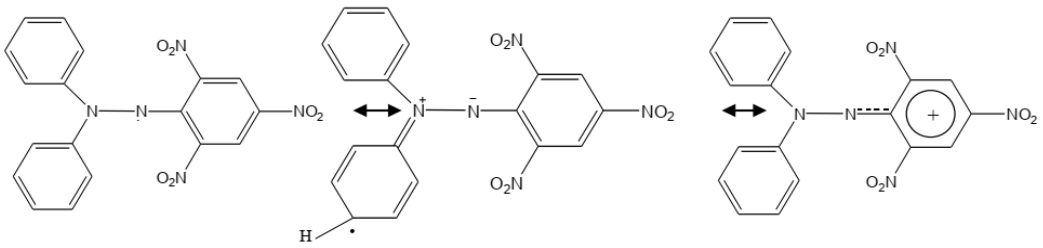


Figure 3: Structure of stable DPPH free radical

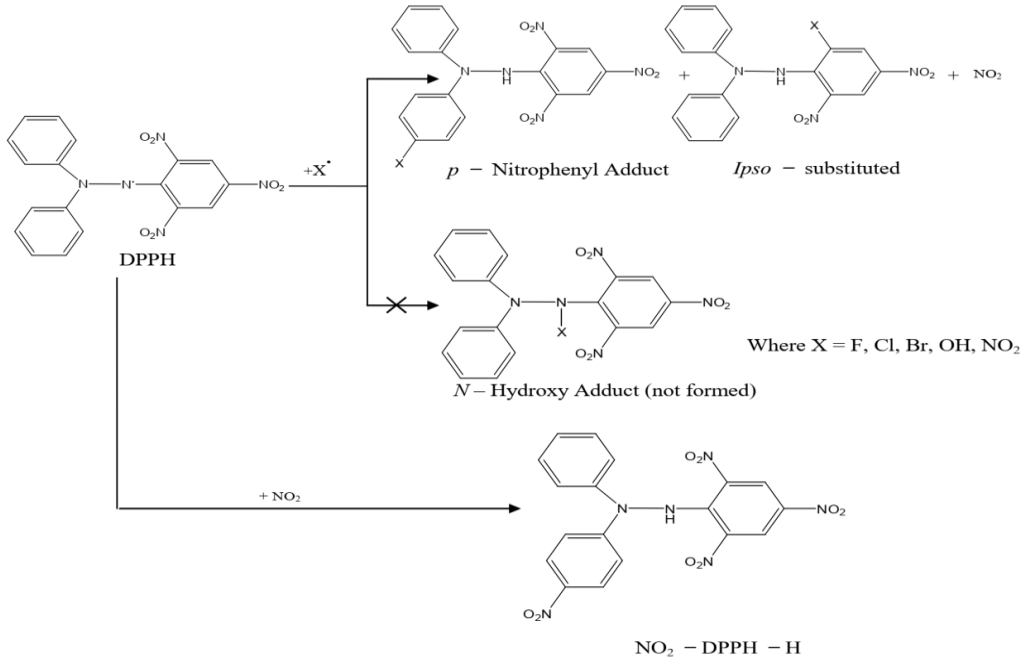


Figure 4: The reaction between the free radical species X· with DPPH (the key reaction product is NO2– DPPH – H) (Ionita P, 2005).

3.4.3.5. ABTS-Scavenging Analyses

ABTS-scavenging analyses were done according to Adedapo and water soluble trolox was used as standard (Adedapo et al., 2009).

The procedure for the preparation of ABTS-reagent:

1. 7mM ABTS solution was made in distilled water
2. Potassium per sulphate (2.45 mM) was made in distilled water
3. Both ABTS and potassium per sulphate were mixed in a ratio of 1:0.5 V/V and incubated in dark at room temperature for 12-16 hrs.
4. The absorbance of the above mixture was taken at 734 nm &0.1M phosphate buffer (the PH value 7.4) was used to bring its absorbance at 0.7 ± 0.02.
5. This ABTS mixture was used for the ABTS test of the crude extracts

1 mL of ABTS mixture was added to various concentrations; such as 50, 100, 150, 200, 250, and 300µg/mL of a test sample, then absorbance was taken at 734 nm in a spectrophotometer and finally percentage of free radical scavenging activity of extracts was determined.

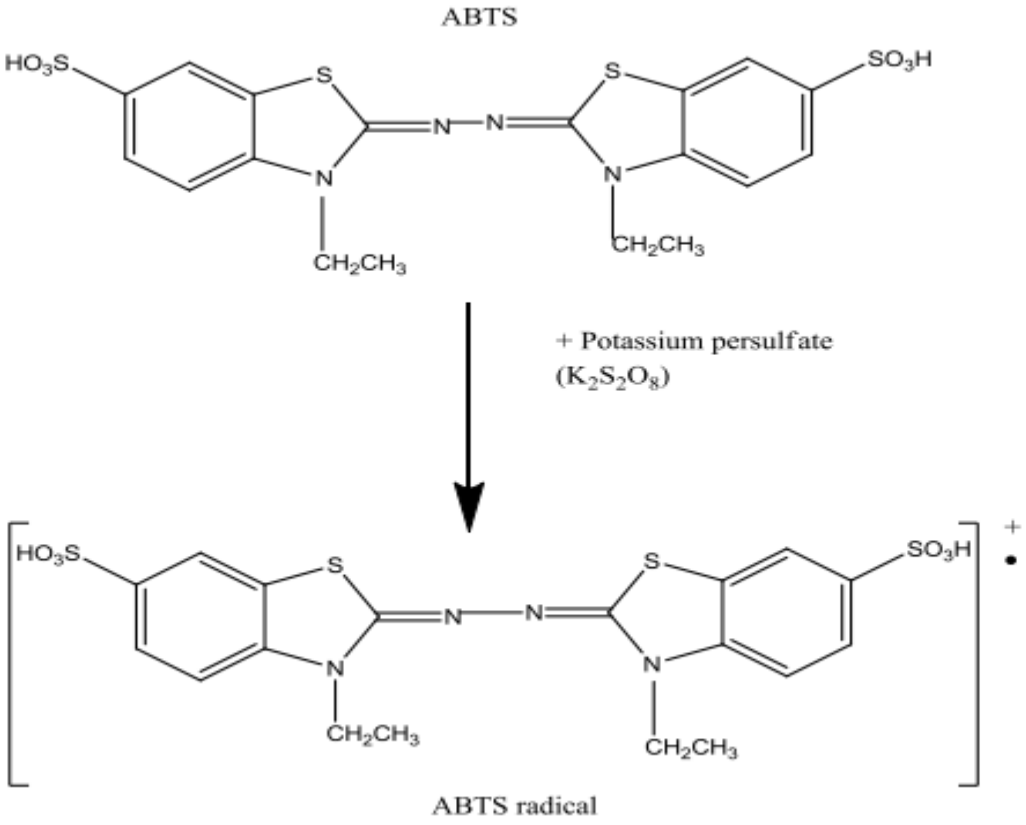


Figure 5: Schematic representation of the formation of ABTS radical after the addition of potassium persulfate

3.4.3.6. Antibacterial Assay

Solvents extracts were evaluated in vitro for anti-bacterial assay by utilizing the paper disc diffusion method. The bacterial species had been used for this analysis which had been collected from the department of Microbiology, Indian Agricultural Research Institute, New Delhi, India. Those bacterial strains were grown in nutrient broth (NB) media at 37°C for 24 hrs prior to the experiment and planted on agar plates through the pour plate technique and plates had been incubated for twenty-four hrs at 37°C. After an incubation period, zone of inhibition was measured (Jagtap & Bapat, 2013).

3.4.3.7. Protocols for Antiviral Assay

3.4.3.7.1. MTT Assay

Prepare a monolayer of Vero cells by seeding 10,000 cells per well in 96 well plate and incubate it for overnight i.e. 18 hrs. Prepare the dilution of drug in a separate round bottom 96 well plate. Aspirate the media from the plate, wash it with 1 x PBS and transfer the diluted drug into the plate. Incubate the plate at 37℃ and 5% CO_2 for 48 hrs. Aspirate the media and wash the plate with 1 X PBS. Use freshly prepared MTT (5mg/mL, Cat. – RM1131 HiMedia, Maharashtra, India) stock and add 10 µL in each well having 90 µL sera free DMEM (Cat. – AL007A HiMedia, Maharashtra, India) in the plate to make it a 0.5 mg/mL working solution. Incubate it for 4 hrs at 37℃ and 5% CO_2. Carefully aspirate the supernatant, add 150 µL DMSO (Cat. - TC185 HiMedia, Maharashtra, India), wait for 30 minutes to dissolve the Formazan complex or directly add 100 µL of 10% SDS – 0.01M HCl solution and leave for overnight. Take reading at 562nm (Microplate Reader, Wuhan USCNK Life Science Inc.).

3.4.3.7.2. MTT based DENV CPE - Inhibition Assays

3.4.3.7.2.1. Pre-treatment Assay

Make a monolayer of VERO cells in a 96 well plate by seeding 10,000 cells per well and incubated for overnight (around 18 hours). Next day, treat the cells with 100 µL serially diluted non-cytotoxic concentrations of plant extract for 2 h in advance. After treatment, infect the cells with 10 MOI of DENV-2 (ATCC, New Guinea C strain) in 100 µL sera free DMEM and incubate them for another 2 hours. Aspirate the media and wash the plate with 1X PBS. Add maintenance media (DMEM supplemented with 2% FBS, 2mM Glutamine) incubated for 96 hours at 37°C and 5% CO_2. Now aspirate the media, wash the plate and add 10 µL of freshly prepared MTT (5mg/mL) stock in each well having 90 µL sera free DMEM. Incubate for 3-4 hrs at 37℃ and 5% CO_2. After incubation, carefully aspirate the supernatant, add 150 µL DMSO and wait for 30 minutes to dissolve the Formazan complex. Take reading at 562nm (Microplate Reader, Wuhan USCNK Life Science Inc.).

3.4.3.7.2.2. Post-treatment Assay

Make a monolayer of VERO cells in a 96 well plate by seeding 10,000 cells per well and incubated for overnight. Infect the cells with 10 MOI (multiplicity of infection) of virus, incubate for 2 h at 37°C and 5% CO_2. Now, treat the infected cells with serially diluted non-cytotoxic concentrations of plant extract for 2 hours at 37°C and 5% CO_2. After incubation, wash the plate, add maintenance media and incubate for 96 h at 37°C and 5% CO_2. Now, aspirate the media, wash the plate and add 10 µL of freshly prepared MTT (5mg/mL) stock in each well having 90 µL sera free DMEM. Incubate for 3-4 hrs at 37°C, 5% CO_2. After incubation, carefully aspirate the supernatant, add 150 µL DMSO and wait for 30 minutes to dissolve the Formazan complex. Take reading at 562nm (Microplate Reader, Wuhan USCNK Life Science Inc.).

3.4.3.7.2.3. Co-incubation Assay

Make a monolayer of VERO cells in a 96 well plate by seeding 10,000 cells per well and incubated for overnight. Next day, incubate separately the serially diluted non-cytotoxic concentrations of plant extracts along with 10 MOI of virus to each well of 96 well plate and incubate at 37°C for 1 h. Transfer 200 µL of mix to infect Vero cells in 96-well plate and incubate for 2 hrs at 37°C and 5% CO_2. Now, aspirate the media, wash the plate and add 10 µL of freshly prepared MTT (5mg/mL) stock in each well having 90 µL sera free DMEM. Incubate for 3-4 hrs at 37°C, 5% CO_2. After incubation, carefully aspirate the supernatant, add 150 µL DMSO and wait for 30 minutes to dissolve the Formazan complex. Take reading at 562nm (Microplate Reader, Wuhan USCNK Life Science Inc.)

$$\%\ \text{Cell Survival} = \frac{\text{At}-\text{Ab}}{\text{Ac}-\text{Ab}} \times 100$$

Whereas, At = Absorbance of Test, Ab = Absorbance of Blank (media), Ac = Absorptance of control (cells)

3.4.3.8. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)-Perkin Elmer

3.4.3.8.1. Sample Preparation

All the chemicals used were of supra pure or trace metal grade. 0.25g of the seed's samples were taken in different digestion vessels and 1 mL of dist. H_2O, 2 mL of conc. HNO_3, 1mL of H_2O_2, and 0.2 mL conc. H_2SO_4 were added to it. After 30 minutes of pre-digestion, the vessels were closed and kept inside the digester at 483K. After accomplishment of digestion, the samples were transferred to a volumetric flask and made up to 50 mL. NIST standards were also digested following the same procedure. The blank sample solution was prepared following similar procedure as above without adding the sample. Instrument calibration was done for all the analyzed elements by mixing the standard solutions in the required proportions (Ibrahim et al., 2019).

3.4.3.9. Gas Chromatography-Mass Spectrometry Analysis

Sisymbrium irio L and *Colchicum autumnale* L solvents extracts were injected in GC-MS (Shimadzu GCMS-QP2010) for obtaining the results. The samples were introduced in split mode at 260°C. Oven temperature was planned from 50°C (2 minutes) to 280°C (16 minutes). The column flow rate was 1.21 mL/min and electron Ionization (EI) was used as the ionization mode. The identification of parts was accomplished by comparison of retention time and fragmentation pattern, and like mass spectra within the national institute of standards and Technology spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS. The relative percentage of each extract constituent was expressed as percentage with peak area normalization (Grover & Patni, 2013).

3.4.3.10. UHPLC-QExactive Orbitrap Analysis

3.4.3.10.1. Sample Preparation and Analysis by UHPLC-QExactive Analysis

2g *Colchicum autumnale* L powder was mixed with 10 ml 1% Formic acid (FA) in water and with a waiting time of 10 minutes; 10 ml methanol and 10 ml acetonitrile were added and vortex for one minute and kept on shaker for 40 minutes at 350rpm (at room temperature) and centrifuge at 5000 RPM for 5 min. & 0.5 millilitre of supernatant diluted by 0.5 millilitre acidified water. Then, 5µL of the extract was injected to QE-Orbitrap focus system. In this study, UHPLC-QExactive Focus orbitrap system was used to acquire raw data using Xcalibur software in full scan with a ddMS^2 mode, which offered simultaneously full MS (R=70,000) as well as MSMS (R=17500) spectra in a single acquisition with positive/negative polarity. The data were processed through the compound discoverer software using a non-target approach and to identify the maximum number of compounds. The analysis becomes completed on Vanquish ultra high-performance liquid chromatography (UHPLC Thermo Scientific™), coupled with a QExactive focus (Orbitrap, Thermo Scientific, Bremen, Germany). UHPLC analysis was achieved with Vanquish UHPLC (Thermo Scientific™) equipped with an Accucore aQ™ C18 (100 x 2.1-millimeter, 2.6 micrometre particle size) column maintained at forty degree Celsius. The mobile phase consisted of phase A [water: methanol (90:10, v/v) + 0.2 % HCOOH] and phase B [methanol: water (90:10, v/v) + 0.2% HCOOH] with a constant flow rate (0.4 mL/min). A gradient program was used as follows: 0-1-minute, 2% B, 1-11 minute, 2-100% B, 11-16-minute, 100% B, 16–17-minute 2% B, 17-22-minute, 2% B. The full MS-ddMS^2 mode offered a full MS spectrum with MS/MS simultaneously in a single LC run. For ddMS2, the normalized collision energy was ramped from 10-55v. The data acquisition was performed in Xcalibur 4.1 software. The full MS spectrum provided info about the complete molecular ion (e.g., M^+, M+H+), whereas the ddMS2 discovery generated the product ion spectra with ramped collision energy. The identification and characterization of metabolites were performed by relative comparison of formerly reported data and from online databases (Wishart et al., 2018).

3.4.3.11. Coding System for Isolated Compounds

Coding system of the isolated pure compounds was based on the first letter from the scientific name of the plants *Sisymbrium irio* L and *Colchicum autumnale* L, and this followed the solvents that have used for extraction, and the numbers after the symbols of solvents represented the numbers of fractions eluted from the column chromatography, respectively.

3.4.3.12. Isolation of Compounds

Part of the methanol crude extract of *Sisymbrium irio* L and dichloromethane crude extract of *Colchicum autumnale* L was chromatographed on silica gel (60-120 mesh) with increasing order of polarity of the solvents such as n-hexane, dichloromethane & methanol in n-hexane as eluents. TLC and UV lamps were used to monitor the purity of the fractions. Then the visualization of the TLC plates was done under a UV lamp at 254 nm and 356 nm. Based on UV and TLC analysis, similar fractions were combined to give different fractions.

3.4.3.12.1. Analysis of Crude Extracts and Fractions

The crude extracts were prepared using n-hexane, dichloromethane, methanol, and analyzed using the TLC plate (silica gel). The TLC plate was developed in the mobile phase of chloroform and methanol by slightly changing the solvent system to analyze the crude extracts. The identification of the spot was achieved by a UV lamp at 254 nm and 356 nm. The spectrum of NMR (^{1}H-NMR, ^{13}C-NMR), FT-IR and MS spectral data were used to characterize the structures of isolated compounds. The thin layer chromatographic method was applied to methanol crude extract of seeds of *Sisymbrium irio* and dichloromethane crude extract of *Colchicum autumnale* L using chloroform and methanol $CHCl_3$: MeOH (6:4). The column was packed with silica gel (150g) (# 60-120) in the presence of n-hexane as eluent. The slurry was prepared using silica gel, methanol and dichloromethane and 18 g of the slurry was poured into the column. The column was eluted with increasing order of polarity of solvents; first eluted with n-Hexane (100%) and chloroform (100%), then varying the ratio of chloroform: methanol (9:1: 8:2. 7:3, 6:4 and 5:5); the flasks were changed

every 250 mL collected fractions; and then they were analyzed by thin-layer chromatography, and visualized under UV light.

Figure 6: Isolation of compounds from methanol and dichloromethane crude extracts of plants using Column chromatography

3.4.3.13. Fourier Transform Infrared (FT-IR) Spectroscopy

The Fourier transform-infrared spectrum was recorded on a Shimadzu (Model FT-IR-8400 CE) with absorption given in wave numbers (cm^{-1}). Potassium bromide (KBr) pellet were utilized in the study. The range was recorded after background correction in the range 4000 - 400cm^{-1}.

3.4.3.14. Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR was recorded at 500 MHz and ^{13}C-NMR was detected at 500 MHz. The spectra were documented or recorded using deuterated chloroform as solvent and tetramethyl silane as the internal standard.

3.4.3.15. Mass Spectroscopy (MS) Instrument

Mass spectrometry has turned into an imperative instrument in the hands of natural scientific experts and organic chemistry on account of its capability to supply conclusive, subjective and quantitative data of molecules dependent on their structural compositions.

3.4.3.16. In-silico anti-inflammatory activity

Molecular docking study was carried out to investigate the comparative inhibitory effects of colchicine, colchicoside, deacetamide-5, and deacetyl colchicine compounds present in the extracts of *Colchicum autumnale* on TNF-α, IL-6 and IL-17.

3.4.3.16.1. Retrieving the target and ligand structures

The experimental structures of TNF-α (1TNF.pdb), IL-6 (1IL6.pdb) and IL-17 (4HR9.pdb) were downloaded from Protein Data Bank (PDB) (http://www.rcsb.org/pdb). The retrieved TNF-α (PDB ID: 1TNF) of resolution 2.6Å consists of three chains (A, B and C) with 157 amino acids sequence length. 1IL6 retrieved from PDB was having 185 amino acids consisting of chain A and similarly 4HR9 protein structure at resolution 2.48Å consisting of Chain A and B with sequence length of 122 amino acid. 1TNF and 1IL6 protein structures downloaded were of zero mutation and 4HR9 structure possessed 2 mutations. Structures of identified compounds (using UHPLC) were obtained from PUBCHEM (https://pubchem.ncbi.nlm.nih.gov/) (D. Yadav et al., 2013).

3.4.3.17.2. Docking studies

For docking studies, grid and docking parameter files were prepared by AutoDock 4.2.1. In order to maintain the electrostatics, hydrogen atoms were added and then merged with the non-polar hydrogen. PDBQT ((Protein Data Bank, Partial Charge (Q), & Atom Type (T)) file of ligands were generated after assigning gasteiger charges and kollman charges. 3D box was designed with 126x126x126 Å and spacing 0.81 Å for TNF-α and IL-17 whereas number of points in X, Y, Z dimension for IL-6 was set at 108x98x90 Å with 0.403 spacing. Blind docking was employed to study the best possible conformation in 10 different poses per run. Best binding pose was selected by estimating minimum binding energy and root mean square deviation (RMSD) (Auto Dock Vina) through Lamarkian algorithm (Mann et al., 2015).

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Phytochemicals Investigation of *Sisymbrium irio* L and *Colchicum autumnale* L Extracts

The qualitative phytochemicals analysis showed the existence of phenols, flavonoids and terpenoids in all solvent extracts of *Sisymbrium irio* L and *Colchicum autumnale* L, whereas steroids were identified only in n-hexane, dichloromethane and methanol extracts of both plants but saponins and tannins were not identified in any solvent extracts of plants.

Table 6: Qualitative phytochemical screening results of the extracts of the plants

S. No	Phytochemicals	*Sisymbrium irio* L extracts			*Colchicum autumnale* L extracts		
		n-Hexane	Dichloromethane	Methanol	n-Hexane	Dichloromethane	Methanol
1	Phenols	+	+	+	+	+	+
2	Flavonoids	+	+	+	+	+	+
3	Saponins	-	-	-	-	-	-
4	Terpenoids	+	+	+	+	+	+
5	Steroids	-	+	+	-	+	+
6	Glycosides	-	-	-	+	+	+
7	Tannins	-	-	-	-	-	-

+ = the existence of phytochemicals - = the absence of phytochemicals

The chemical compounds in the plant parts were recognized to be biologically active compounds and they were responsible for various actions- for example, antioxidant, antifungal, antimicrobial, and anticancer (Hossain & Nagooru, 2011). The terpenoids assemble show critical pharmacological activity, for example, anti-viral, antibacterial, anti-malarial, anti-inflammatory, hindrance of cholesterol combination and anti-malignant activities (Gomathi & Kalaiselvi, 2013).

The phenolic compounds have pharmacological properties particularly antimicrobial activity (Raja et al., 2011), antiviral, mitigating and cytotoxic activity; the anti-mutagenic & anti-carcinogenic activities (Mungole et al., 2010). The therapeutic herb is better-quality with phenolic activity that have superb antioxidant agent properties (Narayana et al., 2001). Phenolics are dynamic in restoring kidney and stomach issues just as accommodating as mitigating in action (Shirwaikar et al., 2003). Phenolics have anti-microbial, anti-oxidative, anti-allergic, anti-mutagenic, anti-diabetic, anti-inflammatory and anti-carcinogenic activities (Shirwaikar et al., 2003 ; Sasikumar et al., 2010).

Flavonoids likewise have antioxidant property as they hinder oxidative and hydrolytic chemicals, have impact on radical scavenging, anti-cancerous and anti-inflammatory activity (Y. Li et al., 2019 ; Alsabri et al., 2013).

4.2. Quantitative Estimation of the Total Phenolic Contents

Phenolic compounds are widely distributed in plants parts and are beneficial to human health due to their antioxidant and free radical scavenging activity (Bakari et al., 2018; Govindarajan et al., 2007). Quantitative estimation of the total phenolic contents showed that their level varies from n-hexane crude extract to methanol crude extracts of *Sisymbrium irio* L and *Colchicum autumnale* L extracts and represented as µg gallic acid equivalent (GAE)/mg of dry extracts. Its total phenolic contents varied from n-hexane to methanol extracts of *Sisymbrium irio* L ranging from 2.566, 3.05 to 23.03 µg GAE/mg of dry extracts. *Sisymbrium irio* L examination showed the methanol crude extract contained the highest percentage of the total phenolic components, followed by dichloromethane and n-hexane crude extract. While in case of *Colchicum autumnale* L, dichloromethane extract contained the highest percentage of over-all phenolic components (26.6 µg GAE/mg of dry extract), followed with the aid of methanol extract (17.3) µg GAE/mg of dry extract), and n-hexane extract (6.448 µg GAE/mg of dry extract).

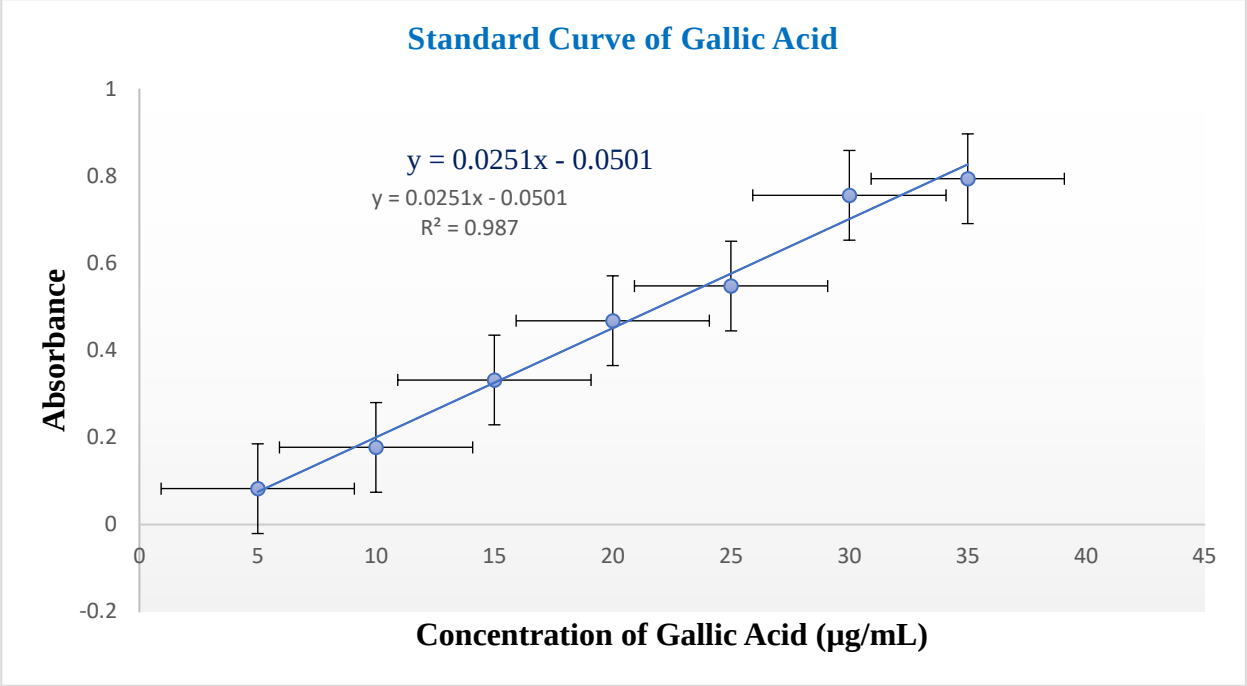


Figure 7: Standard curve of gallic acid to determine the phenolic contents.

Table 7: Quantitative estimation of total phenolic contents in *Sisymbrium irio* L extracts

Sisymbrium irio L Solvent Extracts	Concentration of total phenolic contents (µg GAE/mg of dry extract)
n-hexane extract	2.566
Dichloromethane extract	3.05
Methanol extract	23.03

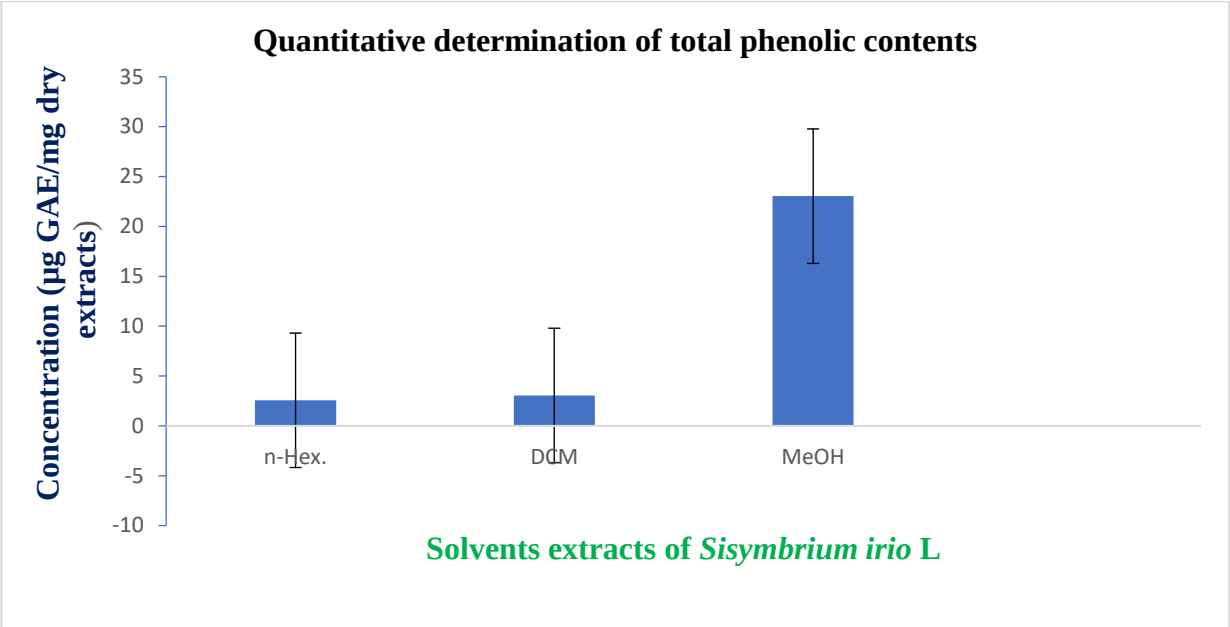


Figure 8: Total phenolic contents in different solvents extracts of *Sisymbrium irio* L.

Table 8: Quantitative estimation of total phenolic contents in *Colchicum autumnale* L extracts

Colchicum autumnale L Solvent Extracts	Concentration of total phenolic contents (µg GAE/mg of dry extract)
n-hexane extract	6.488
Dichloromethane extract	26.6
Methanol extract	17.3

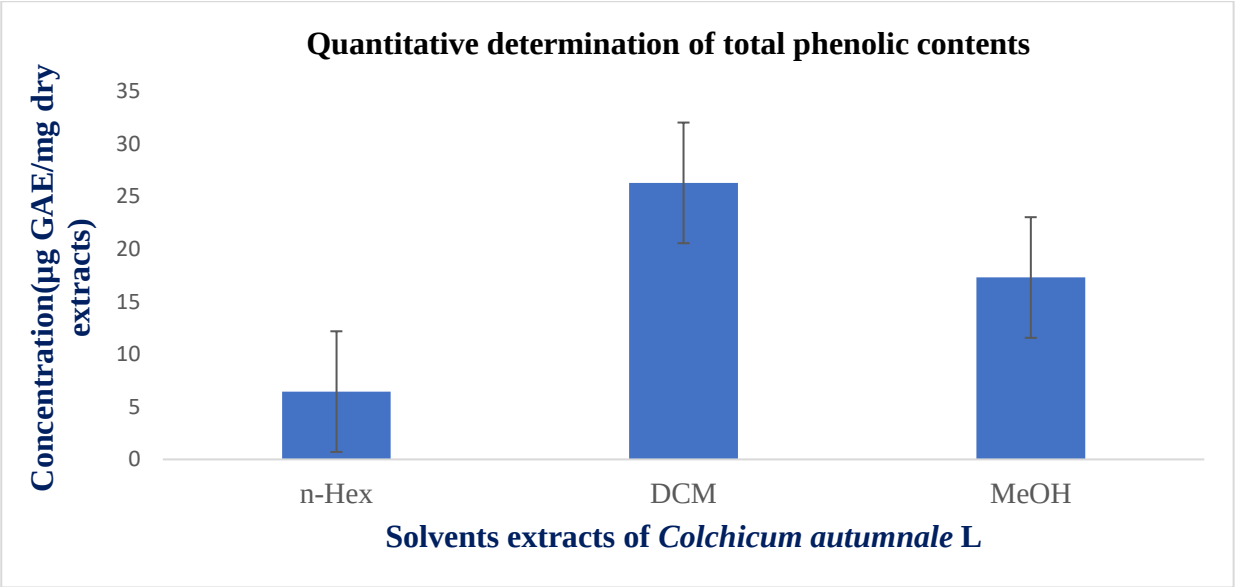


Figure 9: Total phenolic contents in different solvent extracts of *Colchicum autumnale* L.

4.3. Quantitative Estimation of the Total Flavonoid Contents

It is one of the most abundant & considerable group of natural occurring compounds in various parts of plants and thus possesses a large spectrum of chemical and biological activities; for instance, free radical scavenging properties. Using popular plot of quercetin (y= 0.0184x + 0.0154, R^2 = 0.9956), the flavonoid contents of both *Sisymbrium irio* L and *Colchicum autumnale* L extracts were estimated. The result was confirmed that the methanol extract of *Sisymbrium irio* L contained the highest number of flavonoids (6 µg QE/mg of dry extract) in comparison with n-hexane (1.268 µg QE/mg of dry extract), and dichloromethane (1.4 µg QE/mg of dry extract).

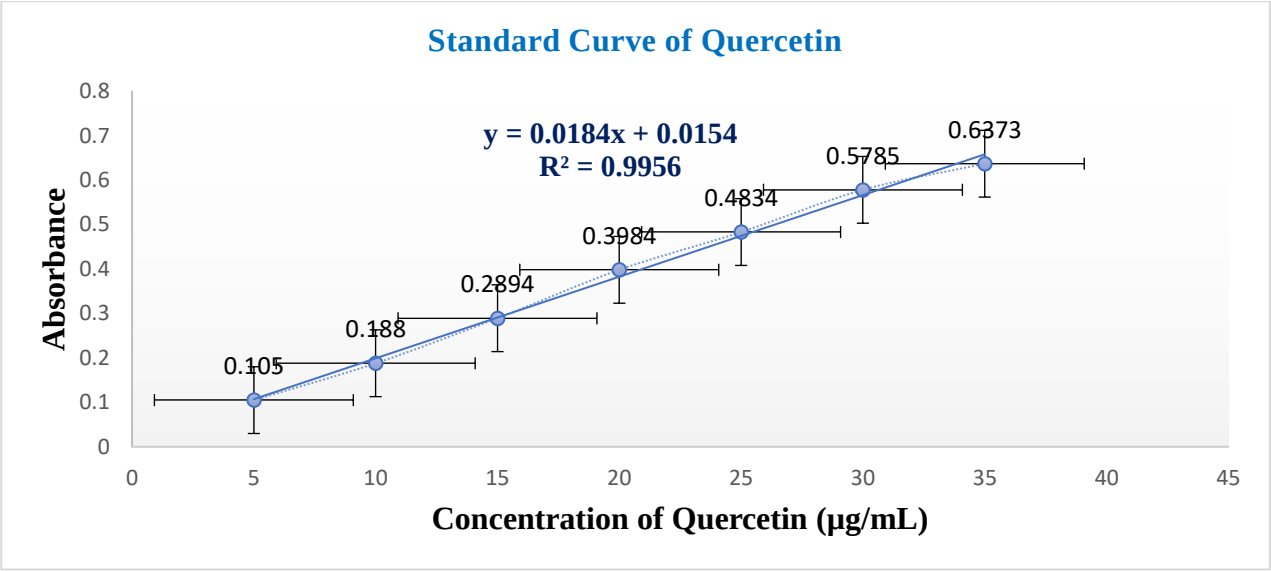


Figure 10: Standard curve of quercetin to determine the flavonoids contents.

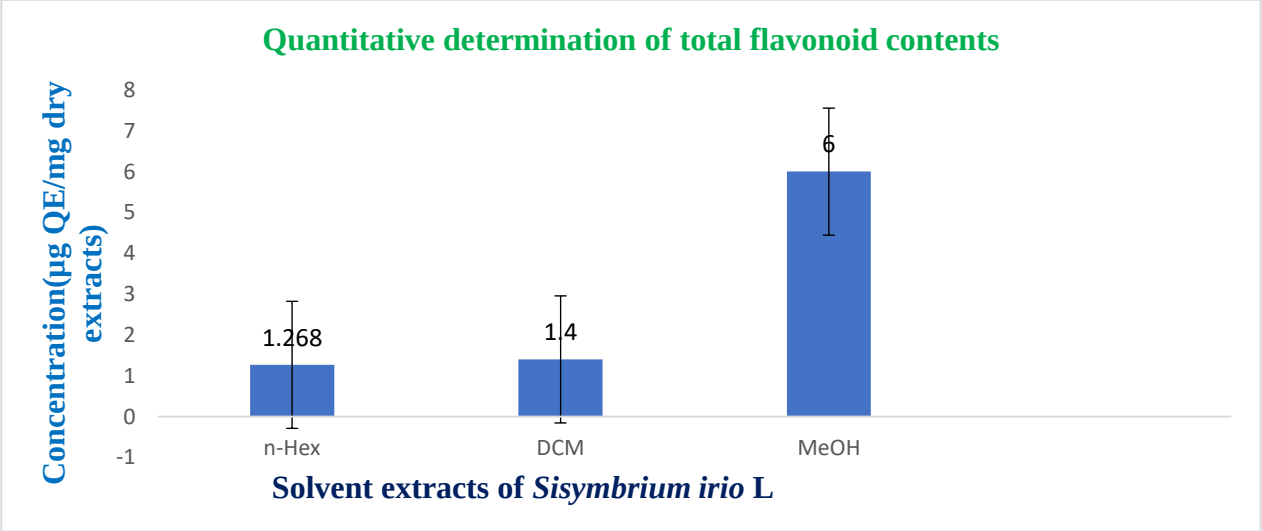


Figure 11: Total estimation of flavonoid contents in solvent extracts of *Sisymbrium irio* L.

Total flavonoids content *Sisymbrium irio* L varied from n-hexane to methanol extracts and are represented as µg QE/mg of dry extract. Methanol extract contained the highest number of flavonoid components like phenolic content, followed by dichloromethane extract and n-hexane extracts.

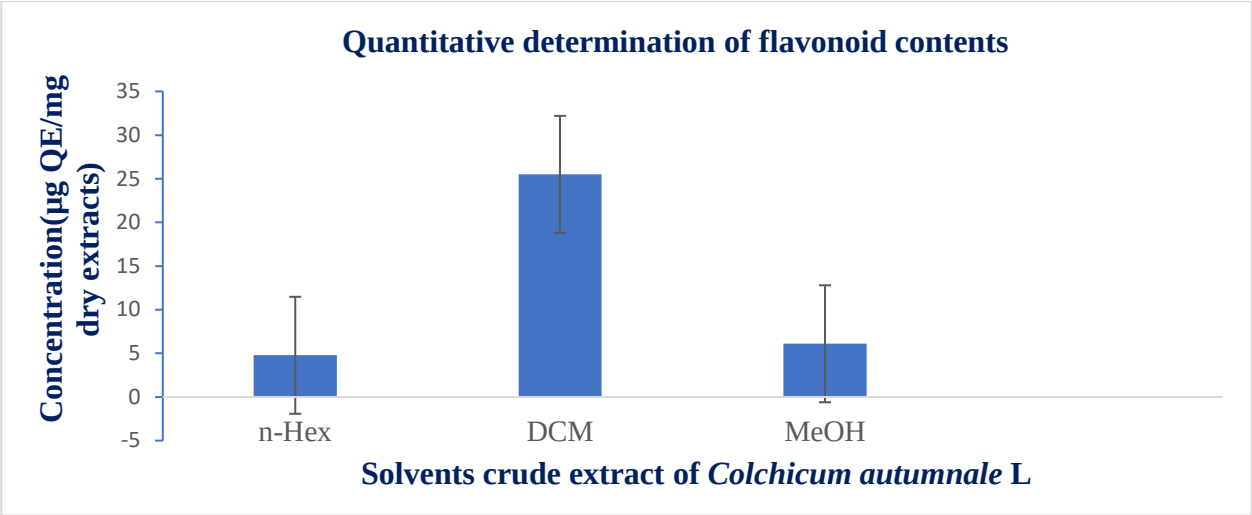


Figure 12: Total estimation of flavonoid contents in solvent extracts of *Colchicum autumnale* L.

Total flavonoids content *Colchicum autumnale* L varied from n-hexane to methanol extracts and are represented as µg QE/mg of dry extract. Dichloromethane extract contained the highest number of flavonoid components like phenolic content, followed by methanol and n-hexane extracts.

4.4. Antioxidant Potential of Plants Extracts

Extracts were assessed in vitro in order to test antioxidant activity by using DPPH free radical scavenging and ABTS free radical scavenging assay. In the current life, products of herbal materials have been extracted & isolated from diverse class of therapeutic plants for the development of new antioxidant tablets. These herbal products are specifically categorized into 3 primary compounds along with terpenoids, alkaloids & phenolic compounds (Bouvier et al., 2005). The research examination on the preparation of medicinal plant extracts could be useful for the isolation & bioassay of bioactive compounds (Harbourne et al., 2013). The extraction method is crucial in the antioxidant assay. The yield of the extract needs to rely on the polarity of the solvent used at some point in preparation (Jadid et al., 2017). Furthermore, the choice of the solvent and solubility of the natural products could also decide the yield. As an instance, lipophilic compounds together with some terpenoids and alkaloids have to use a non-polar solvent which includes n-hexane. A few alkaloids, flavonoids and terpenoid compounds have usually extracted the use of ethyl acetate. In the meantime,

polar solvents together with methanol, ethanol, and acetone are fundamental solvents used to extract a few flavanols, alkaloids, polyphenols and saponins (Monte et al., 2014)

4.4.1. DPPH Radical Scavenging Activity

The antioxidant activity of the extract was analyzed by using DPPH (diphenyl picrylhydrazyl). Diphenyl picrylhydrazyl is a stable free radical compound & absorbance was taken as oxidized form around 515-520nm (Bandoniene & Murkovic, 2002). It is a moderately fast & productive technique to assess the percentage of free radical scavenging activity. In this case, the changes in colour from purple to yellow showed a decline in the absorptance of DPPH radical. This is the confirmation of the antioxidant found in a mixture solution and its interaction with the free radicals (Kedare & Singh, 2011). The percentage of DPPH radical scavenging activity of n-hexane, dichloromethane & methanol extract of *Sisymbrium irio* L & *Colchicum autumnale* L extracts were depicted as: -

Table 9: Effect of solvent extracts of *Sisymbrium irio* L on DPPH assay

Concentration (µg/mL)	DPPH free radical scavenging activity (± SD)*			
	n-Hexane extract	Dichloromethane extract	Methanol extract	Ascorbic acid
50	18.84± 0.006	21.9±0.009	31.13±0.025	10.05±0.0112
100	22.72±0.0935	25.44±0.023	55.5±0.013	22.85±0.006
150	24.74±0.004	27.18±0.006	70±0.0018	36.7±0.0158
200	25.15±0.0025	28.09±0.0019	80.43±0.0017	47.4±0.022
250	26.56±0.013	31.07±0.01	84.89±0.002	68.3±0.023
300	30.76±0.02	31.94±0.0034	87±0.001	86.17±0.014

*All values were expressed as mean ± SD for three determinations.

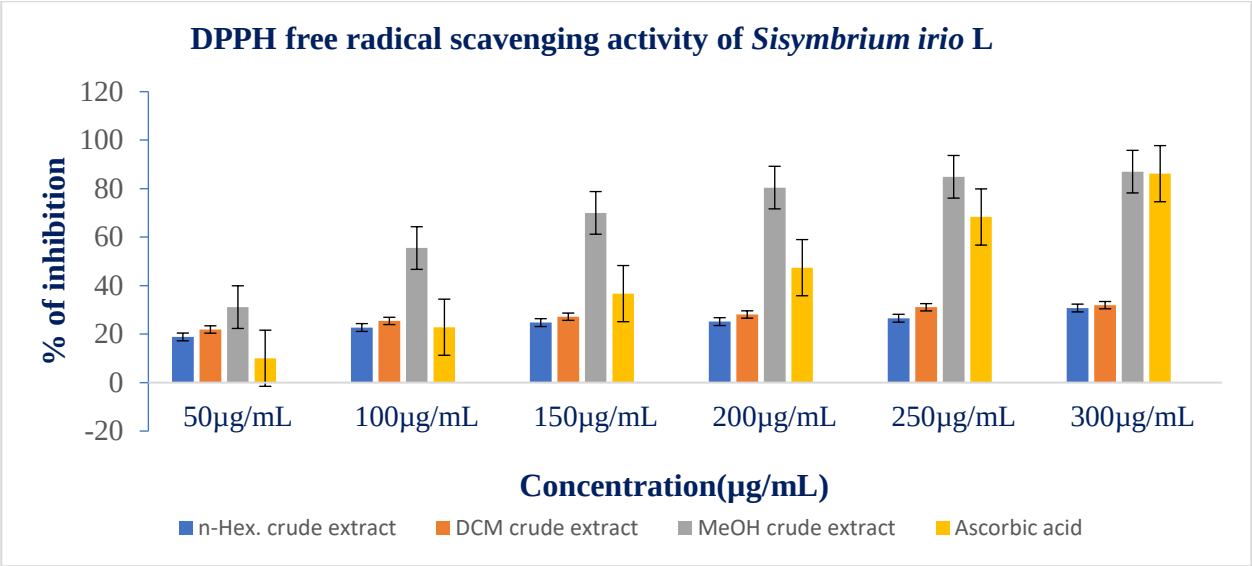


Figure 13: DPPH free radical scavenging assay of solvent extracts of *Sisymbrium irio* L

Table 10: Effect of solvent extracts of *Colchicum autumnale* L on DPPH assay

Concentration (µg/mL)	DPPH free radical scavenging activity (± SD)*			
	n-Hexane extract	Dichloromethane extract	Methanol extract	Ascorbic acid
50	6.86±0.004	26.24±0.0075	14.38±0.0078	10.05±0.0112
100	11.08±0.0058	29.64±0.001	26.325±0.02	22.85±0.006
150	23.18±0.0014	35.6±0.004	33.84±0.0065	36.7±0.0158
200	30.86±0.0009	42.996±0.0013	38.72±0.0046	47.4±0.022
250	37.53±0.004	52.94±0.0037	44.9±0.0185	68.3±0.023
300	40.00±0.064	61.23±0.01	47.785±0.009	86.17±0.014

*All values were expressed as mean ± SD for three determinations.

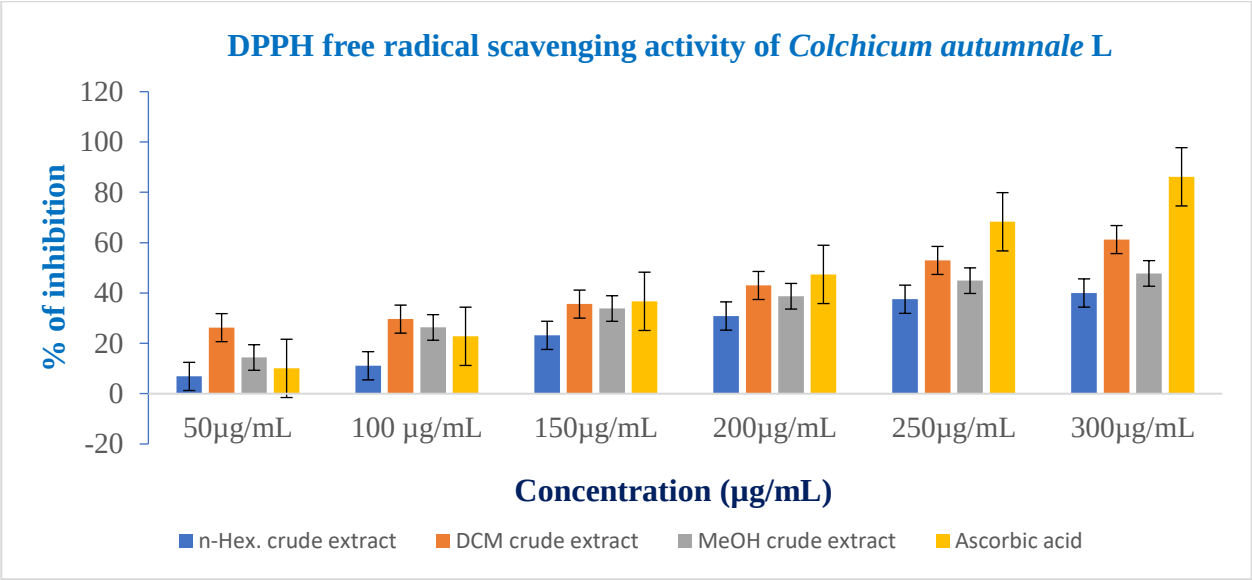


Figure 14: DPPH free radical scavenging activity of solvent extracts of *Colchicum autumnale* L.

Antioxidant analysis was completed in *Sisymbrium irio* L and *Colchicum autumnale* L extracts by utilizing DPPH strategy to examination of antioxidant properties of various organic solvents (n-hexane, dichloromethane, and methanol) extracts at different concentration from 50, 100, 150, 200, 250 and 300 µg/mL were utilized and the result of examination confirmed that the methanol extract of *Sisymbrium irio* L demonstrated the most notable percentage of free radical scavenging activity in comparison with n-hexane and dichloromethane extracts, while dichloromethane extract of *Colchicum autumnale* L showed the highest percentage of free radical scavenging activity in comparison to n-hexane and methanol extracts.

4.4.2. ABTS Radical Scavenging Assay

The function of phenolic compounds is one of the mechanisms of general antioxidant activities. This is predominantly due to their redox residences involved within the plant materials. Typically, the antioxidant mechanisms of the phenolic compounds neutralize lipid oxidation and stop the decomposition of hydroperoxides into free radicals (Deshmukh Krishi Vidyapeeth et al., 2017). This compound has an essential job in balancing out lipid oxidation & that related to an antioxidant activity (Mahdi-Pour et al., 2012).

The percentage of ABTS radical scavenging assay of n-hexane, dichloromethane & methanol extract of *Sisymbrium irio* L and *Colchicum autumnale* L extracts were depicted as:

Table 11: Effect of solvent extracts of *Sisymbrium irio* L on ABTS assay

Concentration (µg/mL)	% of free radical scavenging activity (± SD)*			
	n-Hexane extract	Dichloromethane extract	Methanol extract	Standard (Trolox)
50	1.75± 0.0043	3.796±0.004	18±0.0137	4.65±0.007
100	2.9±0.003	7.75±0.00485	37.37±0.00848	12.2±0.0074
150	8.76±0.002	9.4±0.0078	51.89±0.011	19.15±0.007
200	11.825±0.002	12.4±0.0065	57.95±0.00696	26.7±0.0074
250	14.74±0.00346	15.197±0.0004	70.5±0.0157	34.03±0.008
300	19.7±0.0055	17.8978±0.0015	81.15±0.0023	44.68±0.012

*All values were expressed as mean ± SD for three determinations.

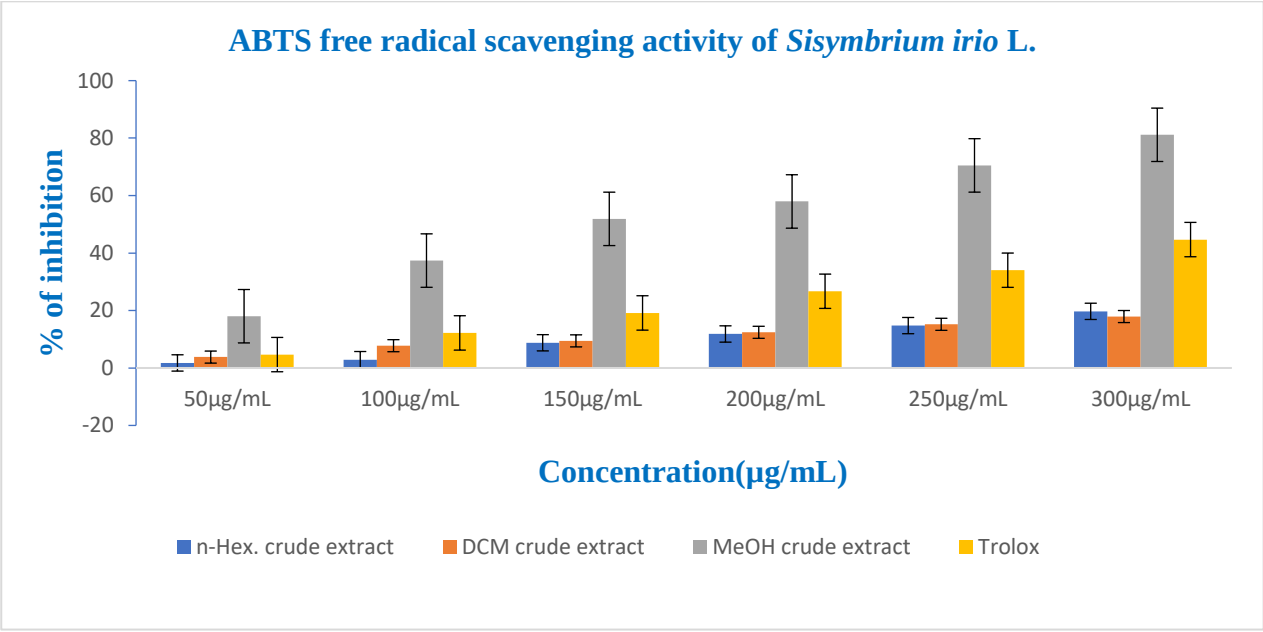


Figure 15: Percentage of inhibition of ABTS free radical scavenging activity solvent extracts of *Sisymbrium irio* L

Table 12: Effect of solvent extracts of *Colchicum autumnale* L on ABTS assay

Concentration (µg/mL)	% of free radical scavenging activity (± SD)*			
	n-Hexane extract	Dichloromethane extract	Methanol extract	Trolox (standard)
50	7.39±0.0025	20.87±0.0137	18.74±0.029	4.65±0.007
100	11.09±0.0115	45.33±0.01245	24.4±0.0036	12.2±0.0074
150	13.197±0.0015	49.13±0.03	31.48±0.0024	19.15±0.007
200	15.98±0.0185	65.94±0.015	38.05±0.0289	26.7±0.0074
250	22.14±0.04	77.2±0.0426	51.52±0.01587	34.03±0.008
300	25.7±0.0138	82.6±0.0168	53.22±0.016	44.68±0.012

*All values were expressed as mean ± SD for three determinations.

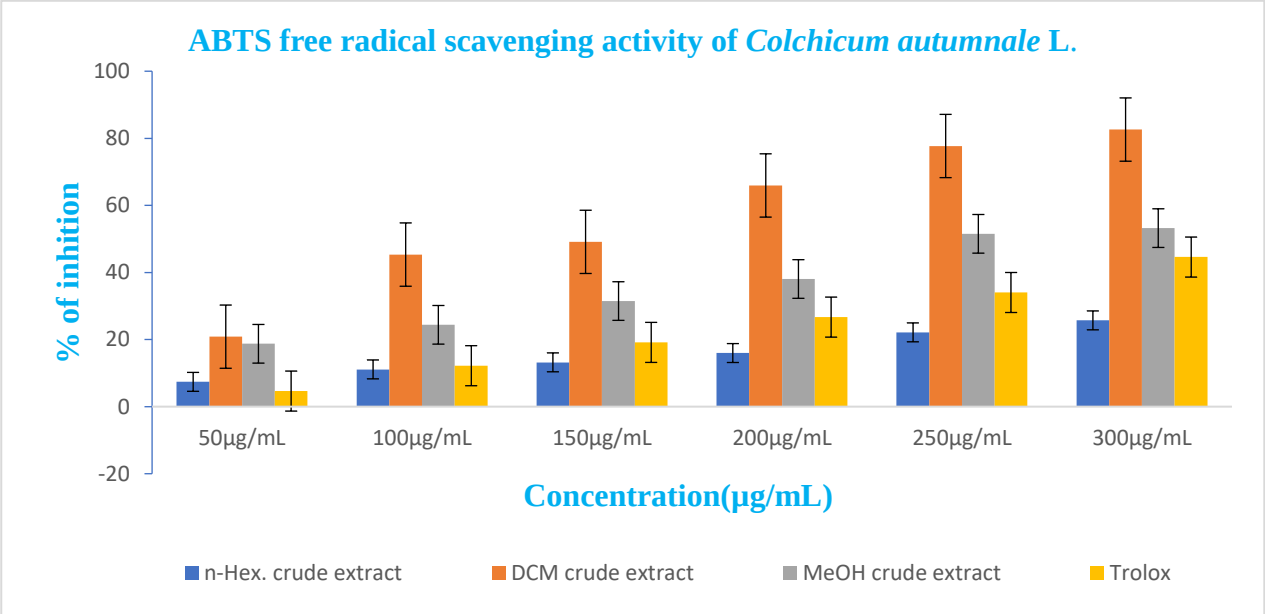


Figure 16: ABTS free radical scavenging activity of solvent extracts of *Colchicum autumnale* L.

The phenolic compounds may have direct role in the antioxidant action for scavenging of free radicals. The polyphenolic compounds recommended that they may display inhibitory impacts on mutagenesis & carcinogenesis in humans (Tanaka *et al*., 1998). The antioxidant phytocompound flavonoid can function as foragers of free radicals by a quickly giving of hydrogen radicals. Huge numbers of the pharmacological impacts of flavonoids are identified with their interaction with several catalysts (Van Acker et al., 1996) and to their antioxidant's activity, which can be because

of their capacity to search free radicals (Bors & Saran, 1987 ; Mira et al., 1999), to search metal ions (Van Acker et al., 1996), and to have synergistic impacts with different cell reinforcements and other antioxidants. Thus, the antioxidant examination of *Sisymbrium irio* L and *Colchicum autumnale* L confirmed the existence of considerable levels of therapeutically important secondary metabolites in the extracts for instance, phenols and flavonoids.

4.5. Antibacterial Activity

Antibacterial activity of n-hexane, dichloromethane & methanol extracts were evaluated in vitro against three bacterial species such as *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Antibacterial activity of *Sisymbrium irio* L, and *Colchicum autumnale* L has been assessed in vitro against bacterial species and a large portion of the tested plant extracts demonstrated some dimension of antibacterial action.

Table 13: Zone of inhibition (mm) of antibacterial activity of solvent extracts of *Sisymbrium irio* L

Sample (plants extracts, positive & negative control)	Concentration (µg/mL)	Zone of inhibition (mm)		
		Bacillus subtilis	*Escherichia coli*	*Pseudomonas aeruginosa*
n-Hexane extract	50	6.5 ± 0.15	-	6.5 ±0.45
	100	7 ±0.23	-	7.5 ±0.08
	150	7.75 ±0.34	-	8 ±0.05
	200	8.75 ±0.43	-	9 ±0.02
Dichloromethane	50	6.25 ±0.13	-	8 ±0.45
extract	100	6.5 ±0.19	8 ±0.27	8.25 ±0.07
	150	8.75 ±0.02	9 ±0.13	9 ±0.02
	200	9.25 ±0.12	9.5 ±0.34	12 ±0.06
Methanol extract	50	6.5 ±0.25	-	7 ±0.01
	100	7 ±0.27	-	8.5 ±0.25

	150	8 ±0.03	-	11 ±0.11
	200	8.5 ±0.16	-	12 ±0.33
Ampicillin (+ve control)	50µg/disc	15	15	40
Methanol (-ve control)	200µL	-		

+ = inhibition - = no inhibition

Table 14: Zone of inhibition (mm) of antibacterial activity of solvent extracts of *Colchicum autumnale L*

Sample (plant extracts, positive & negative control)	Concentration (µg/mL)	Zone of inhibition (mm)		
		Bacillus subtilis	*Escherichia coli*	*Pseudomonas aeruginosa*
n-Hexane extract	50	6.5 ± 0.13	7.5 ±0.03	7 ±0.18
	100	7.5 ±0.36	8 ±0.17	7 ±0.35
	150	7.5 ±0.46	9 ±0.32	8 ±0.42
	200	9.5 ±0.25	10.5 ±0.54	9 ±0.45
Dichloromethane extract	50	7.25 ±0.05	-	-
	100	8.25 ±0.23	-	-
	150	9.25 ±0.13	-	-
	200	10.5 ±0.03	-	-
Methanol extract	50	7 ±0.08	-	7.5 ±0.21
	100	8 ±0.26	-	8.5 ±0.07
	150	8.5 ±0.49	-	9.5 ±0.30
	200	10 ±0.70	-	12 ±0.13
Ampicillin (+ve control)	50µg/disc	15	15	40

Methanol (-ve control)	200µL	-

+ = inhibition - = no inhibition

Antibacterial activity of two medicinal plants has been assessed in vitro against three bacterial species and various extracts of plants showed the inhibition against the bacterial strains while some did not show any activity. However, negative results do not indicate the absence of bioactive constituents, nor do they show that the extracts are inactive. But may be level of dose employed for antimicrobial test was not optimal (Sahafi et al., 2018). The obtained outcomes demonstrated n-Hexane crude extract of *Sisymbrium irio* L revealed a wide antibacterial range against *Bacillus subtilis* and *Pseudomonas aeruginosa* bacterial strains, while n-Hexane crude extract of *Colchicum autumnale* L showed antibacterial activity against all tested bacterial strains; for example, *Bacillus subtilis*, *Escherichia coli,* and *Pseudomonas aeruginosa*. The dichloromethane extract of *Sisymbrium irio* L indicated antibacterial activity against the three tested bacterial strains, while dichloromethane extract of *Colchicum autumnale* L demonstrated inhibition zone against *Bacillus subtilis*, and didn't demonstrate any inhibition zone on *Escherichia coli* and *Pseudomonas aeruginosa*.

The third solvent (methanol) crude extract of both therapeutic plants demonstrated the zone of inhibition on *Bacillus subtilis* and *Pseudomonas aeruginosa*; however, it didn't demonstrate any inhibition zone on *Escherichia coli*. The above results were based on the concentration dependence of the crude extracts. The qualitative and quantitative phytochemicals examination of the crude extracts demonstrated the presence of various kinds of bioactive metabolites and these metabolites has a significant role in prevention of various sorts of microbial illnesses. The phytochemical flavonoids and its subordinates have for quite some time been perceived to work as antimicrobial protection compounds (Dave & Ledwani, 2012). Flavonoids have the capacity to form complex with extracellular and solvent proteins; and also to form complex with bacterial cell wall (R. N. S. Yadav & Agarwala, 2011). Studies have demonstrated that they have antibacterial action against a wide

scope of small-scale organisms (Ghasemzadeh & Ghasemzadeh, 2011). Phenolic compounds have excellent cancer preventive properties (Narayana & Krishna, 2001). They have organic and pharmacological properties; and particularly antimicrobial activity (Raja et al., 2011), antiviral, mitigating and cytotoxic activity (Mungole et al., 2010). Steroids are known to be imperative of their cardiotonic exercises, and also have insecticidal and antimicrobial properties (Majgaine & Verma, 2017). Palmitic acid (n-Hexadecanoic acid) is saturated fatty acid and it showed antimicrobial activity (Chandrasekaran et al., 2011).

4.6. MTT Assay and Antiviral Activity of Solvent Extracts of *Sisymbrium irio* L *Invitro*

The dichloromethane, ethanolic, methanolic, and water extracts of the *Sisymbrium irio* L exhibited cell survivability at varying concentrations. During *invitro* examination of antiviral activity of the *Sisymbrium irio* L extracts against dengue viruses at a maximum nontoxic dose (MNTD) were checked; and with the exception of methanolic extract, the other solvent extracts showed antiviral activity to against dengue viruses. According to Weber, the nontoxic dose was used for antiviral screening (Weber et al., 1991). In the preliminary screening study for anti dengue agent, ethanolic and water extracts of *Sisymbrium irio* L were found to have high potential of being an anti dengue agent. This anti dengue action may be owing to the existence of flavonoid compounds or other compounds such as polyphenols and terpenes that were extractable by ethanol (Chao & Lin, 2010). Numerous flavonoid compounds have been known to possess antiviral properties (Kaul et al., 1985).

4.6.1. MTT Assay- SiDCM (*Sisymbrium irio* Dichloromethane Extract)

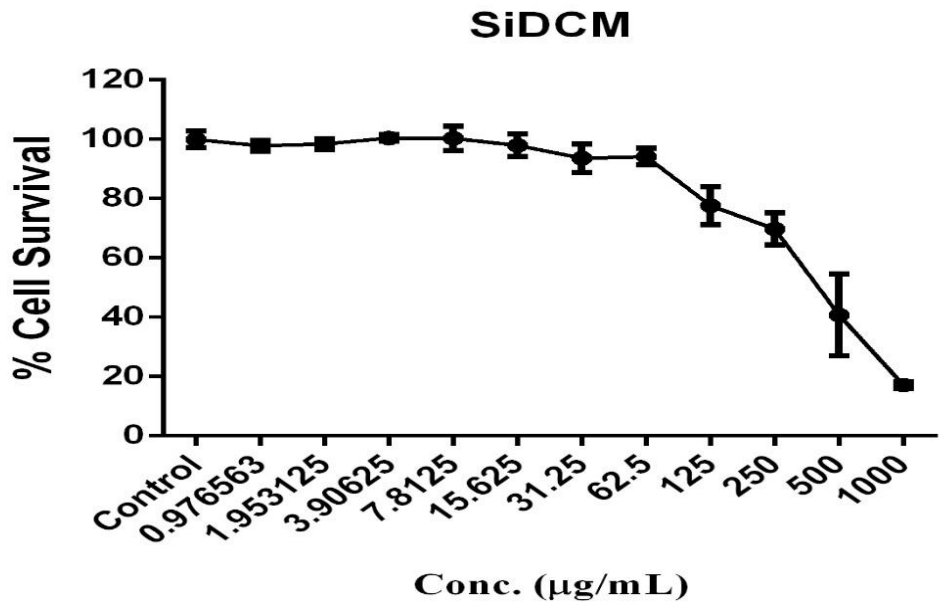


Figure 17: Dichloromethane extract is showing ≥ 80 % cell survival at 62.5 µg/mL

4.6.2. MTT based DENV CPE - Inhibition Assays

4.6.2.1. Pre-treatment Assay – SiDCM (*Sisymbrium irio* Dichloromethane Extract)

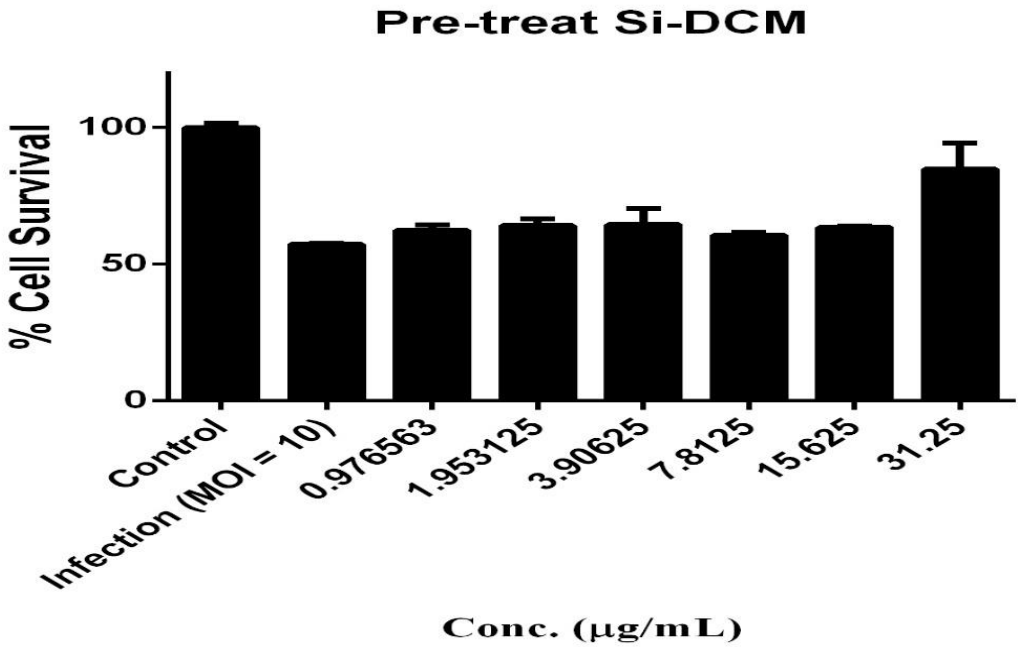


Figure 18: Pre-treatment assay, Dichloromethane extract showing an antiviral effect at the concentration of 31.25 µg/mL.

4.6.2.2. Co-Incubation Assay – SiDCM (*Sisymbrium irio* Dichloromethane Extract)

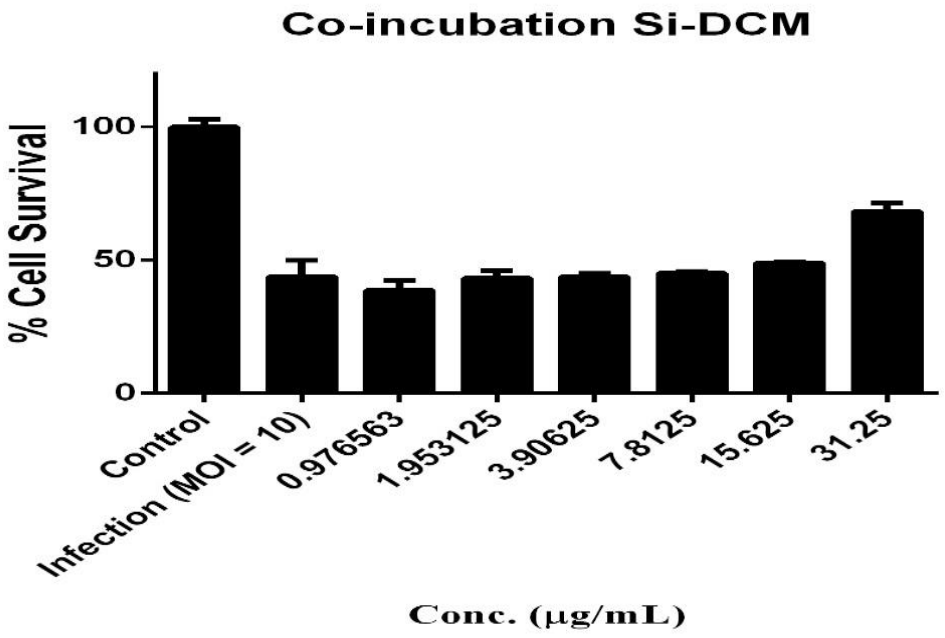


Figure 19: Co-incubation assay, Dichloromethane extract showing an antiviral effect at the concentration of 31.25 µg/mL.

4.6.2.3. Post-treatment Assay – SiDCM (*Sisymbrium irio* Dichloromethane Extract)

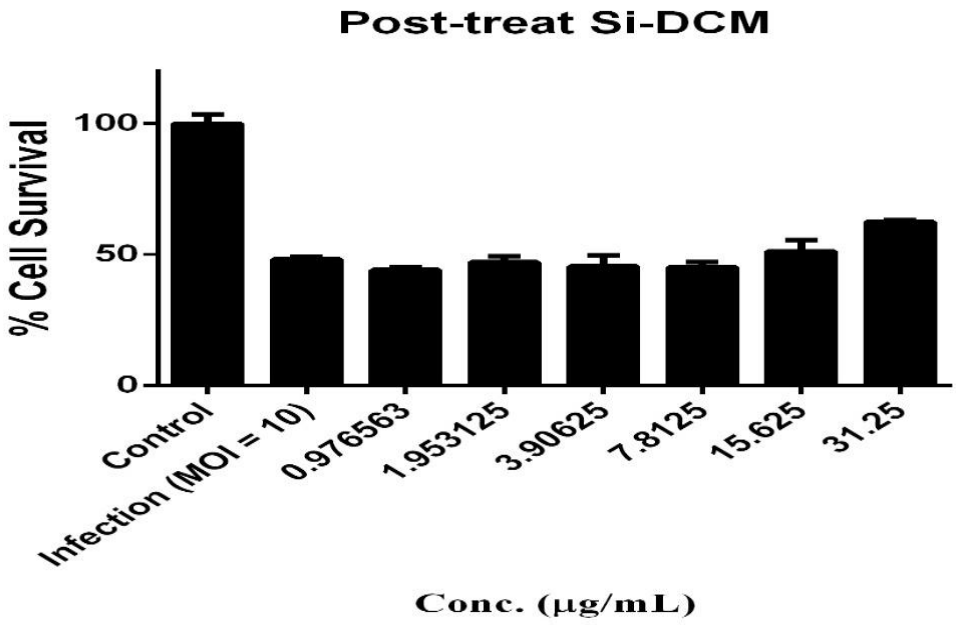


Figure 20: Post-treatment assay, dichloromethane extract showing an antiviral effect at the concentration of 31.25 µg/mL.

4.6.3. MTT Assay - SiEtOH (*Sisymbrium irio* Ethanol Extract)

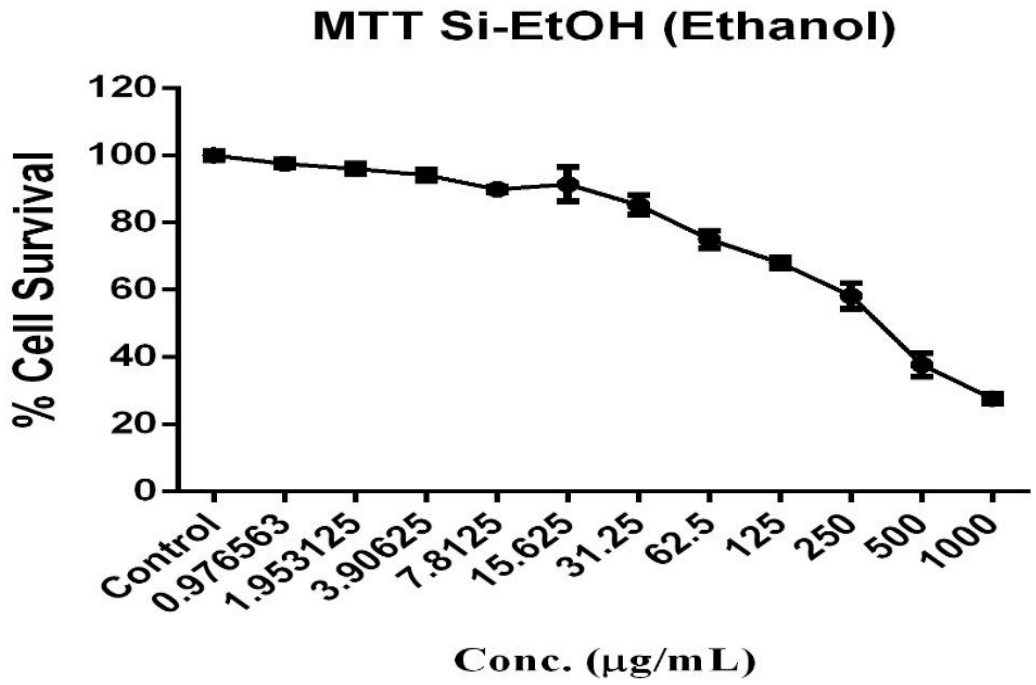


Figure 21: Ethanol extract is showing ≥ 80% cell survival at 31.25 µg/mL

4.6.3.1. Pre-treatment Assay – SiEtOH (*Sisymbrium irio* Ethanol Extract)

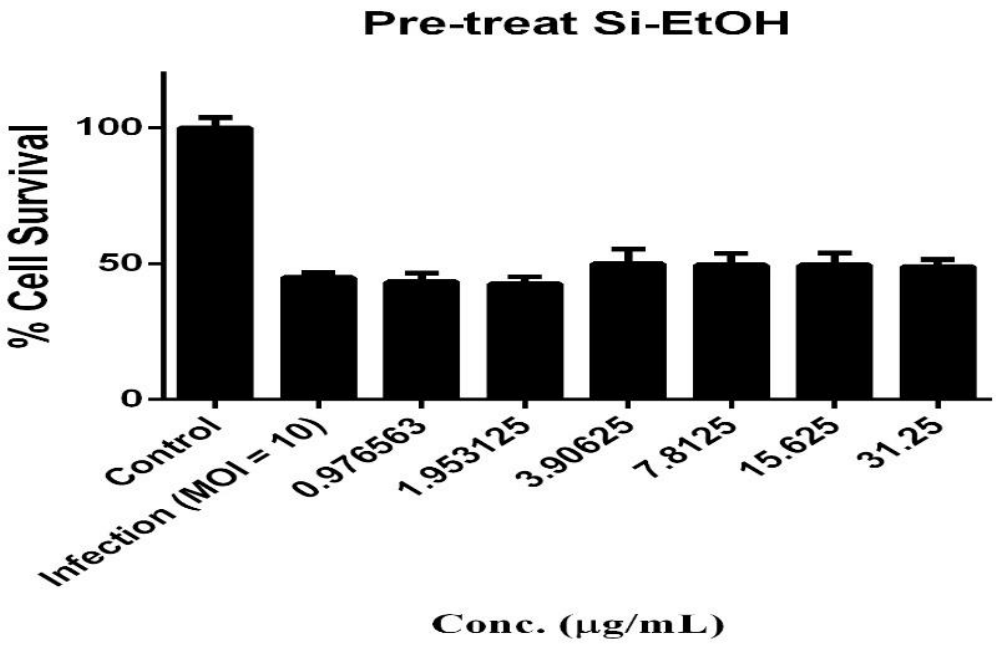


Figure 22: Antiviral Assay

4.6.3.2. Co-Incubation Assay - SiEtOH (*Sisymbrium irio* Ethanol Extract)

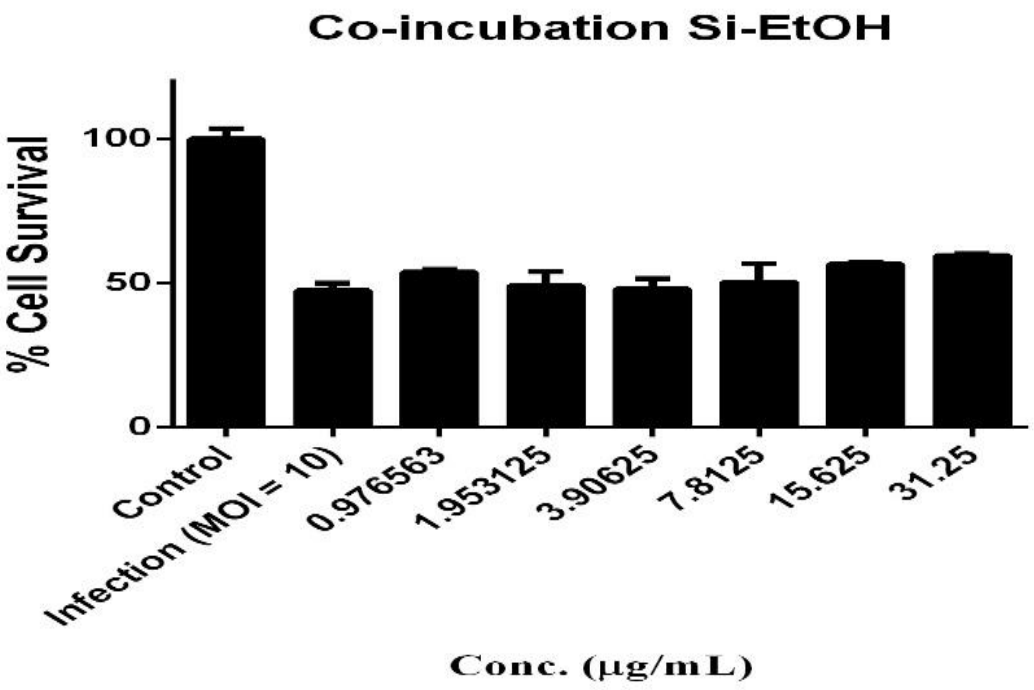


Figure 23: Antiviral Assay

4.6.3.3. Post-treatment Assay – SiEtOH (*Sisymbrium irio* Ethanol Extract)

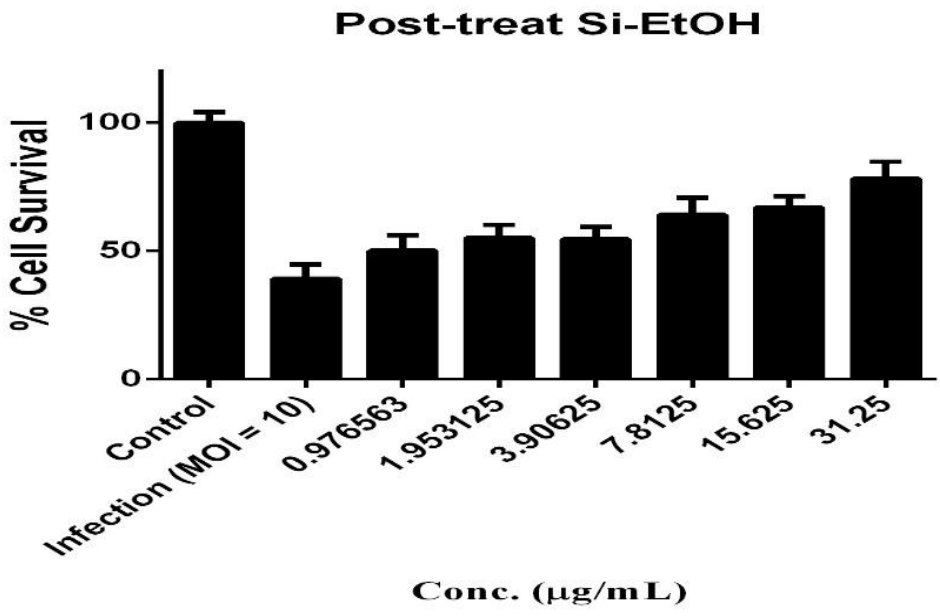


Figure 24: Post-treatment assay, Ethanol extract is showing significant antiviral effect

4.6.4. MTT Assay - SiMeOH (*Sisymbrium irio* Methanol Extract)

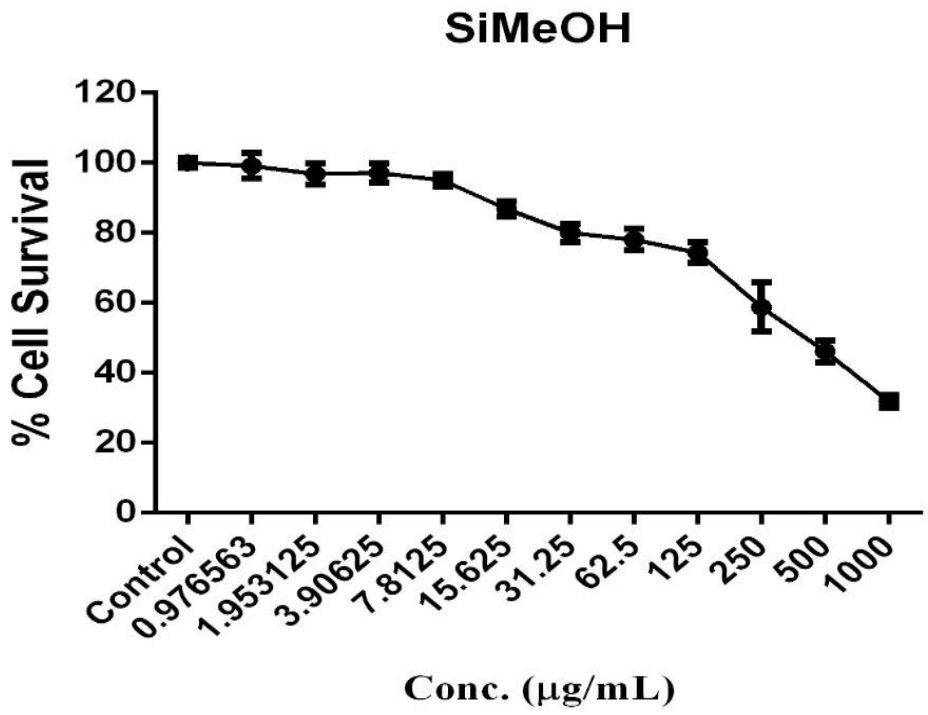


Figure 25: Methanol extract is showing ≥ 80 % cell survival at 15.625 µg/mL

4.6.4.1. Pre-treatment Assay – SiMeOH (*Sisymbrium irio* Methanol Extract)

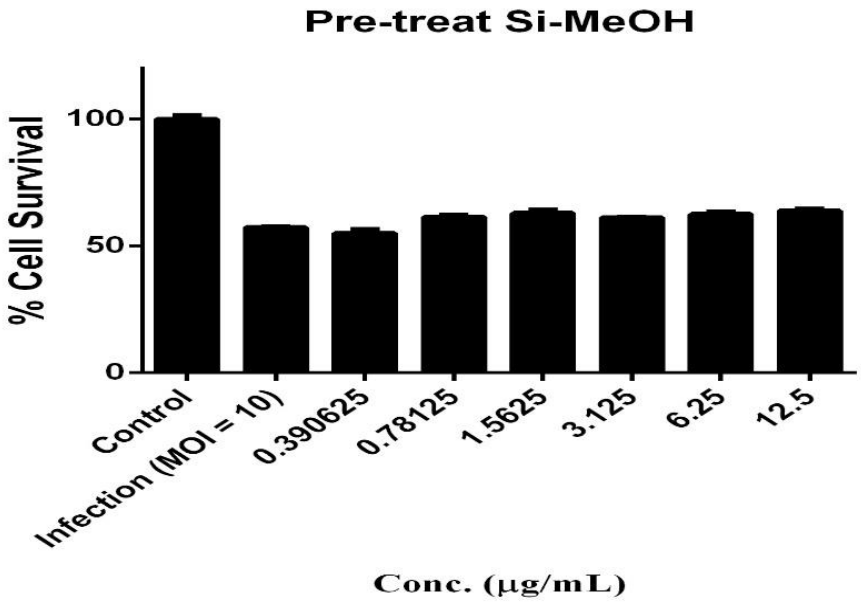


Figure 26: Antiviral assay

4.6.4.2. Co-Incubation Assay – SiMeOH (*Sisymbrium irio* MeOH extract)

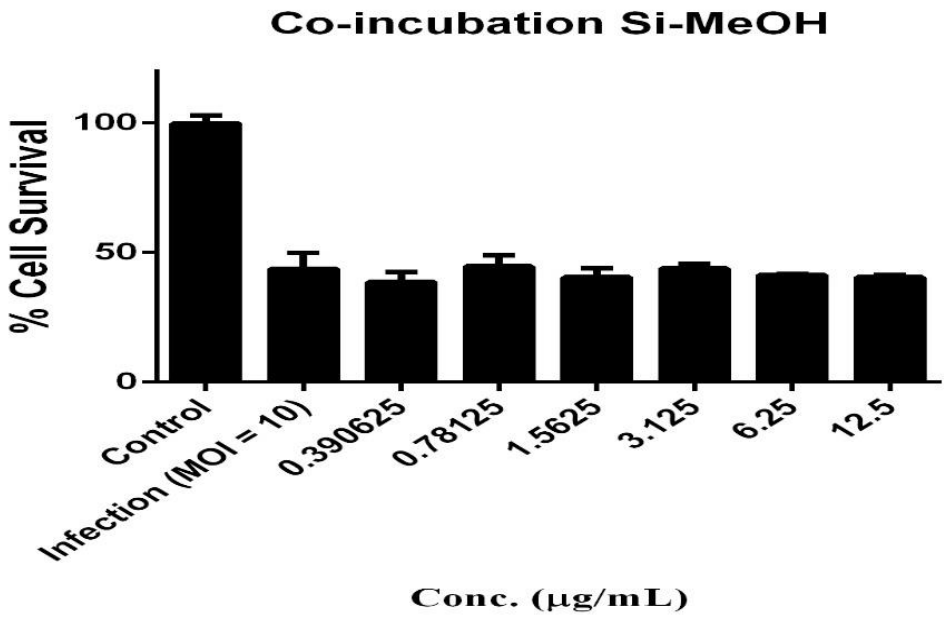


Figure 27: Antiviral Assay

4.6.4.3. Post-treatment Assay - MeOH (*Sisymbrium irio* MeOH extract)

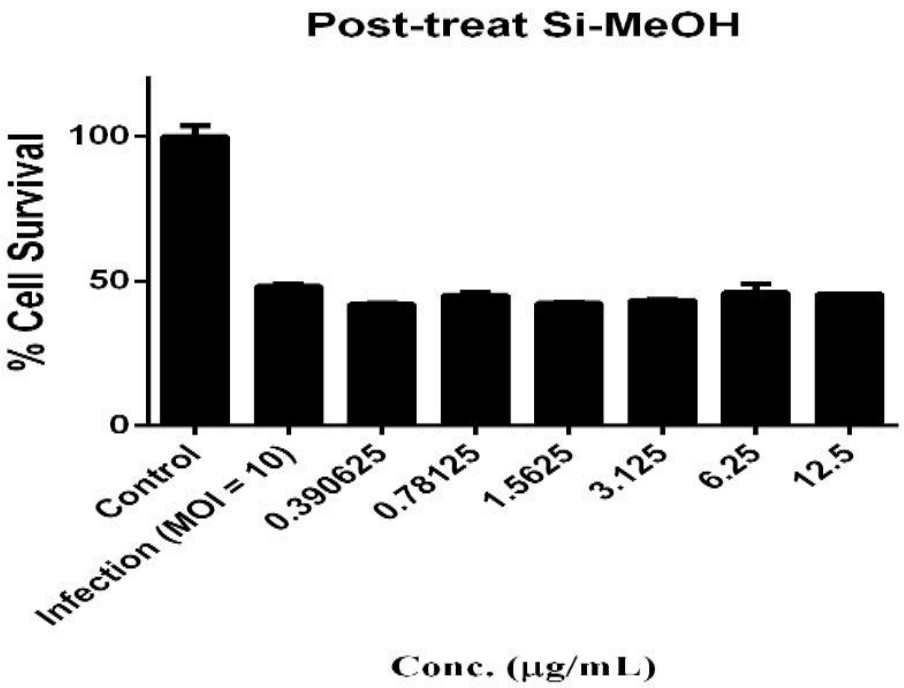


Figure 28: Antiviral Assay

4.6.5. MTT Assay- SiW (*Sisymbrium irio* Water Extract)

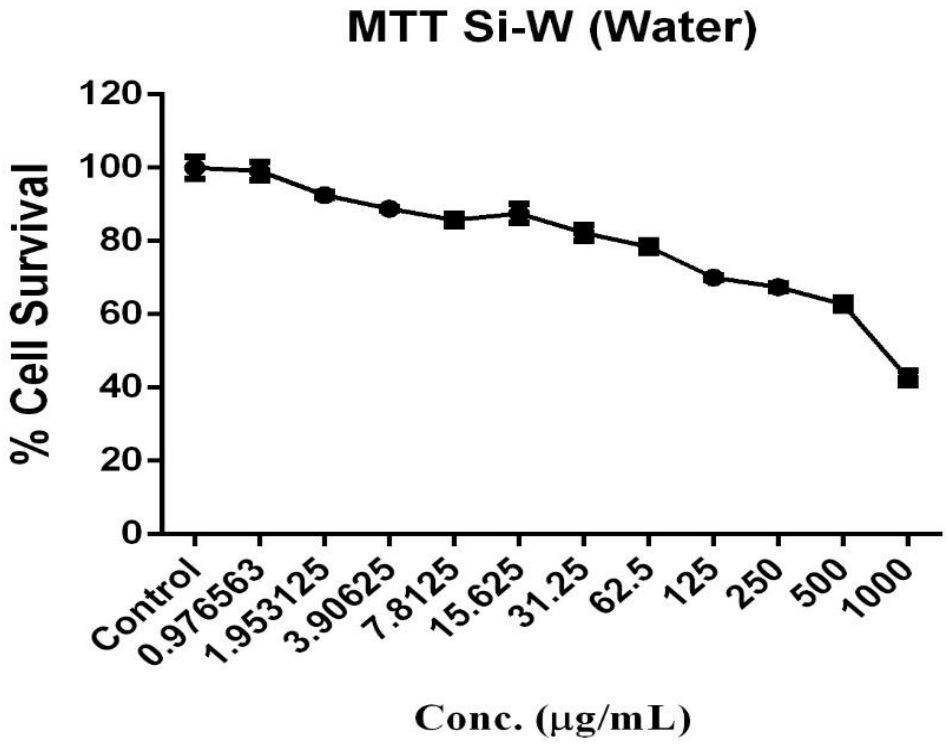


Figure 29: Water extract showing ≥ 80 % cell survival at 31.25 µg/mL

4.6.5.1. Pre-treatment Assay – SiW (*Sisymbrium irio* Water Extract)

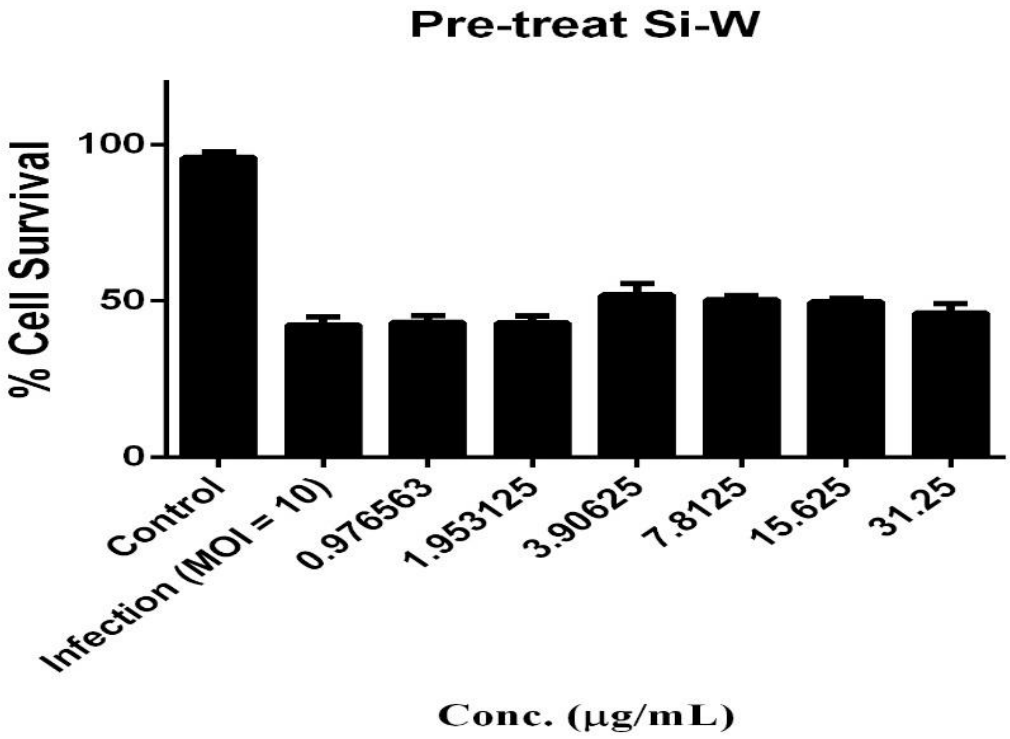


Figure 30: Antiviral Assay

4.6.5.2. Co-Incubation Assay – SiW (*Sisymbrium irio* water extract)

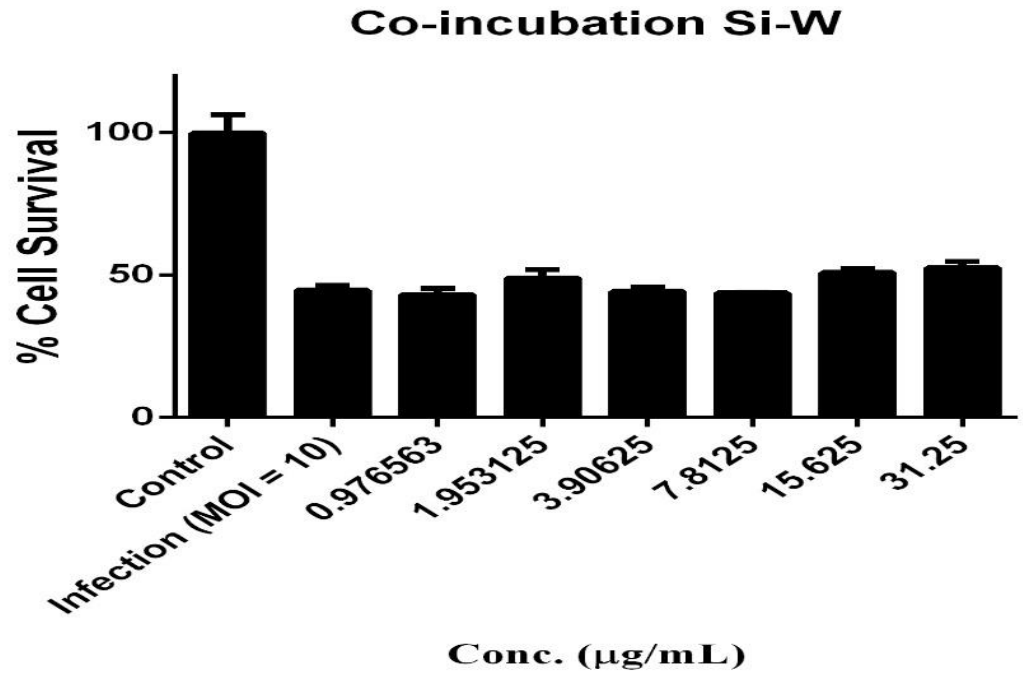


Figure 31: Antiviral Assay

4.6.5.3. Post-treatment Assay – SiW (*Sisymbrium irio* water extract)

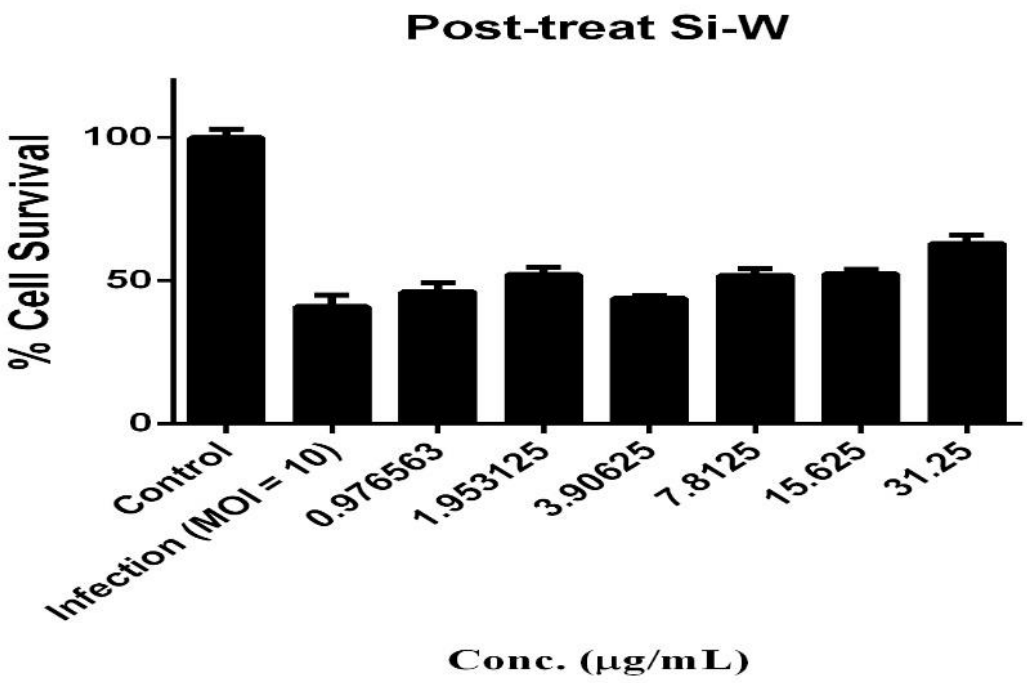


Figure 32: Post-treatment assay, Water extract is showing an antiviral effect

From the above observation and results, it is clearly seen that dengue-infected cells showed CPE and also the structural changes of vero cell because of viral infection. The percentage of inhibition on dengue by the therapeutic *Sisymbrium irio* L extracts showed the antiviral inhibitory effects with the exception of methanolic extract of the *Sisymbrium irio* L. The phytochemicals and antioxidant activity of *Sisymbrium irio* seeds showed the presence of major fatty acids such as: Linolenic acid, Linoleic acid, Oleic acid, cis-11-Eicosanoic acid, Erucic acid, Palmitic acid, phenols, flavonoids, saponins and terpenoids. The other research examination confirmed the content of *Jatropha curcas* as a plant which protects the skin against *Aedes aegypti*. At the same time, it has been found that monounsaturated fatty acid, saturated fatty acid, and octadecanoic acid act as biting deterrent against *Aedes aegypti* (Cantrell et al., 2011). This result showed that there could be a relation between the biting deterrent activity with the repellent activity. Another research examination confirmed the potency of Carica (*Carica pubescens* Lenne and K. Koch) seed extract as repellent against mosquito vector of dengue (*Aedes aegypti* Linn) (Anggraeni & Laela, 2020). In anti-dengue examination the

solvent extracts of *Sisymbrium irio* L were found to have high potential to be an anti-dengue agent. This is might be due to the presence of flavonoids or other phytochemicals, oleic and palmitic fatty acid compounds in the plant extracts.

4.7. Elemental Analysis of *Sisymbrium irio* L and *Colchicum autumnale* L (ICP-MS)

Elemental analysis of *Sisymbrium irio* L and *Colchicum autumnale* L showed the presence of different types of minerals in different concentration.

Table 15: Mineral content of *Sisymbrium irio* L

S. No.	Elements	Concentration (ppm)
1	Mg	2940.1
2	Ca	5844.7
3	Sr	18.2
4	Ti	7.7
5	Cr	2.6
6	Mn	41.8
7	Co	bdl
8	Cu	5.5
9	Ni	bdl
10	Zn	40.7
11	B	1.9
12	Al	315.4
13	Si	101.6
14	Ba	3.7
15	Fe	672.8
16	Mo	bdl
17	Ag	bdl
18	P	6393.4

19	Na	54.7
20	K	8031.9
21	Pb	2.9
22	Bi	bdl
23	Cd	bdl
24	V	bdl

bdl: below detection limit

Table 16: Mineral content of *Colchicum autumnale* L

S.No.	Elements	Sample Concentration
1	Li	bdl
2	Be	bdl
3	B	5.59
4	Na	200.49
5	Mg	638.15
6	Al	76.00
7	P	1164.61
8	K	7368.93
9	Ca	1057.87
10	V	bdl
11	Cr	0.82
12	Mn	3.60
13	Fe	67.77
14	Co	0.04
15	Ni	0.57
16	Cu	6.12

17	Zn	8.25
18	As	0.10
19	Se	0.06
20	Mo	0.07
21	Cd	0.02
22	Sn	bdl
23	Sb	bdl
24	Ba	2.15
25	Hg	bdl
26	Pb	0.34

Bdl: below detection limit

The minerals which were analyzed from *Sisymbrium irio* L and *Colchicum autumnal* L are very useful for the health of human beings. The elements for example, calcium, phosphorus and magnesium may be helpful in the buildings of our bones; potassium and sodium support in the preservation of normal blood pressure. The metal iron is the centre of haemoglobin and also is part of myoglobin. During breakdown of carbohydrates, fats and proteins the elements copper, zinc and manganese play important roles. During the metabolic processes of bone, the elements such as zinc, manganese and copper serve as cofactors for specific enzymes (Saltman & Strause, 1993).

4.8. GC-MS: Fatty Acid profile of *Sisymbrium irio* L cold pressed seed oil

The major fatty acid composition of *Sisymbrium irio* L seed oil was found to be: Linolenic acid (36.29%), Linoleic acid (17.99%), Oleic acid (12.58%), cis-11-eicosenoic acid (9.2%), Erucic acid (9.19%), Palmitic acid (6.66%), Stearic acid (2.2%), Arachidic acid (1.75%) and cis-11, 14 Eicosenoic acid (0.95%). Linoleic acid (omega 6) is one of the fundamental fatty acids that are not created in the human body and should be given to the body from outside and as such *Sisymbrium irio* L seed oil is a good source of linoleic acid. The research examination conducted on the function of

dietary polyunsaturated fatty acid in the nervous system, prostaglandins leukotrienes and essential fatty acid: linoleic acid (omega 6) is well done universally and so is a recognized nutrient for the health of human body (Bourre et al., 1993).

4.9. Gas Chromatography-Mass Spectrometric Analysis (GC-MS Analysis)

In the current scenario, the investigation of the natural compounds from plants & their activity has increased. The combination of a best separation technique (Gas chromatography) with the best recognizable proof method (Mass-spectrometry) makes Gas chromatography-Mass spectrometry a perfect skill for subjective investigation for volatile & semi-volatile bioactive compounds (Grover & Patni, 2013).

The chemical composition of *Sisymbrium irio* L extracts was analysed by GC-MS. The chromatogra m results confirmed the presence of main constituents of the crude extracts of *Sisymbrium irio* L were: 2, 4-Heptadienal, (E, E)-, 2-Decenal, (E)-, 2, 4-Decadienal, (E, E)-, n-Hexadecanoic acid, Ethanol, 2-(9, 12-octadecadienyloxy)-, (Z, Z)-, 9-Eicosenoic acid, (Z)-, Tris (2, 4-di-tert-butylphenyl) phosphate, gamma-Tocopherol, gamma-sitosterol, beta-sitosterol, 3', 5'-Dimethoxyacetophenone, E-14- Hexadecanoic acid, methyl ester, Hexadecenal, Hexadecanoic acid, ethyl ester, 9, 12, 15-Octadecatrienoic acid, methyl ester, Methyl stearate, Cis-11-Eicosenoic acid methyl ester, Docosenoic acid methyl ester, (Z)-, 1-butene, 4-isothiocyanto, Cyano-3, 4-epithiobutane, 9, 12-octadecadienoic acid, methyl ester, 8, 11, 14-Docosatrienoic acid, methyl ester, 9, 12, 15-octatrienoic acid, methyl ester, (Z, Z, Z)-, 9-octadecenoic acid (Z)-, (E)-9-octadecenoic acid ethyl ester, Cis-13-Eicosanoic acid, 3-Butoxy-1, 1, 1, 7, 7, 7-hexamethyl-3, 5, 5-tris (trimethylsiloy) tetrasixone, 9, 12, 15-octadecatrienal, 3-methoxy phenol, 2, 4-bis (1, 1-dimethyethyl) phenol, 1-octadecene, Methyl stearate, bis(2-ethyl hexyl) maleate, Linoleic acid ethyl ester, 7- Tetradecenal, Methyl-18-methylnonadecanoate, Nonacos-1-ene, Decanedioic acid, bis (2-ethylhexyl) ester, alpha, -Tocopheryl acetate, 1- phenyl Ethanone, 3-methoxy phenol, 2, 3-dihydro-6-methyl, 4H-pyran-4-one, 5-hydroxymethyl furfural, 2, 4-ditert-butyl phenol, 4-methoxy-7-methylindan-1-one, Benzene propanoic acid, 3, 5-bis(1, 1-dimethylethyl)-4-hydroxy-, methyl ester. Major chemical constituents

of *Colchicum autumnale* L: 3', 5'-Dimethoxyacetophenone, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid, methyl ester, 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-, 9, 12-Octadecadienoic acid (Z, Z)-, Hexadecanoic acid, methyl ester, 8, 11, 14-Docosatrienoic acid, methyl ester, Tris (2, 4-di-tert-butylphenyl) phosphate, beta. –Sitosterol, beta. -Sitosterol acetate, 3-Methoxy Phenol, 2, 4-bis (1, 1-dimethyl ethyl)-Phenol, Hexadecanoic acid, methyl ester, Hexadecanoic acid, ethyl ester, 9, 12-Octadecadienoic acid, methyl ester, gamma. –Tocopherol, 13-Docosenoic acid, methyl ester, (Z)-.

Table 17: Phytocompounds present in the n-hexane extract of *Sisymbrium irio* L

Peak No	Name of the compounds	R. Time	Area	Area%	Formula	Mol wt
1	2-Heptenal, (E)-	6.323	7285339	0.53	$C_7H_{12}O$	112
2	2,4-Heptadienal, (E, E)-	7.009	13980149	1.02	$C_7H_{10}O$	110
3	2,4-Heptadienal, (E, E)-	7.247	1856818	0.14	$C_7H_{10}O$	110
4	2-Octenal	8.005	916181	0.07	$C_8H_{14}O$	126
5	1-phenyl- Ethanone	8.170	4283537	0.31	C_8H_8O	120
6	Benzene methanol, alpha.,. alpha. - dimethyl-	8.498	2429446	0.18	$C_9H_{12}O$	136
7	Nonanal	8.730	401597	0.03	$C_9H_{18}O$	142
8	1, 5 - Anhydro - 6 - deoxyhexo-2, 3- diulose	9.511	901161	0.07	$C_6H_8O_4$	144
9	Benzene methanol, 4-methoxy-	9.737	392855	0.03	$C_8H_{10}O_2$	138
10		10.078	499175	0.04		
11	5-Hydroxymethylfurfural	10.837	1995522	0.15	$C_6H_6O_3$	126
12	(E)- 2-Decenal	11.180	18629381	1.37	$C_{10}H_{18}O$	154
13	7-Methylene-9-oxabicyclo [6.1.0] non-2-ene	11.575	3860003	0.28	$C_9H_{12}O$	136
14	2,4-Decadienal, (E, Z)-	11.638	3041270	0.22	$C_{10}H_{16}O$	152
15	Formamide, N, N-dibutyl-	11.759	1501875	0.11	$C_9H_{19}NO$	157
16	2,4-Decadienal, (E, E)-	11.999	17094050	1.25	$C_{10}H_{16}O$	152
17	Cyclohexene, 3-(3-methyl-1-butenyl)-, (E)-	12.872	267152	0.02	$C_{11}H_{18}$	150

18	3- Butoxy-1,1,1, 7, 7, 7-hexamethyl-3, 5,5- tris (trimethylsiloy) tetra siloxane	13.805	435674	0.03	$C_{19}H_{54}O_7Si_7$	590
19	6-Methoxycoumaran-7-ol-3-one	15.127	6670501	0.49	$C_9H_8O_4$	180
20	9,17-Octadecadienal, (Z)-	16.315	1178817	0.09	$C_{18}H_{32}O$	264
21		18.191	576617	0.04		
22	Hexadecanoic acid, methyl ester	19.041	3720792	0.27	$C_{17}H_{34}O_2$	270
23	n-Hexadecanoic acid	19.957	304357399	22.30	$C_{16}H_{32}O_2$	256
24		20.779	37416939	2.74		
25	Methyl stearate	21.012	653114	0.05	$C_{19}H_{38}O_2$	298
26	Ethanol, 2-(9,12-Octadeca dienyloxy)-, (Z, Z)-	22.175	870276744	63.77	$C_{20}H_{38}O_2$	310
27	9-Eicosenoic acid, (Z)-	23.576	10583046	0.78	$C_{20}H_{38}O_2$	310
28	9, 12- Octadecadienoyl chloride, (Z, Z)-	28.247	1586869	0.12	$C_{18}H_{31}ClO$	298
29	Pentatriacontane	30.204	655350	0.05	$C_{35}H_{72}$	492
30	delta. -Tocopherol	30.597	830250	0.06	$C_{27}H_{46}O_2$	402
31	gamma. -Tocopherol	31.555	9826642	0.72	$C_{28}H_{48}O_2$	416
32	2-Methyltetracosane	31.954	1427751	0.10	$C_{25}H_{52}$	352
33	Cholest-5-en-3-ol (3. beta.)-	32.290	2455387	0.18	$C_{27}H_{46}O$	386
34	Ergost-5-3-ol	33.487	3387174	0.25	$C_{28}H_{48}O$	400
35	Pentatriacontane	34.179	564561	0.04	$C_{35}H_{72}$	492
36	gamma. -Sitosterol	34.669	18611223	1.36	$C_{29}H_{50}O$	414
36	n-Hexadecanoic acid	38.664	10129269	0.74	$C_{42}H_{63}O_4P$	662
			1364679630	100.00		

Table 18: Phytochemicals present in the dichloromethane extract of *Sisymbrium irio* L

Peak No	Name of the compounds	R. time	Area	Area%	Formula	Mol wt
1	Dimethyl sulfone	5.730	2802920	0.34	$C_2H_6O_2S$	94
2	2-Heptenal, (E)-	6.342	2865543	0.34	$C_7H_{12}O$	112
3	2,4-Heptadienal, (E, E)-	7.016	4209707	0.50	$C_7H_{10}O$	110
4	2-Octenal	8.013	448470	0.05	$C_8H_{14}O$	126
5	1-phenyl- Ethanone	8.183	4120285	0.49	C_8H_8O	120
6	Benzene methanol, alpha.,. alpha. -dimethyl-	8.501	1708351	0.20	$C_9H_{12}O$	136
7	2-butyl-1-Octanol	10.186	461935	0.06	$C_{12}H_{26}O$	186
8	2-Heptyn-1-ol	10.924	860352	0.10	$C_7H_{12}O$	112
9	2-Decenal, (Z)-	11.151	6291012	0.75	$C_{10}H_{18}O$	154
10	1,11-Dodecadiyne	11.556	1187506	0.14	$C_{12}H_{18}$	162
11	2,4-decadienal, (E, Z)-	11.618	1691264	0.20	$C_{10}H_{16}O$	152
12	Formamide, N, N-dibutyl-	11.711	1308922	0.16	$C_9H_{19}NO$	157
13	2,4-Decadienal, (E, E)-	11.968	6602911	0.79	$C_{10}H_{16}O$	152
14	Tetradecane	12.999	497513	0.06	$C_{14}H_{30}$	198
15	2,4-Ditert-butylphenol	14.417	828735	0.10	$C_{14}H_{22}O$	206
16	3',5'-Dimethoxyacetophenone	15.223	62703242	7.52	$C_{10}H_{12}O_3$	180
17	Eicosane	15.504	530639	0.06	$C_{20}H_{42}$	282
18	4-hydroxy-3, 5-dimethoxy-Benzaldehyde	16.275	2515806	0.30	$C_9H_{10}O_4$	182
19	Tetradecanoic acid	17.351	463156	0.06	$C_{14}H_{28}O_2$	228
20	Eicosane	17.743	311277	0.04	$C_{20}H_{42}$	282

21	6, 10, 14-trimethyl-2-Pentadecanol	18.193	321277	0.04	$C_{18}H_{38}O$	270
22	10-Methoxy-NB-. alpha. -methylcorynantheol	18.411	279913	0.03	$C_{21}H_{29}N_2O_2$	341
23	9-Hexadecenoic acid, methyl ester, (Z)-	18.829	1167116	0.14	$C_{17}H_{32}O_2$	268
24	Hexadecanoic acid, methyl ester	19.069	2746143	3.29	$C_{17}H_{34}O_2$	270
25	n-Hexadecanoic acid	19.812	157132192	18.84	$C_{16}H_{32}O_2$	256
26	9, 12, 15-Octadecatrienoic acid, methyl ester	20.849	115696416	13.87	$C_{19}H_{32}O_2$	292
27	Methyl stearate	21.010	7625259	0.91	$C_{19}H_{38}O_2$	298
28		21.896	362776968	43.50		
29	Cis-11- Eicosenoic acid, methyl ester	22.668	20581032	2.47	$C_{21}H_{40}O_2$	324
30	Cyclopentane tridecanoic acid, methyl ester	22.899	3070613	0.37	$C_{19}H_{36}O_2$	296
31	9,12-Octadecadienoic acid (Z, Z)-	23.280	1294311	0.16	$C_{18}H_{32}O_2$	280
32	13-Docosenoic acid, methyl ester, (Z)-	25.010	9702317	1.16	$C_{23}H_{44}O_2$	352
33	Cyclopentane tridecanoic acid, methyl ester	25.339	352032	0.04	$C_{19}H_{36}O_2$	296
34	delta. -Tocopherol	30.568	352748	0.04	$C_{27}H_{46}O_2$	402
35	gamma. -Tocopherol	31.508	3716017	0.45	$C_{28}H_{48}O_2$	416
36	Cholest-5-en-3-ol (3. beta)	32.266	1427201	0.17	$C_{27}H_{46}O$	386
37	Ergost-5-en-3-ol	33.457	2815507	0.34	$C_{28}H_{48}O$	400
38	beta. -Sitosterol	34.615	14543739	1.74	$C_{29}H_{50}O$	414

39	Tris (2,4-di-tert butylphenyl) phosphate		38.543	1293007	0.16	$C_{42}H_{63}O_4P$	662
				834021764	100.00		

Table 19: Phyto-components identified in the methanol extract of *Sisymbrium irio* L

Peak No	Name of the compounds	R. time	Area	Area %	Formula	Mol. wt
1		5.718	859033	0.94		
2	2, 5-methyl-Furancarboxaldehyde	6.491	2347049	2.57	$C_6H_6O_2$	110
3	4-isothiocyanato-1-Butene	6.723	1844103	2.02	C_5H_7NS	113
4	(1methylbutyl)- Oxirane	7.970	2013232	2.21	$C_7H_{14}O$	114
5	1-phenyl- Ethanone	8.212	276463	0.30	C_8H_8O	120
6		8.529	422954	0.46		
7	Cyano-3,4-epithiobutane	9.103	1016137	1.11	C_5H_7NS	113
8	1,5-Anhydro-6-deoxyhexo-2,3-diulose	9.574	1474057	1.62	$C_6H_8O_4$	144
9	Benzene, (2-methyl-3-butenyl)-	10.85	2028713	2.22	$C_{11}H_{14}$	146
10	Thieno[3,2-c] pyridin-4(5H)-one	11.12	186493	0.20	C_7H_5NOS	151
11	2-Methoxy-4-vinylphenol	11.901	205191	0.23	$C_9H_{10}O_2$	150
12		12.138	313014	0.34		

13		12.789	486797	0.53		
14	DL-Proline, 5-oxo-, methyl ester	13.110	1511222	1.66	$C_6H_9NO_3$	143
15	Acetic acid, (3methylbutoxy)-, 2-propenyl ester	13.375	1251399	1.37	$C_{10}H_{18}O_3$	186
16	3, 5-Diamino-6-[2-thienyl]-1, 2, 4-triazine	13.78	304379	0.33	$C_7H_7N_5S$	193
17	2-Hydroxy-2,3-dimethylsuccinic acid	14.03	485445	0.53	$C_6H_{10}O_5$	162
18	2, 4-Ditert-butylphenol	14.42	262111	0.29	$C_{14}H_{22}O$	206
19	1,2,3-trimethoxy-5-methyl Benzene	14.59	173566	0.19	$C_{10}H_{14}O_3$	182
20	Thiazolo[4,5b] pyridine 2(3H)-one	14.690	775184	0.85	$C_6H_4N_2OS$	152
21		14.850	879584	0.96		
22	3', 5'-Dimethoxyacetophenone	15.189	33960397	37.24	$C_{10}H_{12}O_3$	180
23	Thiazolo[4,5-b] pyridin-2(3H)-one	15.952	204158	0.22	$C_6H_4N_2OS$	152
24	2-Butanone, 4-(2, 2-dimethyl-6-methylenecyclohexyl)-	16.314	488936	0.54	$C_{13}H_{22}O$	194
25	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	16.832	300885	0.33	$C_{13}H_{22}O_2$	210

26	9-(methylthio)- Nonane nitrile	17.141	411181	0.45	$C_{10}H_{19}NS$	185
27	9 (methylthio)- Nonane nitrile	18.275	405604	0.44	$C_{10}H_{19}NS$	185
28	Hexadecanoic acid, methyl ester	19.040	1908810	2.09	$C_{17}H_{34}O_2$	270
29	9-Octadecenoic acid (Z)-	19.225	283940	0.31	$C_{18}H_{34}O_2$	282
30	n-Hexadecanoic acid	19.482	4811892	5.28	$C_{16}H_{32}O_2$	256
31	9, 12-Octadecadienoic acid, methyl ester	20.68	3091692	3.39	$C_{19}H_{34}O_2$	294
32	8, 11, 14-Docosatrienoic acid, methyl ester	20	3977116	4.36	$C_{23}H_{40}O_2$	348
33	Octadecanoic acid, methyl ester	20	271768	0.30	$C_{19}H_{38}O_2$	298
34	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	21	16565664	18.17	$C_{18}H_{30}O_2$	278
35	9-Octadecenoic acid (Z)-	21.373	557689	0.61	$C_{18}H_{34}O_2$	282
36	Methyl-9-eicosenoate	22.567	447763	0.49	$C_{21}H_{40}O_2$	324
37	Cis-11-Eicosenoic acid	22.986	656605	0.72	$C_{20}H_{38}O_2$	310

38	Ethanamine, 2- [(4chlorophenyl)-2-pyridinylmethoxy] -N, N-dimethyl-	24.894	1615206	1.77	$C_{16}H_{19}ClN_2O$	290
39		28.111	528359	0.58		
40	Retinal	31.917	124260	0.14	$C_{20}H_{28}O$	284
41	beta. -Sitosterol	34.484	460394	0.50	$C_{29}H_{50}O$	414
42	Tris (2, 4-di-tert-butyl phenyl) phosphate	38.520	1000602	1.10	$C_{42}H_{63}O_4P$	662
			91189047	100.00		

Table 20: Phyto-components identified in the n-hexane extract of *Colchicum autumnale* L

Peak No	Name of the compounds	R. Time	Area	Area%	Formula	Mol wt
1	5-methyl-6-Hepten-1-ol	11.189	21377	0.46	$C_8H_{16}O$	128
2	1-bromo- Decane	12.387	967152	20.88	$C_{10}H_{21}Br$	220
3	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5tris(trimethylsiloxy) tetrasiloxane	13.810	84108	1.82	$C_{19}H_{54}O_7Si_7$	590
4		14.122	19440	0.42		
5	2-Propyldecan-1-ol	14.281	11733	0.25	$C_{13}H_{28}O$	200
6	3', 5'-Dimethoxyacetophenone	15.127	82165	1.77	$C_{10}H_{12}O_3$	180
7	3, 4-Dihydroxymandelic acid-tetratms	15.780	62381	1.35	$C_{20}H_{40}O_5Si_4$	472
8	(-)-(4R,5S,6R)-4,5,6-tris-{[(tert-butyl) dimethyl silyl] oxy} cyclohex-2-en	17.478	34341	0.74	$C_{24}H_{50}O_4Si_3$	486
9	Tricyclo [3.3.1.13,7] decane, 1-(Ethynyloxy)-	17.585	32030	0.69	$C_{12}H_{16}O$	176
10		18.126	24063	0.52		
11	Hexadecanoic acid, methyl ester	19.035	385583	8.33	$C_{17}H_{34}O_2$	270
12	n-Hexadecanoic acid	19.428	606902	13.10	$C_{16}H_{32}O_2$	256
13	9, 12-Octadecadienoic acid, methyl ester	20.675	508509	10.98	$C_{19}H_{34}O_2$	294
14	8,11,14-Docosatrienoic acid, methyl ester	20.732	232032	5.01	$C_{23}H_{40}O_2$	348
15	Hexadecanoic acid, methyl ester	20.967	36194	0.78	$C_{17}H_{34}O_2$	270
16	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	21.147	1062065	22.93	$C_{18}H_{30}O_2$	278
17	1, E-11, Z-13-Octadecatriene	21.284	11273	0.24	$C_{18}H_{32}$	248

18		22.510	19920	0.43		
19		22.560	10520	0.23		
20	5-cyclopropylidene-1-Pentanol	23.848	51482	1.11	$C_8H_{14}O$	126
21	10-Undecyn-1-ol	24.453	42445	0.92	$C_{11}H_{20}O$	168
22	9,12,15-Octadecatrienal	24.551	34927	0.75	$C_{18}H_{30}O$	262
23		24.891	43652	0.94		
24	1 (2, 2dibromo cyclopropyl)- Pentane	29.975	10858	0.23	$C_8H_{14}Br_2$	268
25		30.051	9185	0.20		
26		31.100	38613	0.83		
27		31.571	6605	0.14		
28	beta. -Sitosterol acetate	31.919	103473	2.23	$C_{31}H_{52}O_2$	456
29	Methanol, [5,7,9-trimethyl-4-(1-propenyl)-3-oxabicyclo [3.3.1] non-6-en-	34.480	78149	1.69	$C_{15}H_{24}O_2$	236
			4631177	100.00		

Table 21: Phytochemicals present in the dichloromethane extract of *Colchicum autumnale* L

Peak No	Name of the compounds	R. time	Area	Area %	Formula	Mol wt
1	1-Dodecene	10.066	676870	0.08	$C_{12}H_{24}$	168
2	6-hydroxy-4-methoxy-2,3-dimethyl-Benzaldehyde	10.225	738855	0.08	$C_{10}H_{12}O_3$	180
3	3-MethoxyPhenol	10.750	57109758	6.33	$C_7H_8O_2$	124
4	3-Methoxyphenol, TMS derivative	11.400	480608	0.05	$C_{10}H_{16}O_2Si$	196
5	2-Methoxy-4-vinylphenol	11.886	1302579	0.14	$C_9H_{10}O_2$	150
6	1-Tetradecene	12.903	5018912	0.56	$C_{14}H_{28}$	196
7	2, 4bis(1,1dimethylethyl)-Phenol	14.432	22925101	2.54	$C_{14}H_{22}O$	206
8	E-14-Hexadecenal	15.435	19850159	2.20	$C_{16}H_{30}O$	238
9	2, 6-dimethoxy-4-(2-propenyl)-Phenol	15.519	1152714	0.13	$C_{11}H_{14}O_3$	194
10	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	17.132	1132676	0.13	$C_{11}H_{18}$	150
11	1H-Inden-1-one, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-	17.600	4100368	0.45	$C_{12}H_{18}O$	178
12	1-Octadecene	17.698	17454033	1.93	$C_{18}H_{36}$	252
13	1H-Inden-1-one, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-	17.800	1013852	0.11	$C_{12}H_{18}O$	178
14	Pentadecanoic acid, methyl ester	18.007	1144958	0.13	$C_{16}H_{32}O_2$	256
15	1,2Benzenedicarboxylic acid, bis(2methylpropyl) ester	18.425	2375292	0.26	$C_{16}H_{22}O_4$	278
16	Hexadecanoic acid, methyl ester	19.098	52228333	5.79	$C_{17}H_{34}O_2$	270

17	Hexadecanoic acid, ethyl ester	19.762	100756511	11.17	$C_{18}H_{36}O_2$	284
18		19.825	25899597	2.87		
19	Hexadecanoic acid, 14-methyl-, methyl ester	20.051	1212646	0.13	$C_{18}H_{36}O_2$	284
20	9,12-Octadecadienoic acid, methyl ester	20.840	179697233	19.92	$C_{19}H_{34}O_2$	294
21	Methyl stearate	21.027	13217053	1.47	$C_{19}H_{38}O_2$	298
22	Bis(2-ethylhexyl) maleate	21.158	13704627	1.52	$C_{20}H_{36}O_4$	340
23	Linoleic acid ethyl ester	21.396	1022197233	11.33	$C_{20}H_{36}O_2$	308
24	(E)-9-Octadecenoic acid ethyl ester	21.446	38772838	4.30	$C_{20}H_{38}O_2$	310
25	7-Tetradecenal, (Z)-	21.639	153068604	16.97	$C_{14}H_{26}O$	210
26		22.617	1400675	0.16		
27	Methyl-18-methylnonadecanoate	22.874	7475412	0.83	$C_{21}H_{42}O_2$	326
28	trans-2-Undecen-1-ol	23.081	3773629	0.42	$C_{11}H_{22}O$	170
29	Nonacos-1-ene	23.586	10766982	1.19	$C_{29}H_{58}$	406
30	Docosenoic acid, methyl ester	25.314	2185142	0.24	$C_{23}H_{46}O_2$	354
31	Di-n-octyl phthalate	25.390	986307	0.11	$C_{24}H_{38}O_4$	390
32	Nonacos-1-ene	26.376	2608237	0.29	$C_{29}H_{58}$	406
33	Triacontanoic acid, methyl ester	28.480	935395	0.10	$C_{31}H_{62}O_2$	466
34	Decanedioic acid, bis(2-ethylhexyl) ester	29.241	85220113	0.94	$C_{26}H_{50}O_4$	426
35	Squalene	29.389	741272	0.08	$C_{30}H_{50}$	410
36	Heptacosane	30.179	839745	0.09	$C_{27}H_{56}$	380
37	Octacosyl trifluoroacetate	30.993	2102663	0.23	$C_{30}H_{57}F_3O_2$	506
38	gamma. -Tocopherol	31.496	798830	0.09	$C_{28}H_{48}O_2$	416

39	Dotriacontane	31.928	700282	0.08	$C_{32}H_{66}$	450
40	alpha. -Tocopheryl acetate	32.274	4793677	0.53	$C_{31}H_{52}O_3$	472
41	Ergost-5-en-3-ol	33.453	1033929	0.11	$C_{28}H_{48}O$	400
42	3.beta. -Acetyl-5-cholenic acid	33.765	2083583	0.23	$C_{26}H_{40}O_3$	400
43	beta. -Sitosterol	34.588	9265683	1.03	$C_{29}H_{50}O$	414
44	Methyl hydrogen phthalate	35.683	885288	0.10	$C_9H_8O_4$	180
45	Methyl commate D	36.416	905596	0.10	$C_{31}H_{50}O_4$	486
46	Methyl commate D	37.193	776592	0.10	$C_31H_{50}O_4$	486
47		38.618	21249410	2.36		
			902083747	100.00		

Table 22: Phyto-components identified in the methanol extract of *Colchicum autumnale* L

Peak No	Name of the compounds	R. time	Area	Area %	Formula	Mol wt
1	1-phenyl-Ethanone	8.183	3569321	2.22	C_8H_8O	120
2	Benzene methanol, alpha.,. alpha. -dimethyl-	8.514	2437636	1.52	$C_9H_{12}O$	136
3	2-Cyclohexen-1-one, 3,5,5-trimethyl-	9.083	589338	0.37	$C_9H_{14}O$	138
4	Salicyl alcohol	9.338	364141	0.23	$C_7H_8O_2$	124
5	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	9.639	6297004	3.92	$C_6H_8O_4$	144
6	2,3-Dihydro-benzofuran	10.614	306094	0.19	C_8H_8O	120
7	3-Methoxy Phenol	10.758	10704961	6.67	$C_7H_8O_2$	124

8	5-Hydroxymethylfurfural	11.068	11045521	6.88	$C_6H_6O_3$	126
9	2-Methoxy-4-vinylphenol	11.898	396557	0.25	$C_9H_{10}O_2$	150
10	4hydroxy-3-methoxy Benzaldehyde	13.137	752002	0.47	$C_8H_8O_3$	152
11	2, 4-Ditert-butylphenol	14.422	1417855	0.88	$C_{14}H_{22}O$	206
12	2-hydroxy-6-methoxy Benzoic acid	14.878	357310	0.22	$C_8H_8O_4$	168
13	3',5'Dimethoxyacetophenone	15.120	382855	0.24	$C_{10}H_{12}O_3$	180
14	4-Methoxy-7-methylindan-1-one	15.416	2117508	1.32	$C_{11}H_{12}O_2$	176
15	2, 6-dimethoxy-4-(2-propenyl) Phenol	15.523	479829	0.30	$C_{11}H_{14}O_3$	194
16	Tetradecanoic acid	17.351	285992	0.18	$C_{14}H_{28}O_2$	228
17	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	17.590	396242	0.25	$C_{11}H_{18}$	150
18	Cetene	17.666	108308	0.07	$C_{16}H_{32}$	224
19	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	17.790	224680	0.14	$C_{11}H_{18}$	150
20	Pentadecanoic acid, methyl ester	17.992	312706	0.19	$C_{16}H_{32}O_2$	256
21	1,3,5-Triazine-2,4-diamine,6-chloro-n-ethyl-	18.188	131634	0.08	$C_5H_8ClN_5$	173
22	Pentadecanoic acid	18.425	750284	0.47	$C_{15}H_{30}O_2$	242
23	Hexadecanoic acid, methyl ester	19.054	7918240	4.93	$C_{17}H_{34}O_2$	270
24	Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	19.142	3155622	1.97	$C_{18}H_{28}O_3$	292
25	n-Hexadecanoic acid	19.599	26302608	16.38	$C_{16}H_{32}O_2$	256
26	Hexadecanoic acid, ethyl ester	19.712	834687	0.52	$C_{18}H_{36}O_2$	284

27	Linoelaidic acid	20.161	168858	0.11	$C_{18}H_{32}O_2$	280
28	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	20.706	16054690	10.00	$C_{19}H_{34}O_2$	294
29	8,11,14-Docosatrienoic acid, methyl ester	20.760	7166643	4.46	$C_{23}H_{40}O_2$	348
30		20.865	153960	0.1		
31	Octadecanoic acid, methyl ester	20.974	6394417	0.40	$C_{19}H_{38}O_2$	298
32	9,12-Octadecadienoic acid (Z, Z)-	21.298	50459929	31.42	$C_{18}H_{32}O_2$	280
33	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	21.445	690404	0.43	$C_{22}H_{44}O_4$	372
34	Methyl-9-eicosenoate	22.575	400783	0.25	$C_{21}H_{40}O_2$	324
35	Eicosanoic acid, methyl ester	22.824	347642	0.22	$C_{21}H_{42}O_2$	326
36	13-Docosenoic acid, methyl ester, (Z)-	24.905	215171	0.13	$C_{23}H_{44}O_2$	352
37	9,12-Octadecadienoyl chloride, (Z, Z)-	28.043	518959	0.32	$C_{18}H_{31}ClO$	298
38	beta. -Sitosterol	34.493	956809	0.60	$C_{29}H_{50}O$	414
39	Tris (2,4-di-tert-butylphenyl) phosphate	38.528	1174405	0.73	$C_{42}H_{63}O_4P$	662
			160586593	100.00		

The research examination conducted by Patel identified compounds such as n-Hexadecanoic acid, revealed anti-inflammatory, and anti-oxidant properties (Patel et al., 2017). Hexadecanoic acid, methyl ester exhibited antioxidant, hypocholesterolemic, nematicide, pesticide lubricant, antiandrogenic flavour and hemolytic properties (Balamurugan et al., 2018). Linoleic acid (omega-

6) is one of the fundamental fatty acids, that is not created in the human body and should be given to the body from outside, and thus *Sisymbrium irio* L and *Colchicum autumnale* L extracts were a good source of linoleic acid. The research examination conducted on the function of dietary polyunsaturated fatty acid in the nervous system such as prostaglandins leukotrienes and essential fatty acid: linoleic acid (omega 6) is well done, and so is universally recognized nutrient in for the health of human body (Bourre et al., 1993). The compound β-sitosterol is a plant sterol which displays excellent anti-inflammatory and cholesterol lowering activity (Loizou et al., 2010). Other research examination exhibited that β-sitosterol stimulates antioxidant enzymes by activation of estrogen receptor/P13-kinase-dependent pathway. The GSH & DSH/total glutathione ratio recovered after treatment by β-sitosterol signifies that this phytosterol could be a ROS scavenger (Shi et al., 2013). A research studies confirmed that 9, 12, Octadecadienoic acid (Z, Z)- has the possessions of antiarthritic & anti-inflammatory (Dineshkumar & Rajakumar, 2015). Hexadecanoic acid, ethyl ester own cancer preventive, hypocholesterolemic, anti-coronary anti-inflammatory, nematicide, hepatoprotective, insectifuge, anti-histaminic, anti-acne, alpha reductase inhibitor, anti-androgenic, antiarthritic, ant coronary. Cosmetics/antipsychotic, medication/Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavour, haemolytic and 5-Alpha reductase inhibitory properties. Octadecanoic acid, ethyl ester possesses anti-inflammatory properties. Stigmasterol owns tumor inhibitory effects, anti-HIV reverse transcriptase, and also anti-inflammatory qualities. Gamma -Sitosterol have been used for antimicrobial, anti-angeogenic, anti-cancer, anti-diabetic effects; as a pain killer used in Jaundice and also as an antiviral, anti-inflammatory, and anti-diarrhoeal agent (Karthikeyan *et al*., 2017).

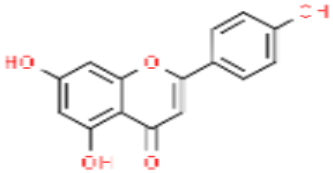
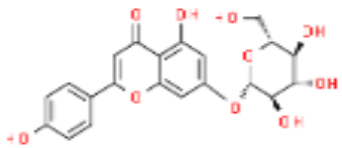
4.10. UHPLC-QExactive Orbitrap Analysis

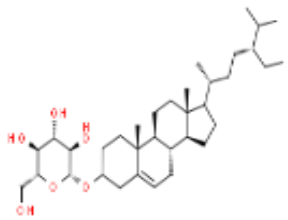
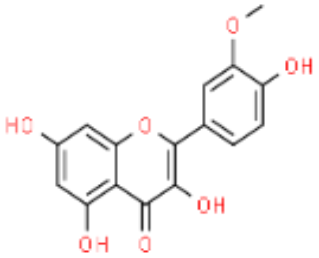
Liquid chromatography coupled with high resolution tandem mass spectrometer, has greatly facilitated explicit identification and highly sensitive quantification of trace component present in complex matrices, which can accomplish qualitative analysis in a short time (Zhou et al., 2011; Shen

et al., 2018). The identification of compounds was carried out using UHPLC-Q Exactive Orbitrap spectrometer for profiling of secondary metabolites from *Sisymbrium irio* L and *Colchicum autumnale* L extracts using mobile phase A [water: methanol (90:10, v/v) + 0.2 % HCOOH] and phase B [methanol: water (90:10, v/v) + 0.2% HCOOH]. The chemical compounds identified from both *Sisymbrium irio* L and *Colchicum autumnale* L extracts were: Isorhamnetin, Isorhamnetin-3-O-neohesperidine, Isorhamnetin-7-O-beta-D-glucopryanoside, Isorhamnetin-7-glucoside, Isorhamneti n-3-Laminariobioside, Colchicine, (R/S) Deacetyl Colchicine, 3-demethyl Colchicine, and Colchicoside (3-demethyl colchicine glucoside.)

According to the review paper of Mohammed Zakir Siddiqui and Sada Akhtar on *Suranjan Shirin* (*Colchicum autumnale* L) showed that colchicine chiefly possesses anti-inflammatory, analgesic and anti arthritic activity. It also possesses expectorant, deobstruent, antidote and aphrodisiac activity. It is chiefly used to relieve the pain and inflammation and to shorten the duration of acute gout and certain gouty infection. It is also prescribed to treat myeloid leukemia. It increases the secretions of the skin, liver and kidneys and also increases the flow of bile. In ascites due to liver disease, it is a very efficacious remedy. In cerebral and hepatic congestions, it acts as a purgative with benefit. It is also found to be efficacious in genital infections like gonorrhea. It is employed orally in tablet form for arthritis, familial Mediterranean fever while corms and seeds are used to treat enlarged prostate, dropsy, gout, rheumatism and arthritis (Mohammad Zakir Siddiqui & Sada Akhtar, 2018). The phytochemical analysis through UHPLC confirmed the *Sisymbrium irio* L and *Colchicum autumnale* L extracts contained the various types of bioactive compounds, and these bioactive compounds have an important role in the therapeutic system of medicine for treatment of diseases.

Table 23: List of identified compounds from *Sisymbrium irio* L, including molecular formula, molecular weight, retention time, adduct [M+H]+, observed mass, error in parts per million (ppm), and fragments ion.

Sr. No.	Name of the compounds	Molecular Formula	Molecular wt (g/mol)	R. time	Adduct	Observed mass	Error (PPM)	Structure	Fragments and Molecular ion
1	Apigenin	$C_{15}H_{11}O_5$	270.0528	8.32	$[M+H]^+$	271.0594	-2.4042		84.9603, 102.9706, 153.0181, 234.9608, 242.9064 & 271.0594
2	Apigenin 7-O-glucoside	$C_{21}H_{21}O_{10}$	432.4	6.88	$[M+H]^+$	433.1122	-1.6395		116.9861, 125.9863, 134.9964, 158.0123, 167.0225, 204.8886, 214.9173, 24.9435, 285.1092, 310.1643, 342.8278, 397.0963 & 433.1122

3	(17ξ) - Stigmast-5-en-3-yl-β-D-glucopyranoside	$C_{35}H_{61}O_6$	576.85	12.60	$[M+H]^+$	577.4496	-		413.2658, 431.3128, 441.2519, 447.2693, 497.3441, 522.3550, 549.3755 & 577.4496
4	Isorhamnetin	$C_{11}H_{17}O_1$ 1	316.26	6.36	$[M+H]^+$	317.0651	-1.6265		254.9254, 256.9223, 264.9541, 270.9105, 272.8764, 278.9698, 284.8897, 291.1112, 296.0909, 302.9003, 305.0963, 309.0277 & 317.0651

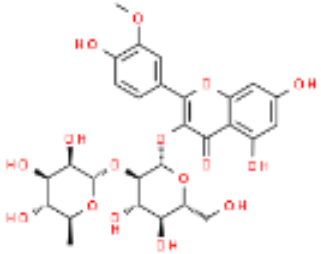
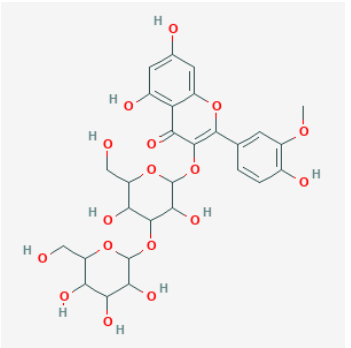
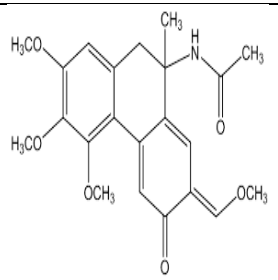
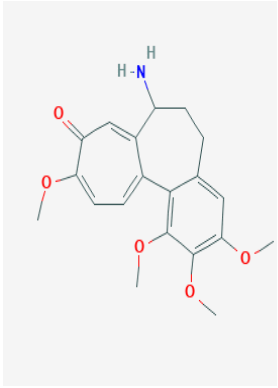
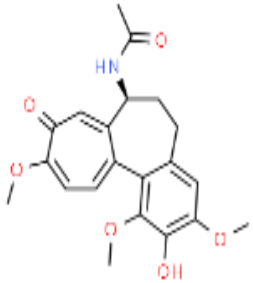
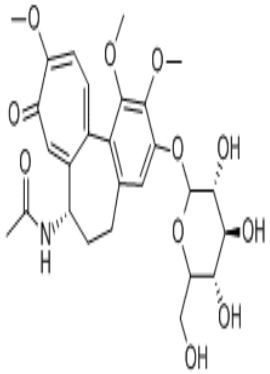
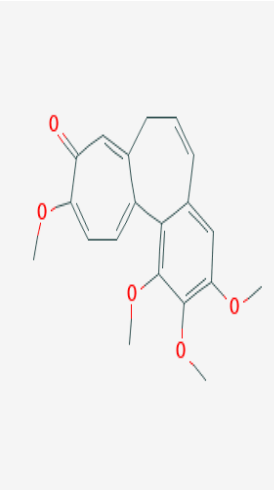
5	Isorhamnetin-3-O-neohesperidine	$C_{28}H_{33}O_{16}$	624.5	7.00	$[M+H]^+$	625.1758	-0.7619		621.8384, 622.1107, 623.1118, 624.0725 & 625.1758
6	Isorhamnetin-7-O-beta-D-glucopryanoside, Isorhamnetin 7-glucoside	$C_{22}H_{23}O_{12}$	478.4	6.99	$[M+H]^+$	479.1181	-0.5600		441.2984, 455.1317, 469.1440 & 479.1181
7	Isorhamnetin-3-Laminaribioside	$C_{28}H_{33}0_{17}$	640.5	6.22	$[M+H]^+$	641.1704	-1.2736		630.8198, 633.1403, 637.1788 & 641.1704

Table 24: List of identified compounds from *Colchicum autumnale* L with their accurate mass, formula, retention time, and product ions.

Sr. No.	Name of compounds	Molecular Formula	Molecular wt (g/mol)	R. time	Adduct	Observed mass	Error (PPM)	Structure	Fragments and Molecular ion
1	Colchicine	$C_{22}H_{26}NO_6$	399.437	7.36	$[M+H]^+$	400.1753	-1.0374		68.7195,98.4443, 127.2563, 170.5951, 239.1045, 239.1045, 267.1000, 310.1177, 358.1649, 382.1639 & 400.1753
2	(R/S)-Deacetyl Colchicine	$C_{20}H_{24}NO_5$	357.4	5.07	$[M+H]^+$	358.1647	-0.5075		110.0095, 116.9872, 125.9871, 137.1074, 141.9587, 173.9847, 213.1022, 232.9297, 248.9004, 295.0490, 313.1672 327.1226, 344.1855 & 358.1647

3	3-demethyl Colchicine	$C_{21}H_{23}NO_6$	385.410		$[M+H]^+$	386.1594	-1.0892		68.8835, 84.9604, 102.9704, 158.9273, 176.9383, 207.0796, 232.9276, 250.9386, 295.0956, 344.1490, 368.1474 & 386.1594
4	Colchicoside (3-demethylcolchicine glucoside)	$C_{27}H_{34}O_{11}N$	547.5 g/mol	5.27	$[M+H]^+$	548.2120	-1.4305		84.9598, 114.1461, 149.0593, 235.0750, 267.1013, 295.0945,344.1479, 368.1482, 386.1591, 405.0515 & 548.2120

5	Deacetamido-5, 6 Dihydrocolchicine	$C_{20}H_{21}O_5$	340	6.0 1	[M+H]$^+$	341.138 2	-0.5779		102.9707, 116.9862, 125.9864, 134.9965, 149.0122, 158.0125, 167.0225, 198.9399, 204.887, 214.9175, 232.9281, 264.9542 & 341.1382

4.11. Isolation of the Compounds

The isolation of pure compounds was done using column chromatography by increasing the polarity of the solvent systems, and different fractions were collected. Then, the purity of each of the collected fractions was monitored using TLC. Fraction SM-9, SM-17, SM-19 and CDCM-8, and CDCM-12 showed a single spot on TLC when developed with $CHCl_3$: MeOH (6:4), concentrated and labelled as SM-9, SM-17, SM-19 and CDCM-8, and CDCM-12. Then, these isolated compounds were analysed using FT-IR, NMR and MS. The other fractions were obtained to be impure during the TLC analysis and they showed more than one spots.

4.12. Characterization of Isolated Compound SM-9

4.12.1. FT-IR Characterization of Isolated Compound SM-9

FT-IR spectroscopic technique is used to identify the functional groups in the plant sample when run under IR region. The vibrational bands between 3000 & 2800 cm^{-1} represent Sp^3 C-H stretching vibrations that are mainly generated by lipids (Wolkers and Hoekstra, 1995; Wei et al., 2009). The weak absorption band of 796.60, 700.16, 617.22 cm^{-1} indicate the presence of chloride, bromide in our plant extract (Muruganatham, 2009). This OH stretching indicates the phenolic compound, that have excellent antioxidant properties (Shirwaikar et al., 2003). Functional groups of isolated compounds from *Sisymbrium irio* L and *Colchicum autumnale* L were identified by FT-IR spectroscopy.

FT-IR spectral data of isolated compound of SM-9 (Appendix figure 6) showed the presence of different functional groups at different wave numbers. A band at 3383.87cm^{-1} in its IR spectrum was suggested to an alcohol functional group (O-H stretch) in isorhamnetin derivative. IR spectrum exhibited the absorption bands at 2997.39 and 2853.59 cm^{-1} asymmetric & symmetric stretching vibrations of C-H of methylene or methyl group. The bands at 1660.98 cm^{-1} signal the presence of carbonyl group of flavonoids. The absorption bands at 1635.65 and 1517.17 cm^{-1} showed the presence of aromatic rings in the compound. The band at 1461.80 and 1367.95 cm^{-1} are due to the characteristic

methylene and methyl bending vibrations respectively while the medium absorption band at 1115.89 cm^{-1} stands for C-O stretching vibration. The bands at 925.33, 881.80, 800.90 and 630.79 cm^{-1} were due to substituents in aromatic rings.

Table 25: IR absorption frequencies of functional groups in SM-9

Peaks (Wave Number)	Functional groups
3383.87	O-H stretching of alcohol
2997.39	Aliphatic Sp^3C-H (stretching)
2853.59	Alkyl Sp^2C-H stretching
1660.98	Carbonyl stretching
1635.65	Carbon-Carbon double bond of aromatic ring
1517.17	Aromatic ring
1461.80	CH_2 bending vibration
1367.95	CH_3-bending vibration
1115.89	C-O stretch
925.33, 881.80, 800.90 and 630.79	Substituent on the aromatic ring

4.12.2. NMR Characterization of Isolated Compound of SM-9

4.12.2.1. ^{1}H-NMR Characterization of Isolated Compound of SM-9 ($CDCl_3$, TMS)

The ^{1}H-NMR spectra of isolated compound of SM-9 showed four clearly separated regions of protons in which the hydroxyl proton was in the middle, whereas the three others separated regions constituted the aromatic proton, olefinic and the aliphatic protons. The signals at δH 6.233, 6.265, 6.754, 7.549 and 7.581 indicated the presence of the aromatic protons. Those signals seen δH from 4.371 to 5.337 were due to olefinic protons. The signal at chemical shift 3.883 was due to hydroxyl proton. Those signals from 3.245 to δH 3.541 were due to the existence of methyl

protons. The peaks appeared from 0.644 to δH 1.219 confirmed the existence of methyl protons in the up-field region.

4.12.2.2. ^{13}C-NMR Characterization of Isolated Compound of SM-9 ($CDCl_3$, TMS)

The spectrum in the (Appendix figure-8) consisted of many signals. The signal at δC 147.435, indicated the presence of the carbonyl carbon. Those signals seen from 145.796, to δC 100 were due to the presence of olefinic carbons. The signals from 75.626, to δC 29.048 in the spectrum were the characteristic of methine, and methylene carbons. The peaks appeared from 19.263 to δC 11.781 shown the existence of methyl carbon atoms in the up-field region.

4.12.2.3. Mass Spectrum of Isolated Compound of SM-9

Isolated compound showed the different types of fragments and molecular ion at m/z- 308.2350, 351.2284, 485.3675, 507.3504, 523.3252, 551.3790, 567.3533, 611.3818, 655.4094, 699.4377, 743.4657 & 787.4937.

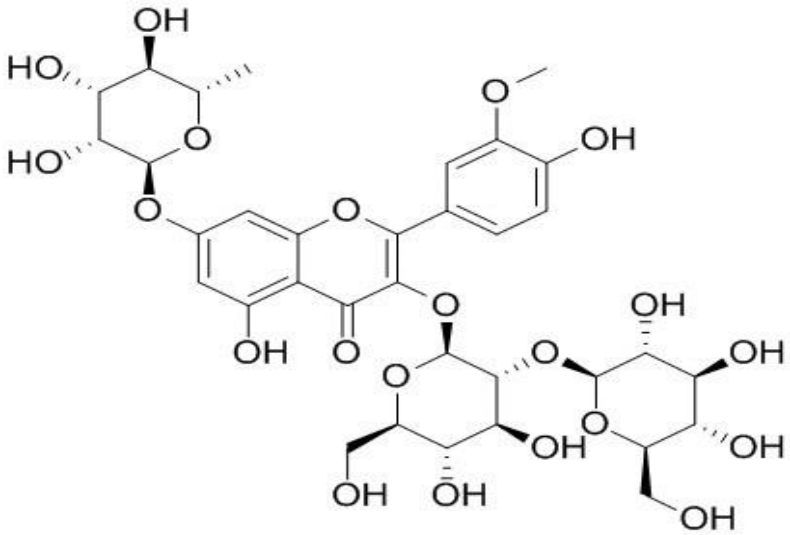


Figure 33: The proposed structure of isolated compound is Isorhamnetin 3-sophoroside-7-rhamnoside derivative

4.13. Characterization of Isolated Compound SM-17

4.13.1. FT-IR Characterization of Isolated Compound of SM-17

FT-IR spectral data of isolated compound in the (Appendix figure 10) showed the presence of the different types of functional groups. Absorption band at 338.87 cm^{-1} in its IR spectrum was suggested to an alcohol functional group (O-H stretch) and the absorption band at 2977.39 cm^{-1} was as a result of aliphatic Sp^3 C-H stretching. FT-IR spectrum showed the absorption bands at 1633.12, 1600.09, and 1409.88 cm^{-1} were due to the presence of carbon-carbon double bonds and aromatic groups. The absorptions bands at 1459.82 and 1377.22 cm^{-1} were due to the characteristic methylene and methyl bending vibrations respectively while the medium absorption band at 1147.13 cm^{-1} stand for C-O stretching vibration. Vibrational band at 925.34, 666.23, and 635.54 cm^{-1} were as a result of the substituents on the aromatic ring.

Table 26: IR absorption frequencies of functional groups in SM-17

Peaks (Wave Number)	Functional groups
3383.87	Hydroxyl (O-H stretching)
2997.39	Alkyl C-H (stretching)
1633.12	Carbon- Carbon double bond
1600.09	Aromatic ring
1459.82	CH_2 bending vibration
1409.88	Aromatic group
1377.22	CH_3-bending vibration
1147.13	C-O stretch
925.34, 666.23, 635.54	Substituent on the aromatic ring

4.13.2. NMR Characterization of Isolated Compound of SM-17

4.13.2.1. ^{1}H-NMR Characterization of SM-17

The ^{1}H-NMR spectra of isolated compound SM-17 (Appendix figure 11) shows three clearly separated regions of protons in which the hydroxyl proton is in the middle, whereas the two others separated regions constitute the aromatic proton and the aliphatic protons.

4.13.2.2. ^{13}C-NMR ($CDCl_3$, TMS)

The ^{13}C-NMR spectrum of SM-17 (Appendix figure-12) contains various types of signals. The signal at δC 173.32 indicates the presence of the ester carbonyl carbon atoms. Those signals seen from 132.45, to δC 127.89 indicate the presence of olefinic carbons. The signals from 76.70 to δC 22.58 in the spectrum are characteristic of methine and methylene carbon atoms in glycerol moiety of a triglyceride respectively. The peaks which appeared from 10.96 to δC 14.12 indicate the presence of methyl carbon atoms in the up-field region.

4.13.2.3. Mass Spectrum of Isolated Compound SM-17

Isolated compound showed the various types of fragmentation and molecular ion at m/z 683.4604, 705.4185, 720.3650, 721.3679, 722.3695, 756.5867, 760.6172, 778.5682, 780.5852, 782.6006, 784.6176 & 786.6332.

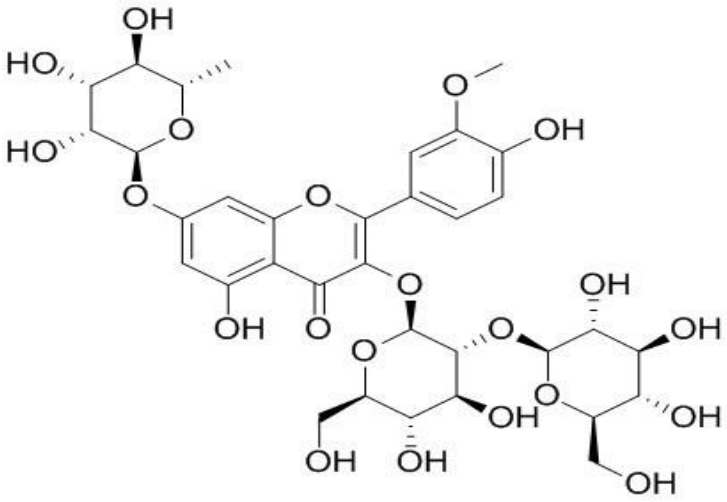


Figure 34: Proposed structure of isolated compound is Isorhamnetin 3-sophoroside-7-rhamnoside derivative

4.14. Characterization of Isolated Compound SM-19

4.14.1. FT-IR Characterization of Isolated Compound of SM-19

FT-IR spectral data in the (Appendix figure 14) showed the presence of different types of functional groups which absorb at different wave number. The absorption band at 3437.72 cm^{-1} in its FT-IR spectrum was suggested to an alcohol functional group (O-H stretch). The vibrational bands at 1722.42 cm^{-1} indicated the existence of carbonyl functional group & other vibrational bands at 1655.10, 1531.07 & 1398.71 cm^{-1} were due to the presence of carbon-carbon double bond and aromatic ring. Absorption band at 1116.51 cm^{-1} stands for C-O stretching vibration. The bands at 802.00, 623.28 & 584.35 cm^{-1} were due to presence of substituents on aromatic ring.

Table 27: IR absorption frequencies of functional groups in SM-19

Peaks (Wave Number)	Functional groups
3437.72	Hydroxyl (O-H stretching)
1722.42	Carbonyl of ketone
1655.10	Carbon-Carbon double bond
1531.07, 1398.71	Aromatic ring
1116.51	C-O stretch
802.00, 623.28, 584.35	Substituent on the aromatic ring

4.14.2. NMR Characterization of Isolated Compound of SM-19

4.14.2.1. ^{1}H-NMR Characterization of SM-19

The ^{1}H-NMR spectra of SM-19 (Appendix figure 14) shows the presence of three clearly separated regions of protons in which the hydroxyl proton being in the middle, whereas the two others separated regions constitute the aromatic proton and the aliphatic protons.

4.14.2.2. ^{13}C-NMR ($CDCl_3$, TMS)

The ^{13}C-NMR spectra of SM-19 (Appendix figure-15) consisted of many signals. The signal at δC 173.32 indicates the presence of the ester carbonyl carbon atoms. Those signals seen from 132.45 to δC 127.89 indicate the presence of olefinic carbons. The signals from 76.70, to δC 22.58 in the spectrum are characteristic of methine and methylene carbon atoms in glycerol moiety of a triglyceride respectively. The peaks which appeared from 10.96 to δC 14.12 indicate the presence of methyl carbon atoms in the up-field region.

4.14.2.3. Mass Spectrum of SM-19

The mass spectrum and other spectroscopic elucidation confirm that the isolated compound SM-19 was isorhamnetin-3-sophoroside-7-rhamnoside derivative. The estimated molecular formula of this compound was $C_{34}H_{38}O_{21}$ (protonated molecule with m/z 118.09, 213.13, 308.12, 365.12, 431.16, 518.34, 544.36, 558.30, 667.24, 720.36, 721.36, 772.37 and 782.6).

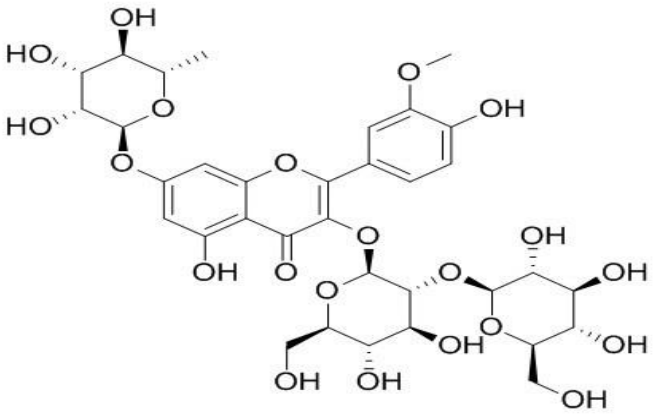


Figure 35: The proposed structure of isolated compound is isorhamnetin-3-sophoroside-7-rhamnoside derivative

4.15. Characterization of Isolated Compound CDCM-8

4.15.1. FT-IR Characterization of Isolated Compound of CDCM-8

FT-IR spectral data of isolated compound in the (Appendix figure 18) showed the presence of different types of functional groups which absorb at different frequency. A band at 3435.14 cm^{-1} in its IR spectrum was suggested to the NH stretching vibration (N-H stretch) of the compound (3-Demethylthiocolchicine). The FT-IR spectrum showed the absorption band at 2928.60 cm^{-1}, (SP^3 C-H aliphatic stretching vibrations of methyl group). The absorptions at 1713.83 cm^{-1} was because of the keto carbonyl group of amides and the bands at 1594.17, 1489.58, and 1403.40 cm^{-1} could be assigned to the aromatic ring. The bands at 1462.42 and 1322.28 cm^{-1} were due to the characteristic methylene and methyl bending vibrations respectively while the medium absorption band at 1137.90 cm^{-1} stands for C-O stretching vibration.

Table 28: IR absorption frequencies of functional groups in CDCM-8

Peaks (Wave Number)	Functional groups
3435.14	Hydroxyl, phenolic groups
2928.60	Alkyl C-H (stretching)
1713.83	Carbonyl group of amides
1594.17, 1489.58, 1403.40	Aromatic rings
1462.42	CH_2 bending vibration
1322.28	CH_3-bending vibration
1137.90	C-O stretch
765.02, 635.54, 617.93	Substituent on the aromatic ring of Colchicine

4.15.2. NMR Characterization of Isolated Compound of CDCM-8

4.15.2.1. ^{1}H-NMR Characterization of CDCM-8

The ^{1}H-NMR spectra of isolated compound in the (Appendix figure19) showed three clearly separated regions of protons in which the amide proton was in the middle, whereas the two others separated regions constituted the aromatic proton and the aliphatic protons. The ^{1}H-NMR spectrum showed a singlet at δ 1.289 due to $COCH_3$ group. Methylene protons at C5 and C6 showed multiplets between δ 1.997-2.306. The methoxy protons showed the signals at δ 3.443, 3.627, 3.657, 3.859, 3.896, 3.929, 3.988 and 4.062. The aromatic protons at C4 and C8 showed singlets at δ 6.55 and 7.63, respectively while those at C11 and C12 showed doublets and quartet at δ6.9 and 7.4, respectively. The signal due to NH proton appears as a doublet at δ 6.7.

4.15.2.2. ^{13}C-NMR ($CDCl_3$, TMS)

The isolated compound of CDCM-8 (Colchicine) spectrum in the (Appendix figure 20) consisted of many signals. The signals at δC 185.657, 171.251 and 170.251 were due to the presence of the carbonyl carbon of ketone, amide, and aromatic carbon. Those signals seen from 162.679, to δC 112.483 were because of the carbon-carbon double bonds in the isolated compound. The signals from

81.020 to δC 50.495 in the spectrum were the characteristic of carbon-oxygen single bond and those signals from 44.646 to δC 44.227 were as a result of the carbon-carbon single bonds in the compound.

4.15.2.3. Mass Spectrum of CDCM-8

The isolated compound CDCM-8 was colchicine and the estimated molecular formula of this compound was $C_{22}H_7NO_6$ (fragments and molecular ion at m/z 341.15, 372.19, 400.19 and 401.19). The proposed structure of isolated compound of CS-8 was: -

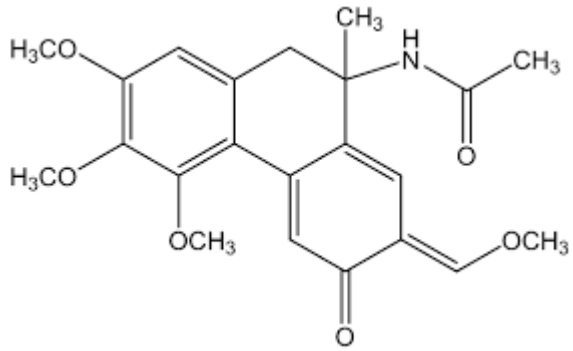


Figure 36: The proposed structure of isolated compound is Colchicine

Molecular Weight: 401.19 g/mol and molecular formula: $C_{22}H_7NO_6$

4.16. Characterization of Isolated Compound CDCM-12

4.16.1. FT-IR Characterization of Isolated Compound CDCM-12

FT-IR spectral data of isolated compound in the (Appendix figure 22) showed the presence of different types of functional groups which absorb at different wave numbers. The broad band at 3381.09 cm^{-1} in its FT-IR spectrum was suggested to the O-H functional group in the isolated compound. The absorption band at 2963.69 cm^{-1} was because of methyl C-H vibrations stretching in the isolated compound. The absorption at 1639.10 cm^{-1} was due to the presence of carbonyl group and the band at 1412.35 cm^{-1} was because of the characteristic methyl bending vibration; and the absorption bands at 1146.25 cm^{-1} was because of the C-O stretching vibration. Similarly, vibrational absorption bands at 866.39, 703.86 and 631.62 cm^{-1} were due to substituents in the isolated compound.

Table 29: IR absorption frequencies of functional groups in CDCM-12

Peaks (Wave Number)	Functional groups
3381.09	Hydroxyl, phenolic groups
2963.69	Alkyl C-H (stretching)
1639.10	Carbonyl group
1412.35, 1261.74	CH_3 bending vibration
1146.25	C-O stretch
866.39, 703.86, 631.62	Substituent on the aromatic ring of the compound

4.16.2. NMR Characterization of Isolated Compound of CDCM-12

4.16.2.1. ^{1}H-NMR Characterization of CDCM-12

The proton spectra of isolated compound in the (Appendix figure 23) shows three clearly separated regions of protons in which the hydroxyl proton is in the middle, whereas the two others separated regions constitute the aromatic proton and the aliphatic protons.

4.16.2.2. ^{13}C-NMR ($CDCl_3$, TMS)

The ^{13}C-NMR spectrum of CDCM-12 (Appendix figure-24) consisted of many signals. The signal at δC 140 and 115 indicated the presence of the carbonyl carbon and aromatic ring. Those signals seen at δC 50.322 were due to the presence of carbon-oxygen single bonds. The signals from 31.893 to δC 0.971 in the spectrum were characteristic of methine and methylene carbon atoms in the compound.

4.16.2.3. Mass Spectrum of Isolated Compound CDCM-12

The isolated compound showed the different kinds of fragmentation and molecular ion at m/z 136.0676, 203.0630, 268.1152, 316.1369, 327.0918, 372.1955, 420.1914, 401.1962, 474.2305, 485.3667, 546.4237 & 548.2352.

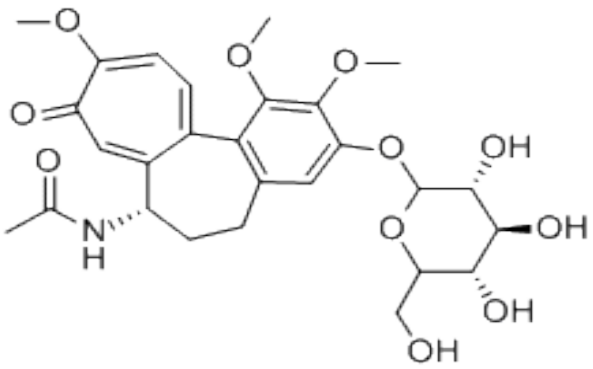


Figure 37: The proposed structure of isolated compound is 3-demethylcolchicine glucoside

Molecular Weight: 548.4237 g/mol and Molecular Formula: $C_{27}H_{34}O_{11}N$

4.17. Docking studies

Various interaction conformations of both the targets and compounds were studied by AutoDock 4.2.1. The *in-silico* studies of present analysis indicated that *colchicoside* is strong inhibitor of IL-6 having binding energy -7.1 kcal/mol with RMSD value of 0.00 for both lower and upper bound (Figure 38), compared to diclofenac having binding energy -6.1 kcal/mol. In case of IL-17, *colchicoside* again showed minimum binding energy (-6.5 kcal/mol) as compared to standard drug diclofenac which showed binding affinity of -4.7 kcal/mol. There are polar contacts between protein and the ligand. List of binding energy and RMSD values has been summarized in Table 23. These results indicated that *Colchicum autumnale* L and equal amount of methanolic: acetonitrile: water (with 1:1:1 combination of three solvents) extract was having the great potential to inhibit main instigator cytokines (IL-6, TNF-α, IL-17) of inflammation and hence may be investigated for future drug design study.

Table 30: Representation of binding energy and RMSD

	Affinity (kcal/mol)			RMSD* (l.b./u.b.) **		
	TNF-α	IL-6	IL-17	TNF-α	IL-6	IL-17
Colchicine	-5.9	-6.4	-6.2	0.00/0.00	0.00/0.00	0.00/0.00

Colchicoside	-6.2	-7.1	-6.5	0.00/0.00	0.00/0.00	0.00/0.00
Deacetamido-5, 6-Dihydrocolchicine	-5.7	-6.5	5.1	0.00/0.00	0.00/0.00	0.00/0.00
Deacetyl Colchicine	-6.1	-6.4	-5.3	0.00/0.00	0.00/0.00	0.00/0.00
Declofenac	-5.6	-6.1	-4.7	0.00/0.00	0.00/0.00	0.00/0.00

*Root mean square deviation; **l.b. = lower bound; u.b. = upper bound

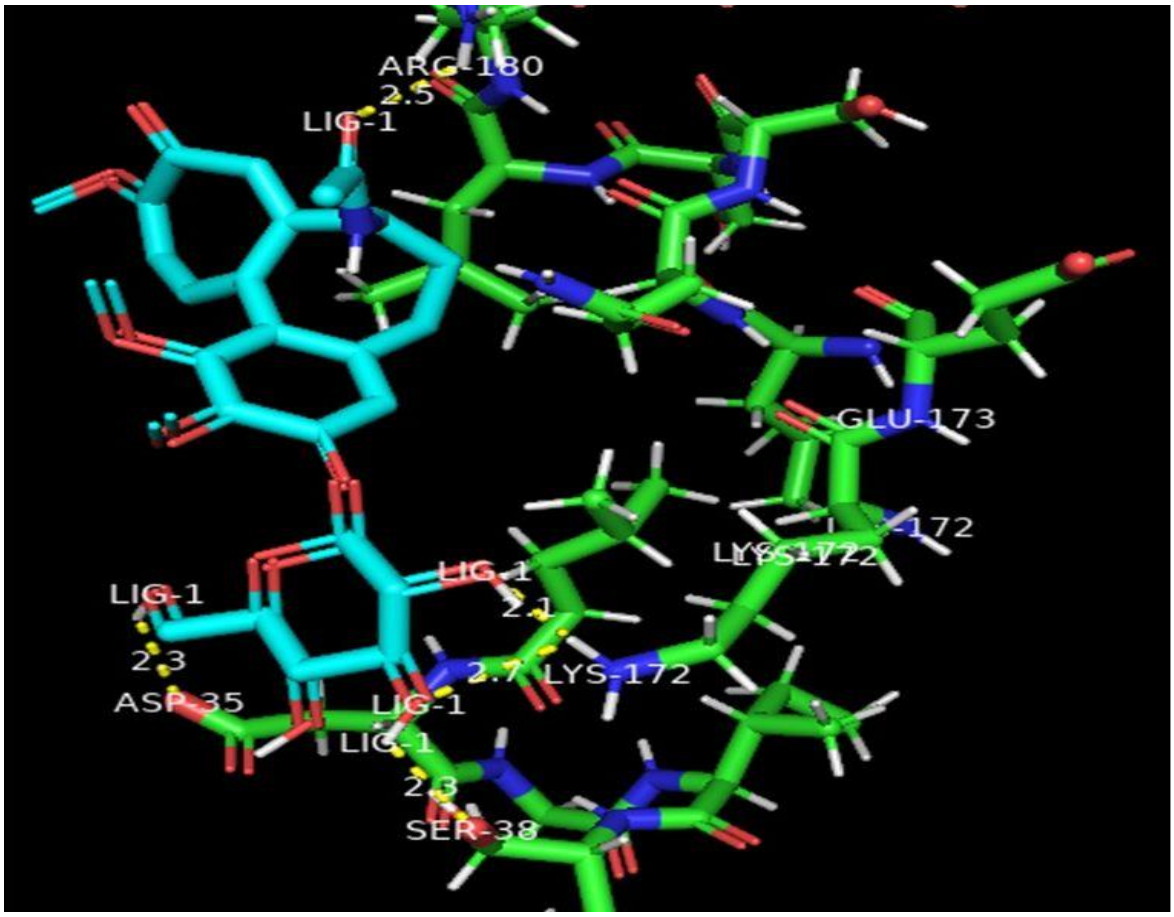


Figure 38: Representative image of binding of compound *colchicoside* (Ligand) with protein IL-6 (Receptor) showing polar contacts with distance in ºA and residues of binding.

CHAPTER 5: CONCLUSION AND FUTURE WORK

Medicinal plants have been used in daily life to treat different types of diseases all over the world and they are the major source of secondary metabolites and an important source of pharmaceutical drugs. The current research work was conducted to carry out phytochemicals and pharmacological investigation of *Sisymbrium irio* L and *Colchicum autumnale* L collected from New Delhi, India. From literature reviews both the plants have a wide use in Unani medicine. The plants material of *Sisymbrium irio* L and *Colchicum autumnale* L were collected from Indian Drugs House, Khari Baoli, Delhi. The Botanical specimens of the plant were identified by Dr. Mokhtar Alam, Central Council for Research in Ayurvedic Sciences, Ministry of Ayush, Government of India, and National Institute of Science Communication and Information Resources, New Delhi and the voucher specimen/file number 6238 and SC-0171/15 were deposited at the Ministry of Ayush, Government of India and Raw Material Herbarium and Museum, Delhi (RHMD).

500 g powdered seed of *Sisymbrium irio* L and *Colchicum autumnale* L were extracted with 5 L of n-hexane, dichloromethane and methanol for 5-8 hrs in Soxhlet apparatus consecutively. Then, the extracts were dried with 5 g of anhydrous sodium sulfate and filtered & concentrated with a rotary evaporator under reduced pressure at 40℃. Then, all extracts were kept at 4℃ until investigation.

The medicinal properties of *Sisymbrium irio* L and *Colchicum autumnale* L served as a good candidate for pharmacological investigation of all extracts to test, antioxidant, antibacterial, antiviral activity using DPPH, ABTS, paper disc diffusion and MTT assay, whereas phytochemicals analysis or screening of extracts were performed using both qualitative and quantitative test, GC-MS, UHPLC-MS/MS for metabolites analysis and structure elucidation and characterization of isolated compounds were done via FT-IR, ^{1}H-NMR, ^{13}C-NMR and MS.

Qualitative phytochemicals investigation of the extracts of plants seeds exhibited the existence of flavonoids, phenols, saponins, terpenoids & another bioactive metabolite. Quantitative investigation of *Sisymbrium irio* L seed extracts confirmed that methanol extract contained the highest number of flavonoid contents and phenolic contents, followed by dichloromethane and n-hexane, whereas, the dichloromethane extract of *Colchicum autumnale* L contained the highest amount of flavonoid and phenolic contents, followed by methanol and n-hexane extracts. The phytochemicals constituents are responsible for its antioxidant, antimicrobial and anti dengue activity and other medicinal uses. Antioxidant analysis of the methanol extract of *Sisymbrium irio* L exhibited the highest percentage of free radical scavenging activity in comparison to n-hexane & dichloromethane extracts, while dichloromethane extract of *Colchicum autumnale* L exhibited the highest percentage of free radical scavenging activity in comparison to n-hexane and dichloromethane extracts. Over all, antioxidant results demonstrated that both plants are a good source of phenolic, flavonoid and other phytochemicals that have a significant role in free radical scavenging. Hence, they serve as antioxidants agents for the scavenging of free radicals. Antibacterial activity of n-hexane, dichloromethane, and methanol extracts were evaluated *in vitro* against three bacterial species such as *E. coli*, *B. subtilis*, and *P. aeruginosa*, and they showed dose dependent antibacterial activity. The *Sisymbrium irio* L research study exhibited that dichloromethane, ethanol and water extracts derived from *Sisymbrium irio* L vero cell line *in vitro* possess clear antiviral activity. The results suggested *Sisymbrium irio* L could be an interesting source of natural antiviral substances with potential use in medicine and this therapeutic plant showed anti-dengue activity. The elemental analysis (ICP-MS analysis) of the *Sisymbrium irio* L and *Colchicum autumnale* L showed the presence of magnesium, calcium, strontium, titanium, chromium, manganese, copper, zinc, boron, aluminium, silicon, barium, iron, phosphorus, sodium, potassium and lead and also useful for the health of human beings.

Phytochemical examination of the different extracts namely n-hexane, dichloromethane and methanol identified by GC-MS revealed the presence of: :- 3', 5'-Dimethoxyacetophenone, n-

Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 3-Methoxy Phenol, 2, 6-Dimethoxy-4-(2-propenyl)-phenol, 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z), 9, 12-Octadecenoic acid, methyl ester, 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester, (E)-9-Octadecenoic acid ethyl ester, Linoleic acid ethyl ester, 7-Tetradecenal, (Z)-, 3-Methoxy-5-Hydroxymethylfurfural phenol, 8, 11, 14-Docosatrienoic acid, methyl ester, beta- Sitosterol, etc. While the chemical compounds identified from *Sisymbrium irio* L and *Colchicum autumnale* L extracts by using UHPLC were: - Apigenin (fragment and molecular ion at m/z (84.9603, 102.9706, 153.0181, 234.9608, 242.9064 & 271.0594), Apigenin 7-O-glucoside at m/z (116.9861, 125.9863, 134.9964, 158.0123,167.0225, 204.8886, 214.9173, 24.9435, 285.1092, 310.1643, 342.8278, 397.0963 & 433.1122), (17ξ)-Stigmast-5-en-3-yl β-D-glucopyranoside at m/z (413.2658, 431.3128, 441.2519, 447.2693, 497.3441, 522.3550, 549.3755 & 577.4496), Isorhamnetin at m/z (254.9254, 256.9223, 264.9541, 270.9105, 272.8764, 278.9698, 284.8897, 291.1112, 296.0909, 302.9003, 305.0963, 309.0277 & 317.0651), Isorhamnetin-3-O-neohesperidine at m/z (621.8384, 622.1107, 623.1118, 624.0725 & 625.1758), Isorhamnetin 7-O-beta-D-glucopryanoside, Isorhamnetin 7-glucoside at m/z (441.2984, 455.1317, 469.1440 & 479.1181), Isorhamnetin-3-Laminaribioside at m/z (630.8198, 633.1403, 637.1788 & 641.1704). The compound colchicine was detected at Rt = 7.36min and it exhibited a protonated molecular ion $[M+H]^+$at m/z 400.1753; and it also generated different types of fragment ion at m/z of 68.7195, 98.4443, 127.2563, 170.5951, 239.1045, 239.1045, 267.1000, 310.1177, 358.1649 and 382.1639. The compound (R/S)-Deacetyl colchicine was detected at Rt = 5.07min; it displayed a protonated molecular ion $[M+H]^+$ at m/z 358.1647 and it also generated fragmention m/z 110.0095, 116.9872, 125.9871, 137.1074, 141.9587, 173.9847, 213.1022, 232.9297, 248.9004, 295.0490, 313.1672, 327.1226 and 344.1855. The compound 3-Demethyl colchicine was detected at Rt = 6.50min and it exhibited a protonated molecular ion $[M+H]^+$ at m/z 386.1594; it also generated fragment ion at m/z 68.8835, 84.9604, 102.9704, 158.9273, 176.9383, 207.0796, 232.9276, 250.9386, 295.0956, 344.1490, 368.1474. The compound Colchicoside (3-Demethyl colchicine glucoside) was detected at

Rt = 5.27min; it displayed a protonated ion $[M+H]^+$at m/z 548.2120 and it also generated fragment ion at 84.9598, 114.1461, 149.0593, 235.0750, 267.1013, 295.0945, 344.1479, 368.1482, 386.1591 and 405.0515. The compound Deacetamido-5, 6-Dihydrocolchicine was detected at Rt = 6.01min; it showed a protonated ion $[M+H]^+$at m/z of 341.1382, and it also generated the fragment ion at m/z of 102.9707, 116.9862, 125.9864, 134.9965, 149.0122, 158.0125, 167.0225, 198.9399, 204.887, 214.9175, 232.9281, and 264.9542. Phytochemicals GC-MS and UHPLC-QExactive Orbitrap analysis and molecular docking results revealed the *Sisymbrium irio* L and *Colchicum autumnale* L extracts were accountable for numerous therapeutic uses such as anti-oxidant, anti-bacterial and anti-inflammatory activities. The anti-inflammatory activity suggests that *Colchicum autumnale* L can be used for relieving the symptoms of inflammation. Comparative docking studies revealed colchicoside as most potent anti-inflammatory compound with minimum binding energy (affinity) as compared to other compounds and the standard drug diclofenac. Bioassay-guided effort yielded a compound which after characterization using UHPLC-QExactive Orbitrap, FT-IR, ^{1}H-NMR, ^{13}CNMR and MS identified as a class of apigenin, isorhamnetin derivative, and colchicine derivative. Thus, spectroscopic techniques play a pivotal role in the isolation, identification and investigation of natural products for the discovery of new drugs. Hence, phytochemical analysis confirmed that *Sisymbrium irio* L and *Colchicum autumnale* L extracts contained various types of bioactive compounds. Antioxidant, antimicrobial, anti-inflammatory and anti-dengue agents derived from the *Sisymbrium irio* L and *Colchicum autumnale* L source can lead to the development of new drugs. So, it can be concluded that therapeutic medicinal plants and its derivatives are active to against various type of diseases. Herbal drug treatment may be recommended to the rural and poor people to treat effectively the microbial, inflammatory and dengue cases as the overall treatment would be cheaper.

Screening of therapeutic plants for antioxidant, antimicrobial, anti-inflammatory and anti-dengue activity provides a huge space for development of strong agents for the treatment of aforementioned diseases. Further, *Sisymbrium irio* L and *Colchicum autumnale* L must be explored more through *in vivo* tests in order to determine the mechanisms of action.

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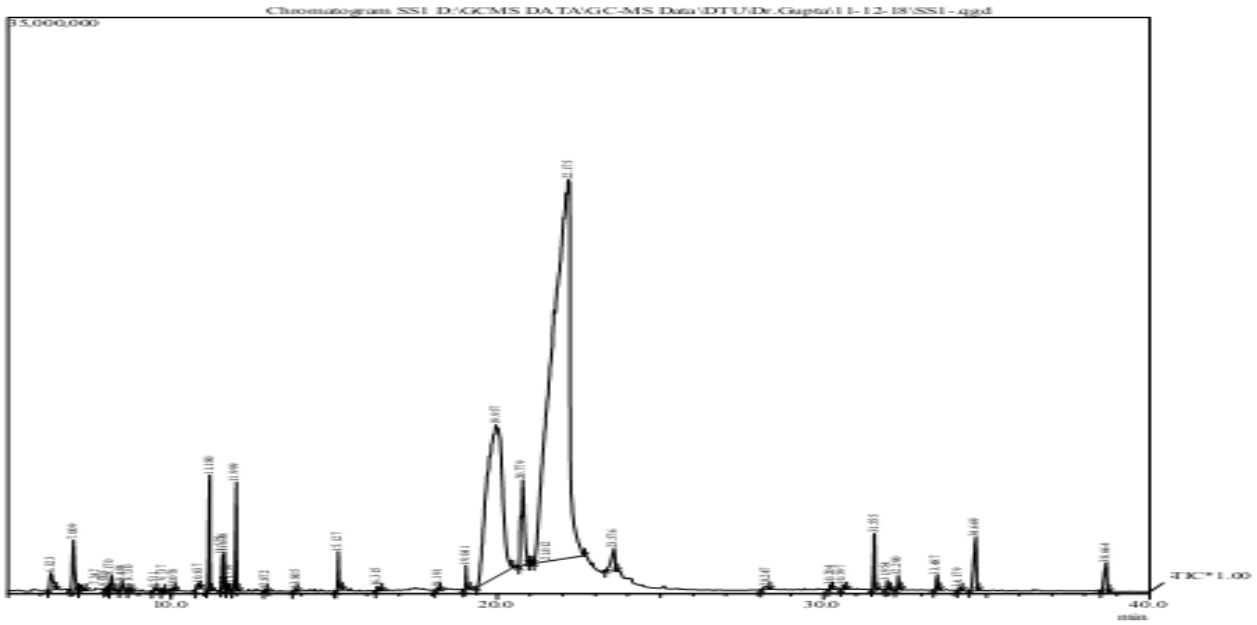
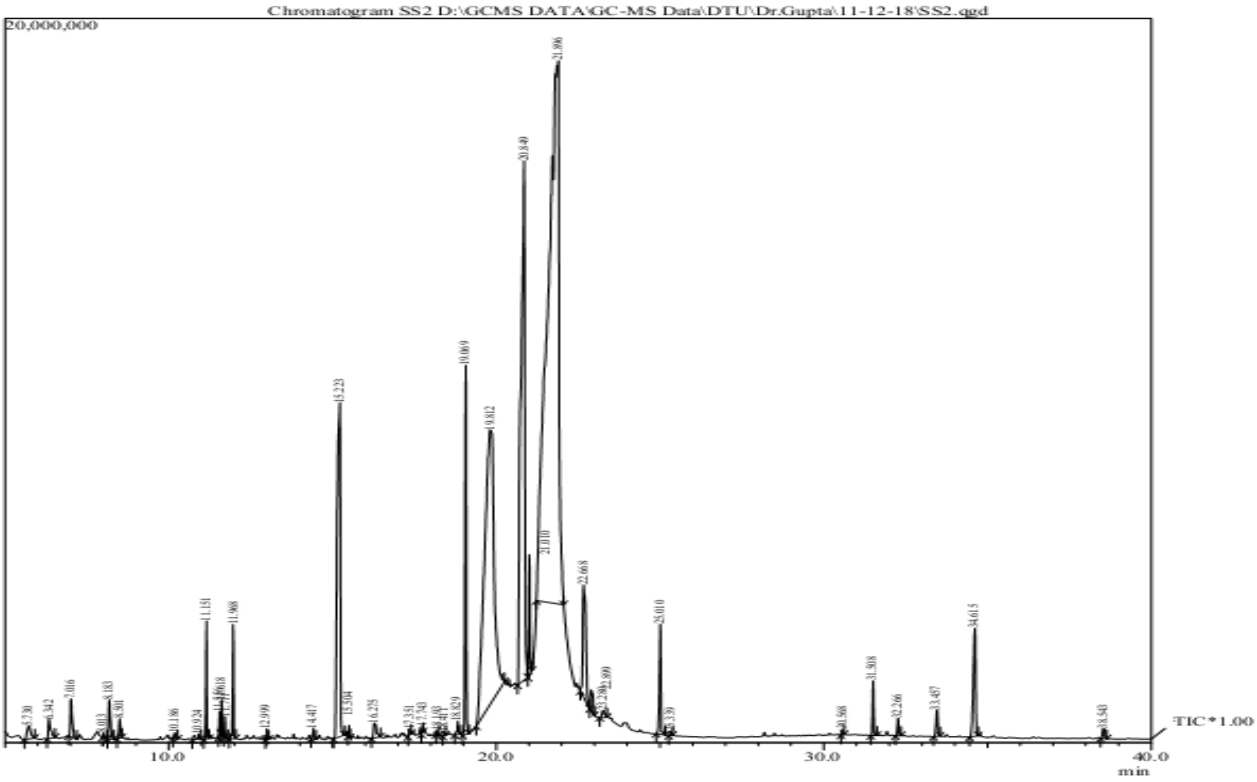
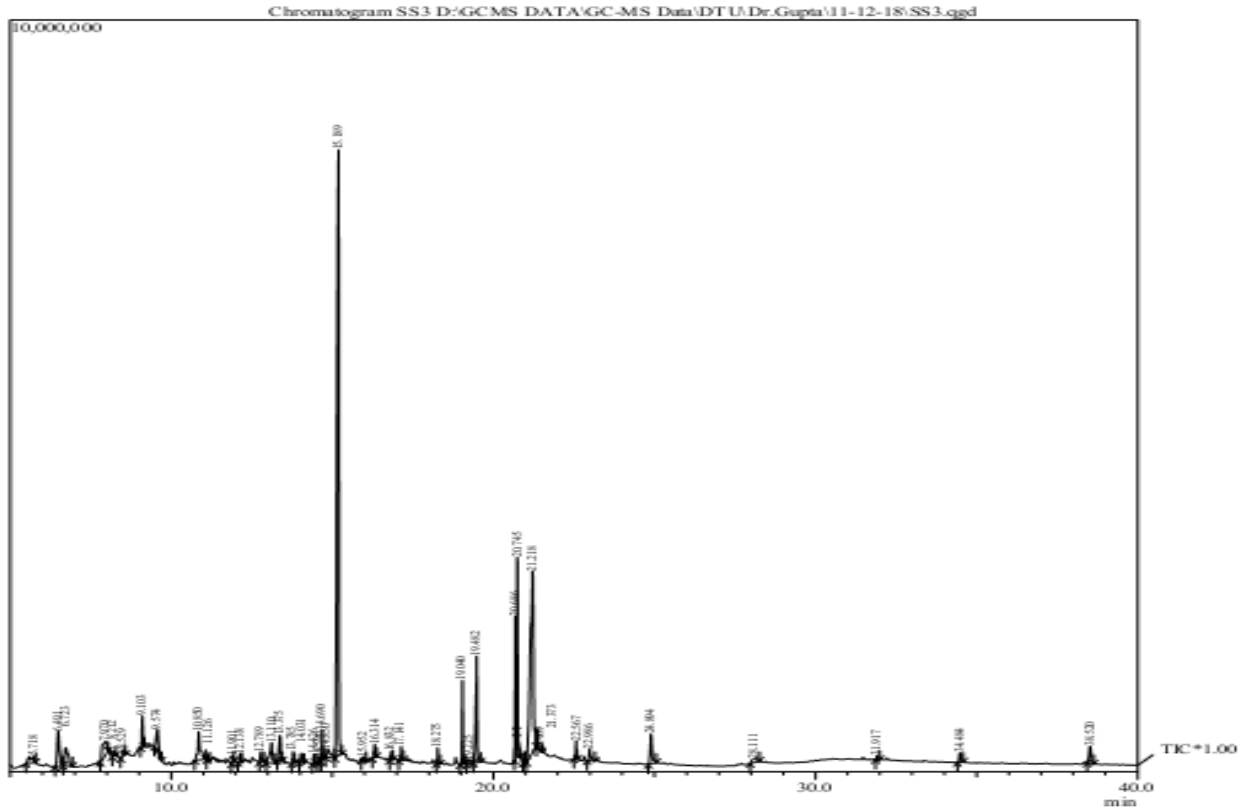
Appendix 7



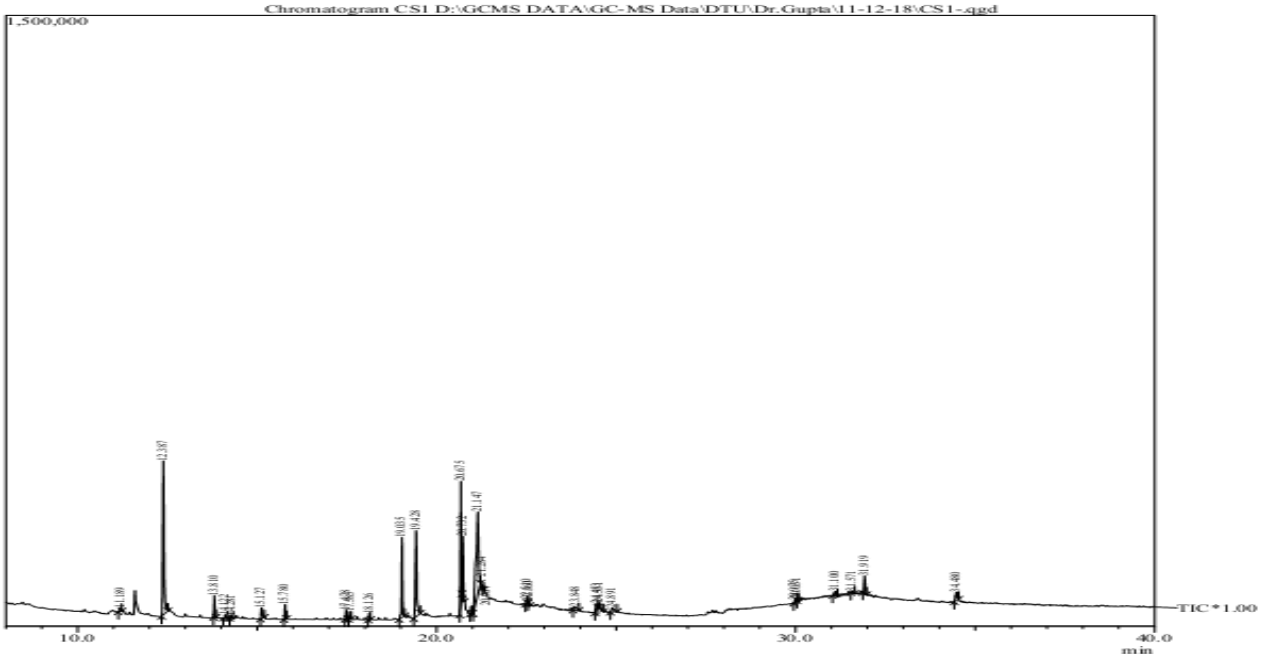
Peak no. 1: GC-chromatogram of n-hexane crude extract of *Sisymbrium irio* L



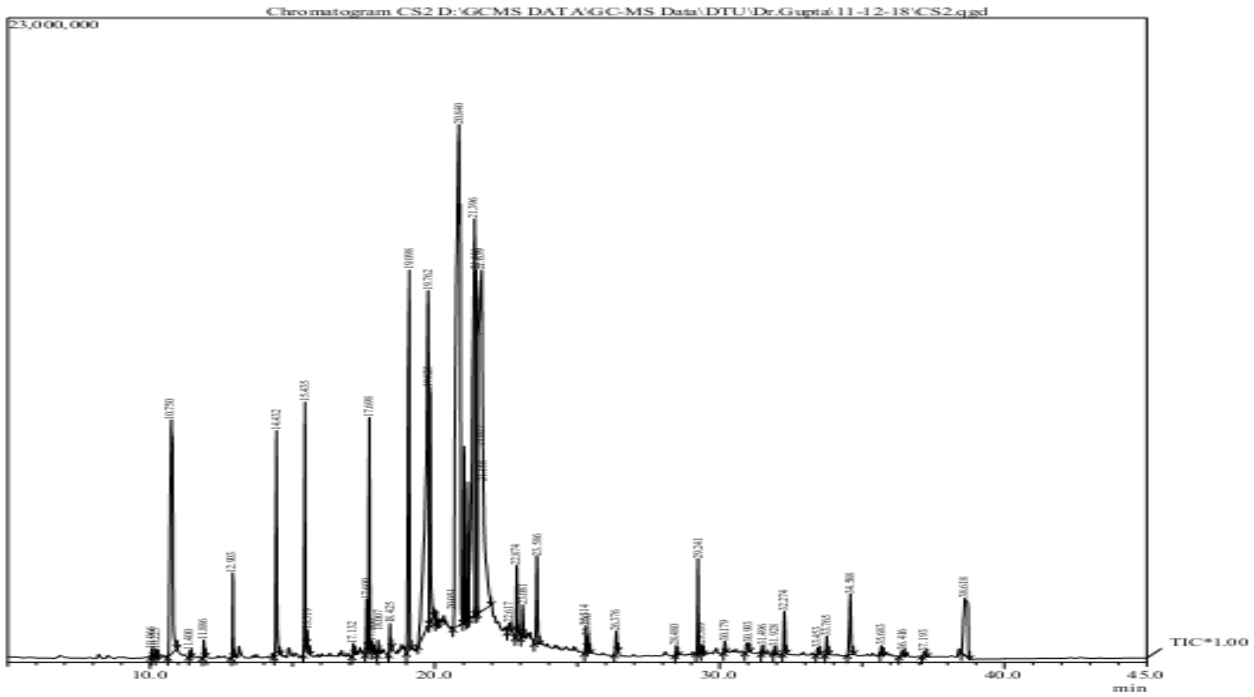
Peak no. 2: GC-chromatogram of dichloromethane crude extract of *Sisymbrium irio* L



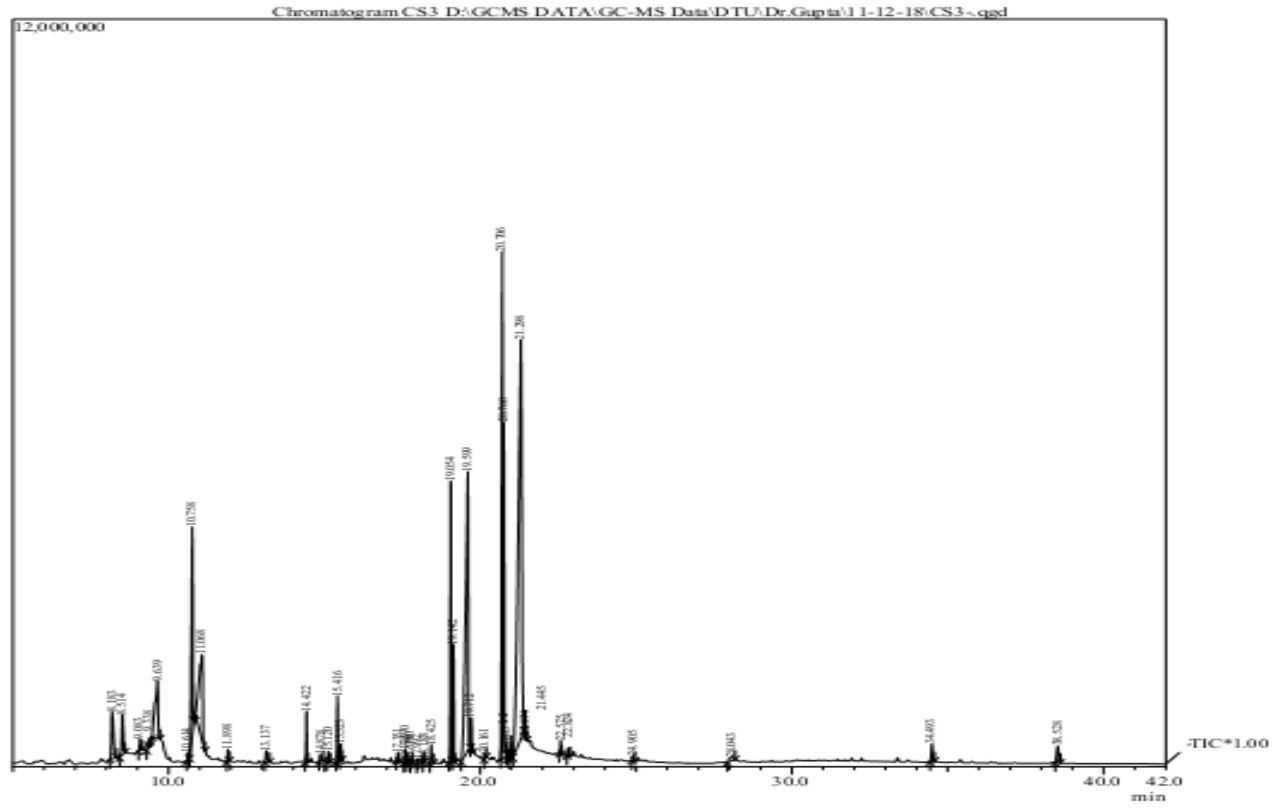
Peak no. 3: GC-chromatogram of methanol crude extract of *Sisymbrium irio* L



Peak no. 4: GC-chromatogram of n-hexane crude extract of *Colchicum autumnale* L



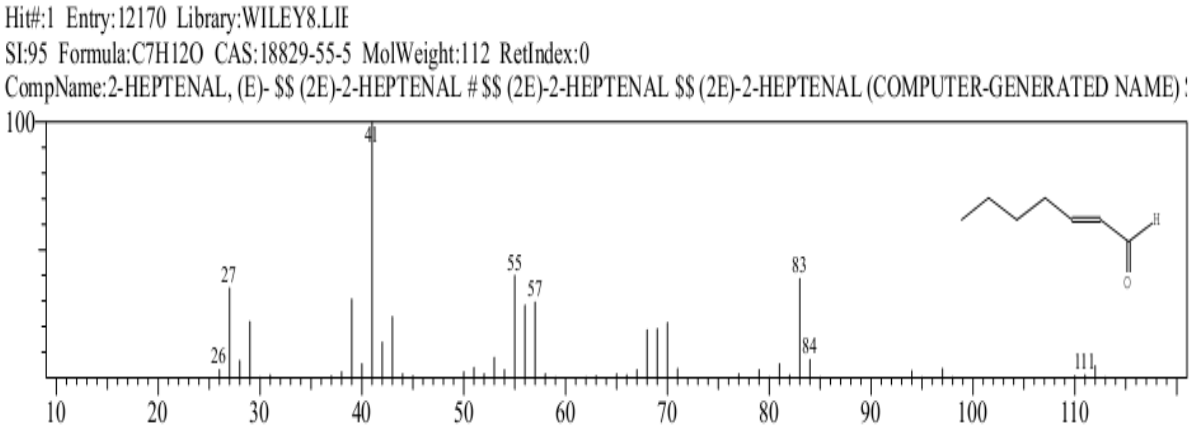
Peak no. 5: GC-chromatogram of dichloromethane crude extract of *Colchicum autumnale* L



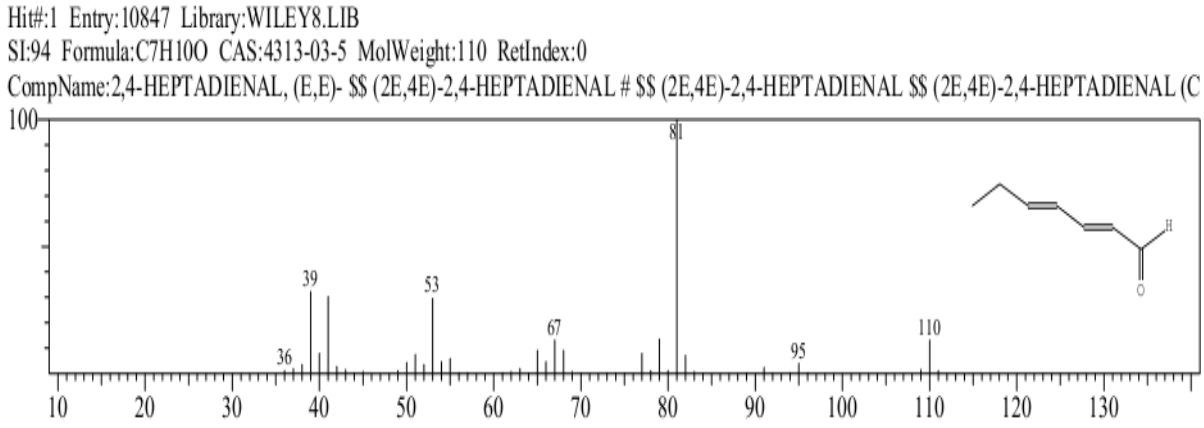
Peak no. 6: GC-chromatogram of methanol crude extract of *Colchicum autumnale* L seed

Appendix figure 1. Gas chromatogram of the components of *Sisymbrium irio* L and *Colchicum autumnale* L

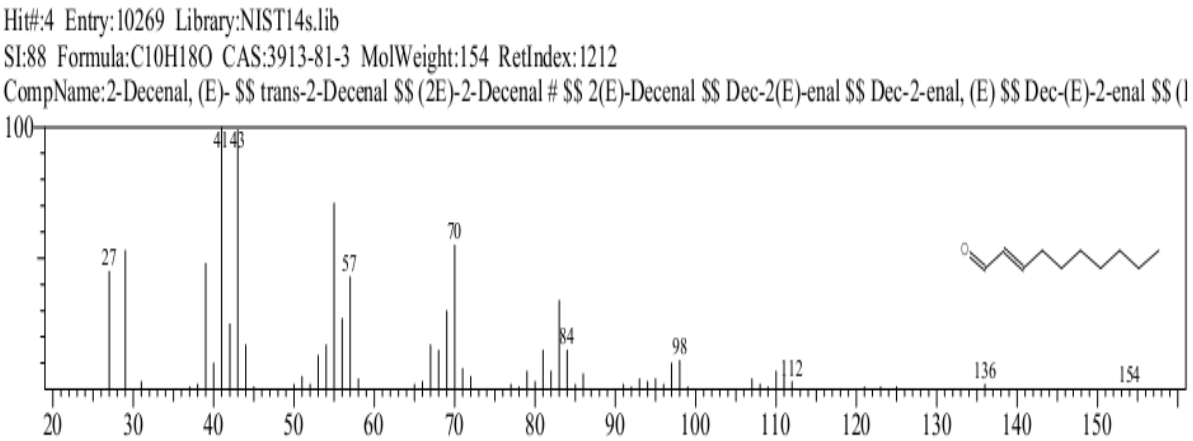
The major mass spectrum (m/z) of n-Hexane seed crude extracts of *Sisymbrium irio* were:



Peak no. 7: 2-Heptenal



Peak no. 8: 2, 4-Heptadienal, (E, E)



Peak no. 9 :2-Decenal

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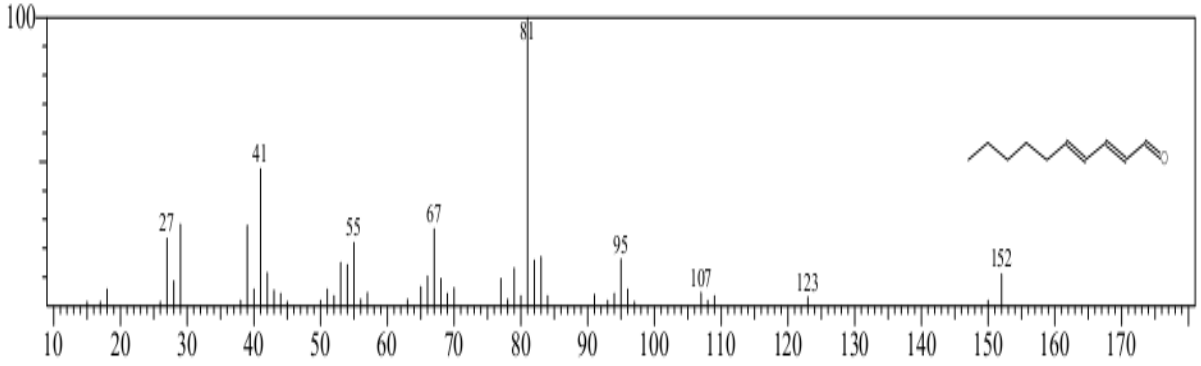

Peak no. 10: 2, 4-Decadienal, (E, E)-

Hit#:1 Entry:25118 Library:NIST14s.lib
SI:94 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
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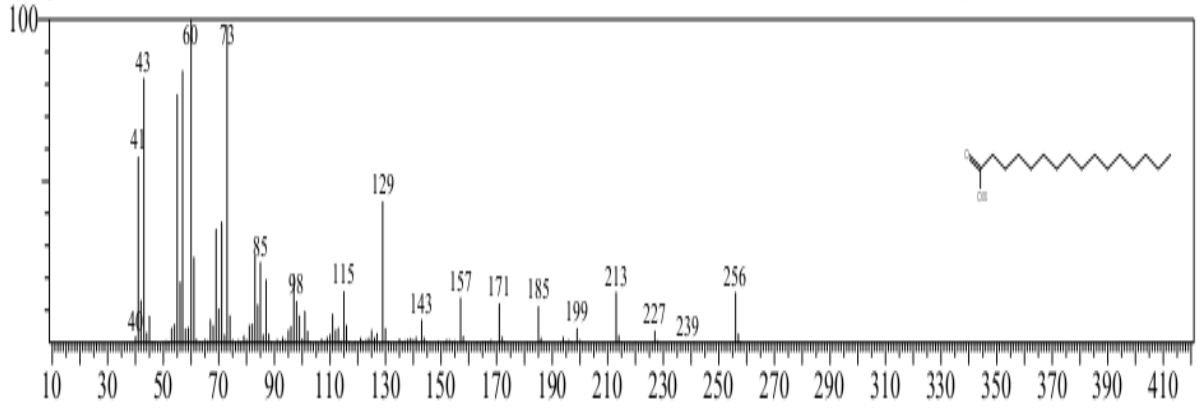

Peak no. 11: n-Hexadecanoic acid

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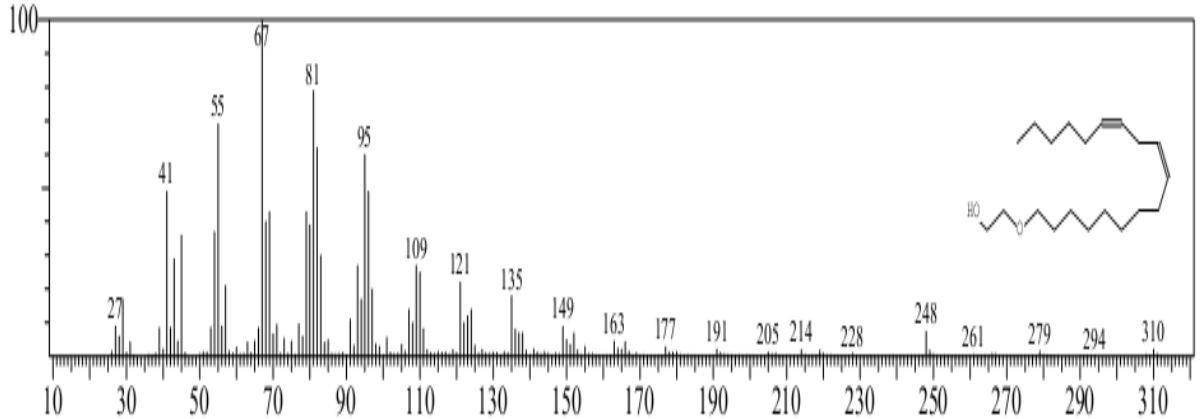
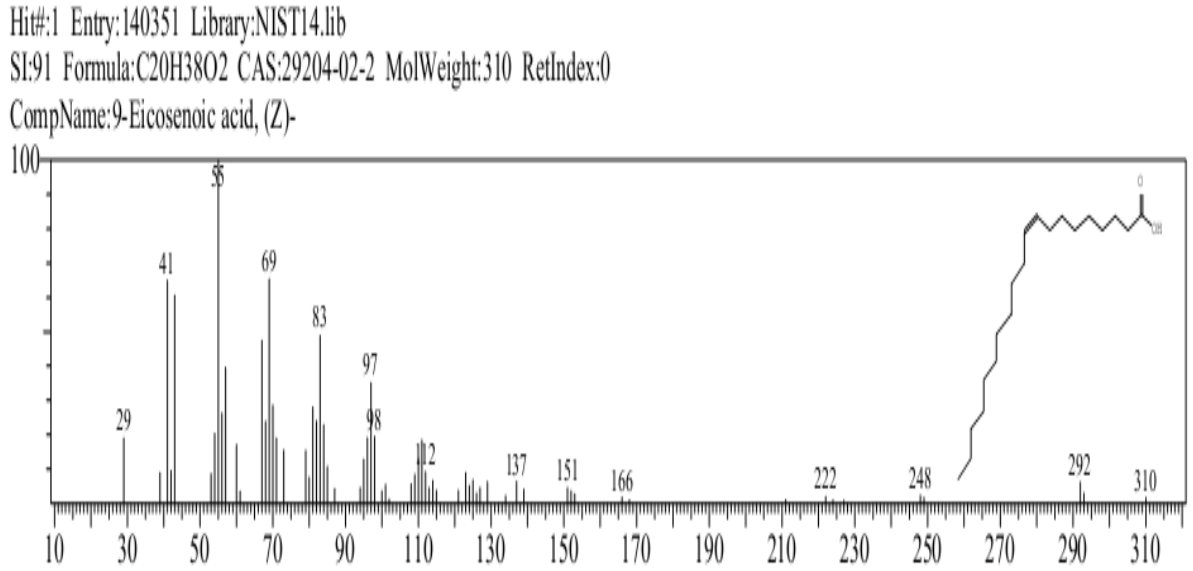
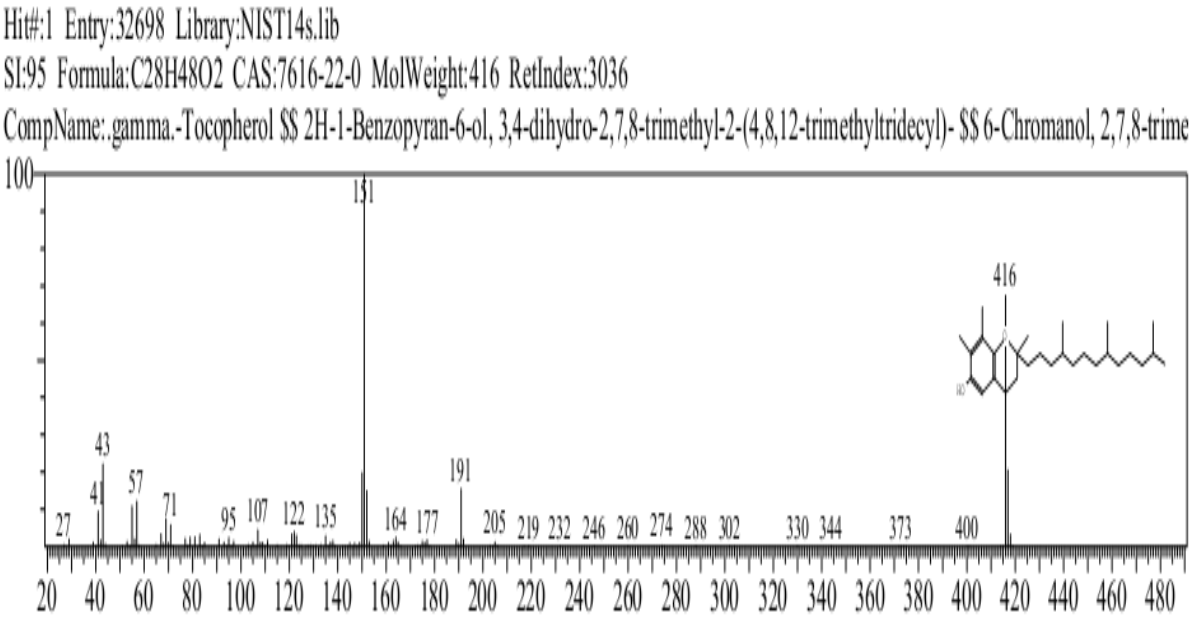
CompName:Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- $$ 2-cis,cis-9,12-Octadecadienyloxyethanol $$ 2-[(9Z,12Z)-9,12-Octadecadienyloxy]eth



Peak no. 12: Ethanol, 2-(9, 12-Octadecadienyloxy)-, (Z, Z)-

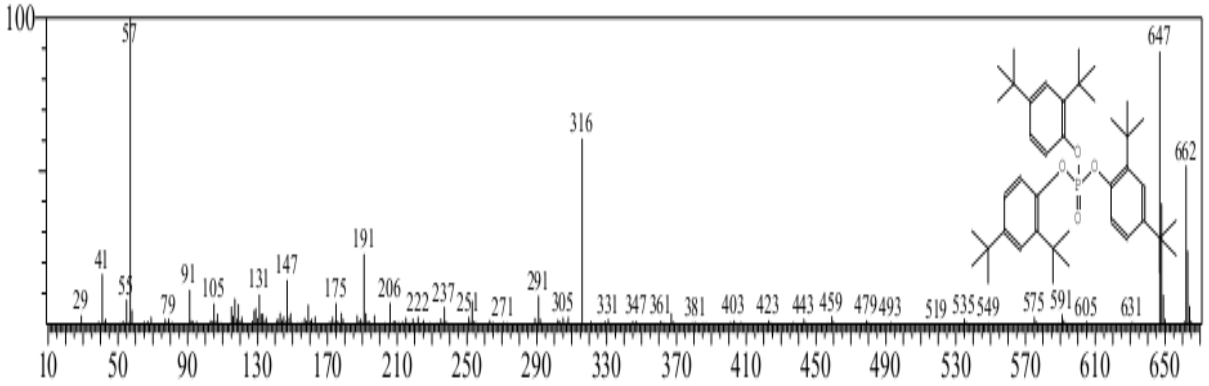


Peak no. 13: 9-Eicosenoic acid, (Z)-



Peak no. 14: gamma, -Tocopherol

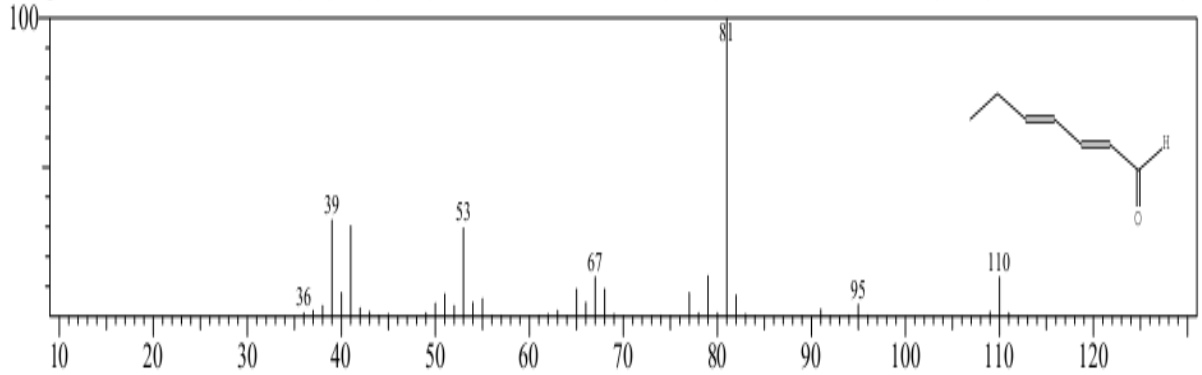
Hit#:1 Entry:240877 Library:NIST14.lit
SI:76 Formula:C42H63O4P CAS:95906-11-9 MolWeight:662 RetIndex:0
CompName:Tris(2,4-di-tert-butylphenyl) phosphate $$ Phenol, 2,4-bis(1,1-dimethylethyl)-, 1,1',1''-phosphate $$ Phenol, 2,4-bis(1,1-dimethylethyl



Peak no. 15: Tris (2, 4-di-tert-buty phenyl) phosphate

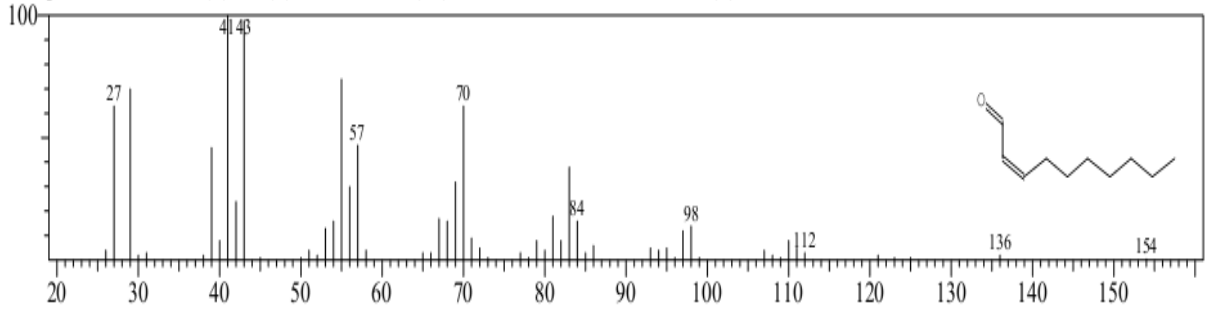
The major mass spectrum (m/z) of dichloromethane seed crude extracts of *Sisymbrium irio* were:

Hit#:1 Entry:10847 Library:WILEY8.LIB
SI:92 Formula:C7H10O CAS:4313-03-5 MolWeight:110 RetIndex:0
CompName:2,4-HEPTADIENAL, (E,E)- $$ (2E,4E)-2,4-HEPTADIENAL # $$ (2E,4E)-2,4-HEPTADIENAL $$ (2E,4E)-2,4-HEPTADIENAL (C

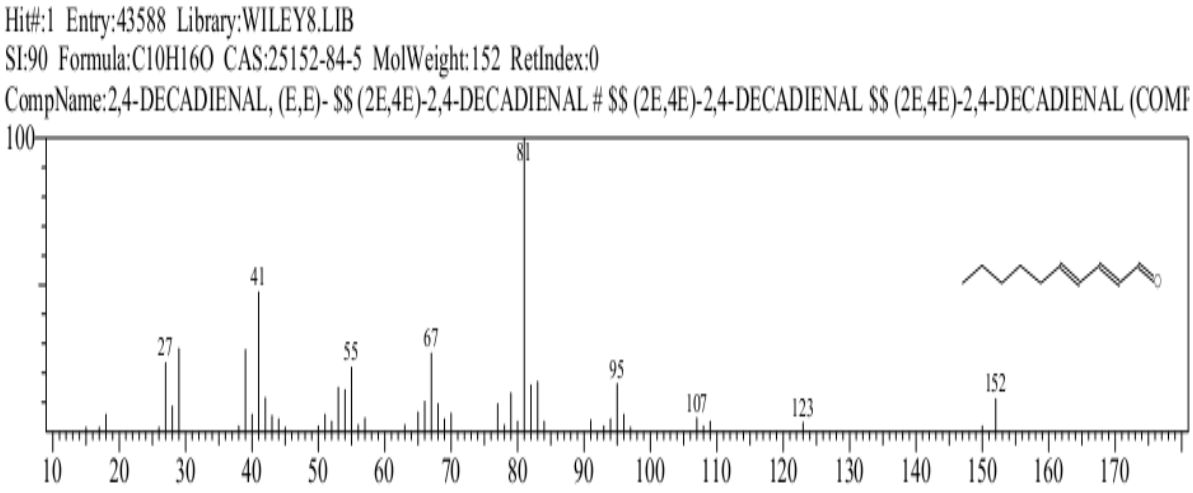


Peak no. 16: 2, 4-Heptadienal, (E, E)-

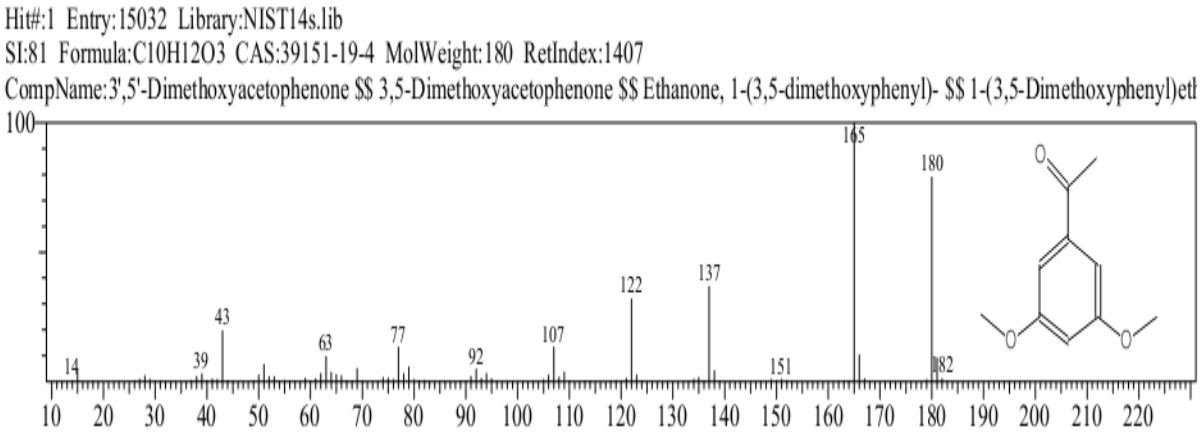
Hit#:1 Entry:17926 Library:NIST14.lib
SI:97 Formula:C10H18O CAS:2497-25-8 MolWeight:154 RetIndex:1212
CompName:2-Decenal, (Z)- $$ (Z)-2-Decenal $$ (2Z)-2-Decenal # $$ 2Z-Decenal $$ (Z)-Dec-2-enal $$



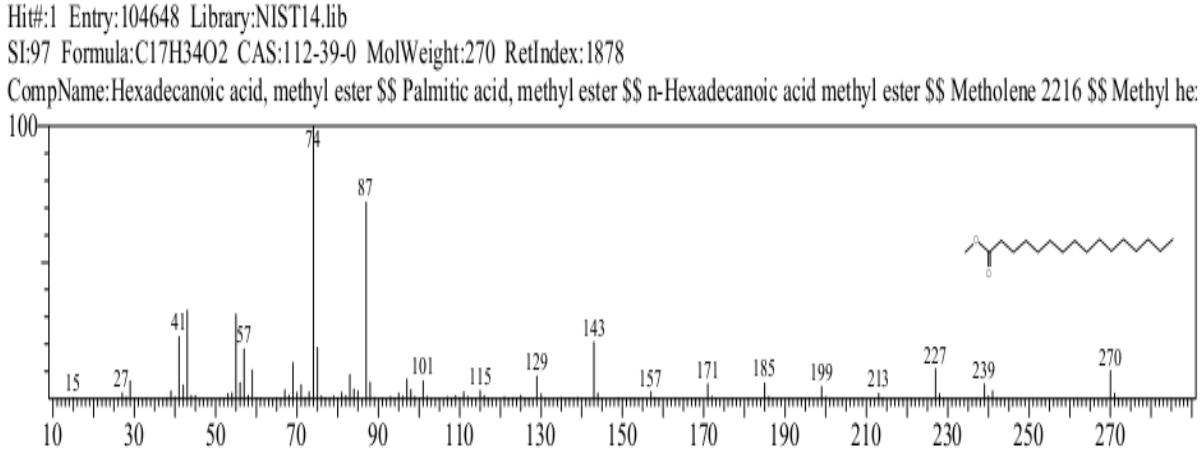
Peak no. 17: 2-Decenal, (Z)-



Peak no. 18: 2, 4- Decadienal, (E, E)-

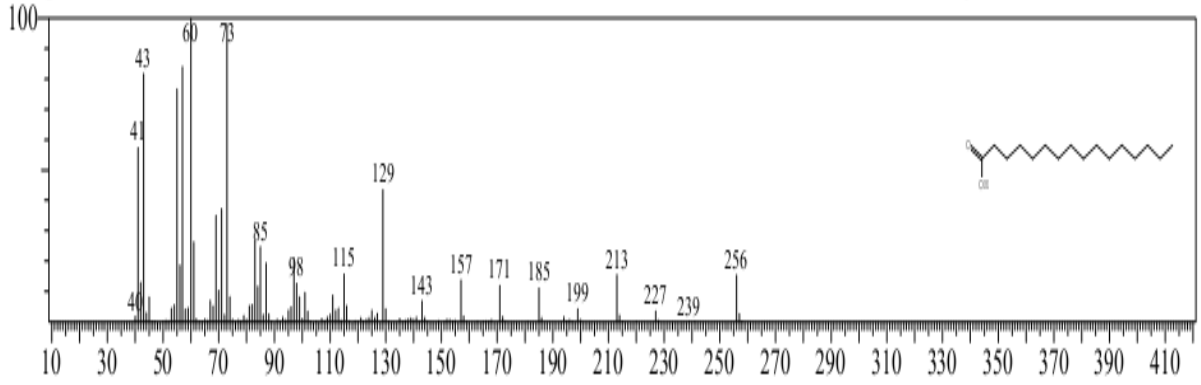


Peak no. 19: 3', 5'-Dimethoxyacetophenone



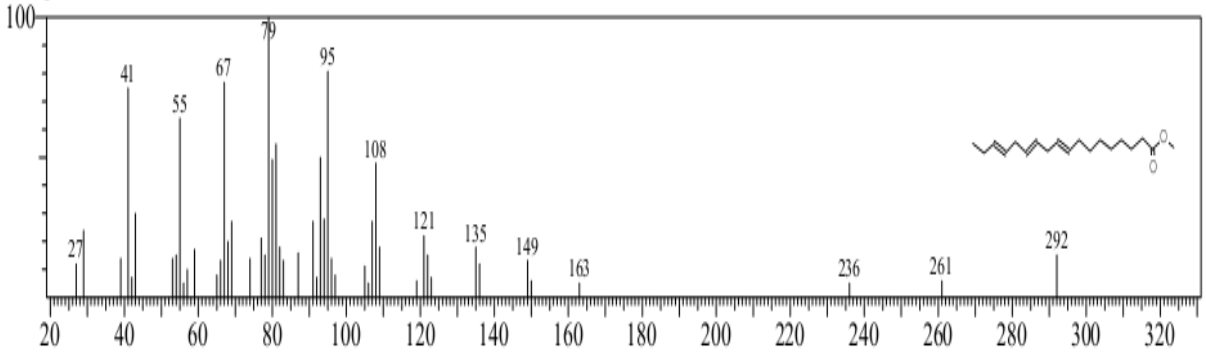
Peak no. 20: Hexadecanoic acid, methyl ester

Hit#:1 Entry:25118 Library:NIST14s.lib
SI:95 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecaneca



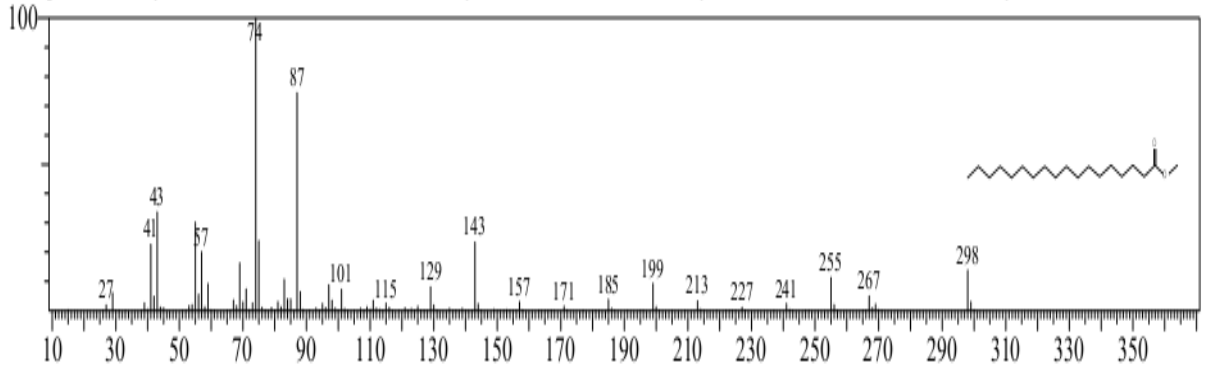
Peak no. 21: n-Hexadecanoic acid

Hit#:1 Entry:230319 Library:WILEY8.LIB
SI:90 Formula:C19H32O2 CAS:7361-80-0 MolWeight:292 RetIndex:0
CompName:9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER $$ METHYL (9E,12E,15E)-9,12,15-OCTADECATRIENOATE # $$ ME



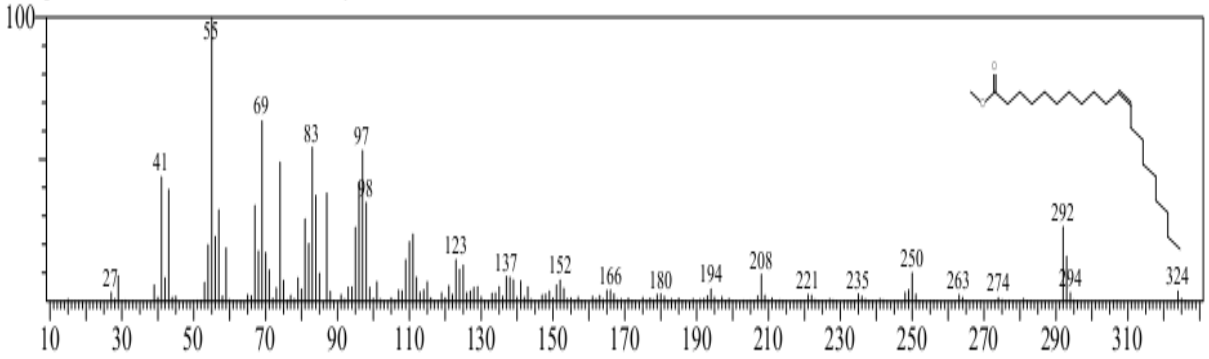
Peak no. 22: 9, 12, 15-Octadecatrienoic acid, methyl ester

Hit#:1 Entry:129694 Library:NIST14.lib
SI:95 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077
CompName:Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemester 971



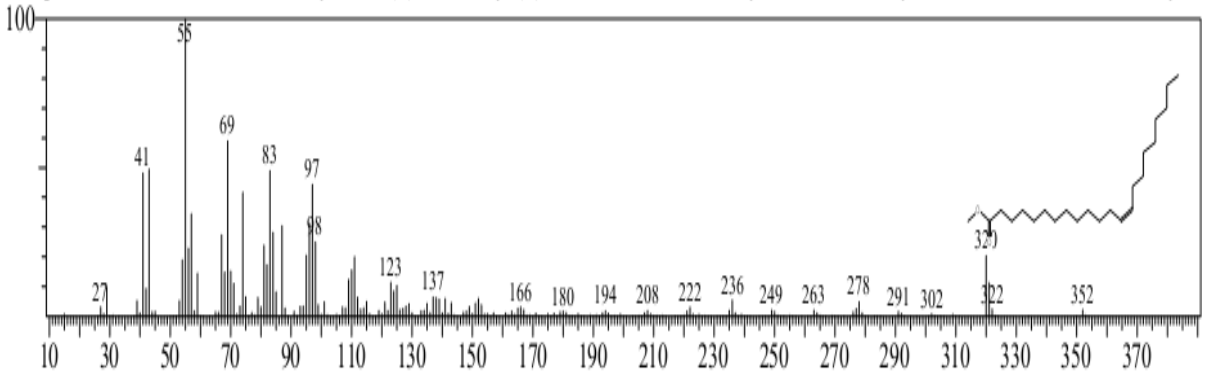
Peak no. 23: Methyl stearate

Hit#:1 Entry:152755 Library:NIST14.lib
SI:91 Formula:C21H40O2 CAS:0-00-0 MolWeight:324 RetIndex:2284
CompName:cis-11-Eicosenoic acid, methyl ester



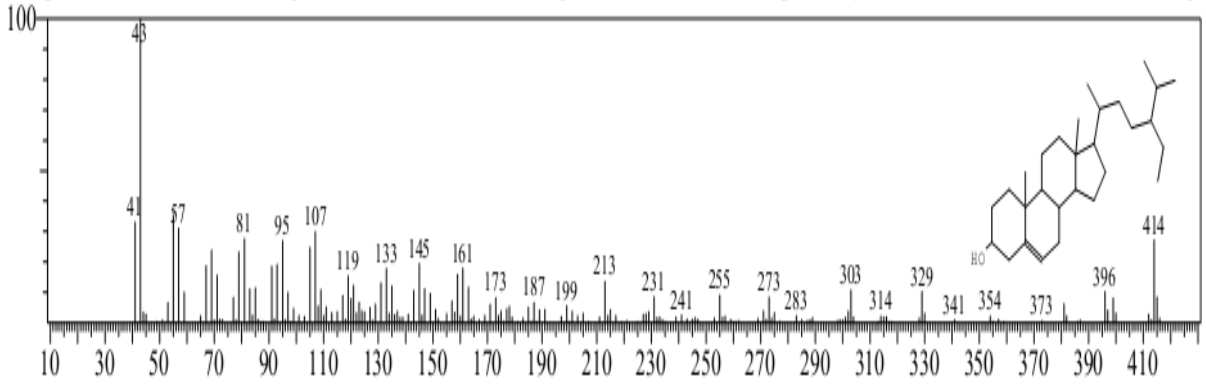
Peak no. 24: Cis-11-Eicosenoic acid methyl ester

Hit#:1 Entry:31014 Library:NIST14s.lib
SI:94 Formula:C23H44O2 CAS:1120-34-9 MolWeight:352 RetIndex:2483
CompName:13-Docosenoic acid, methyl ester, (Z)- $$ Methyl (Z)-13-docosenoate $$ Methyl erucate $$ Methyl 13-docosenoate-, cis- $$ Methyl ci



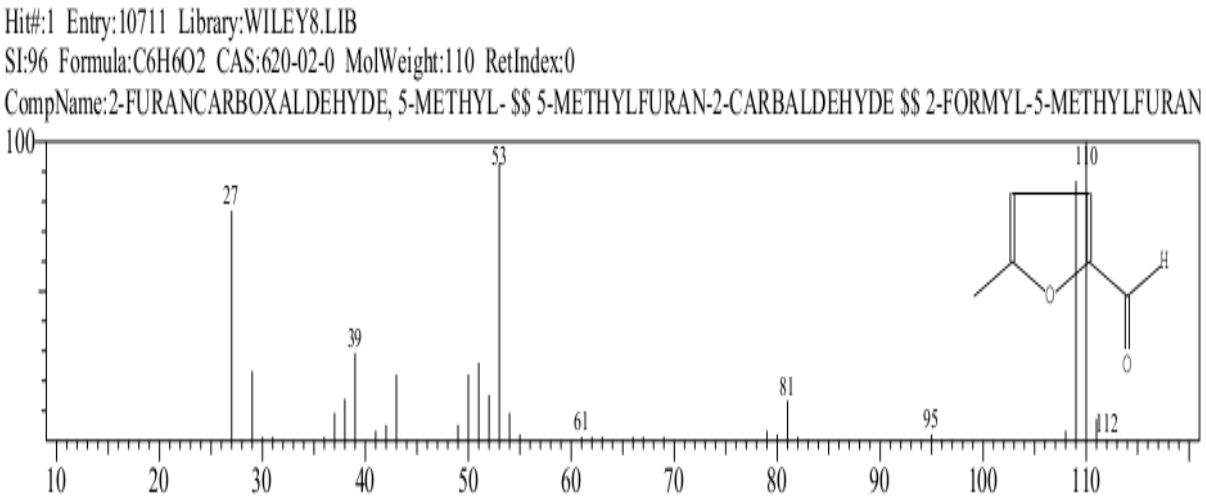
Peak no. 25: Docosenoic acid methyl ester, (Z)-

Hit#:1 Entry:212396 Library:NIST14.lib
SI:92 Formula:C29H50O CAS:83-46-5 MolWeight:414 RetIndex:2731
CompName:.beta.-Sitosterol $$ Stigmast-5-en-3-ol, (3.beta.)- $$ Stigmast-5-en-3.beta.-ol $$ .alpha.-Dihydrofucosterol $$ .beta.-Sitosterin $$ Ange

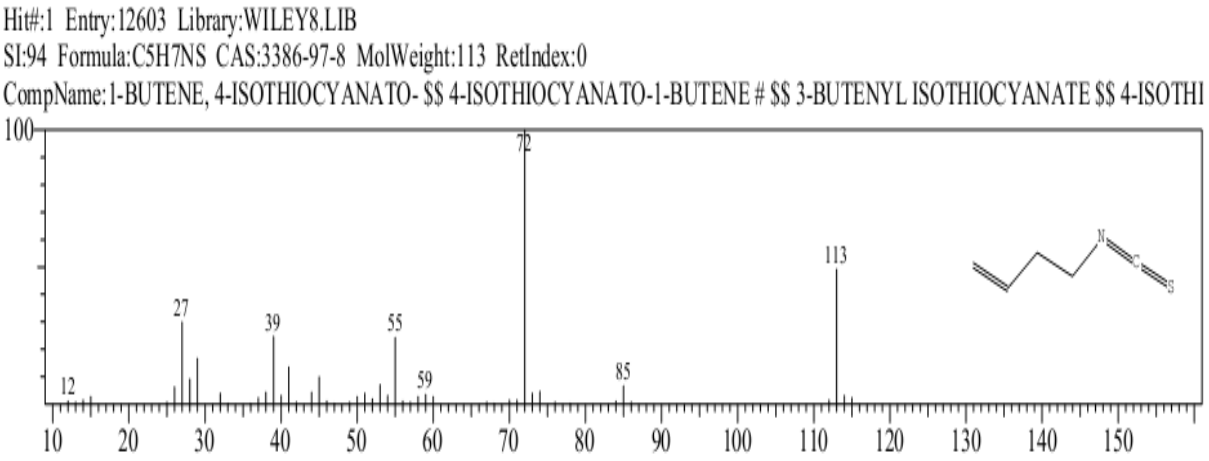


Peak no. 26: beta. -Sitosterol

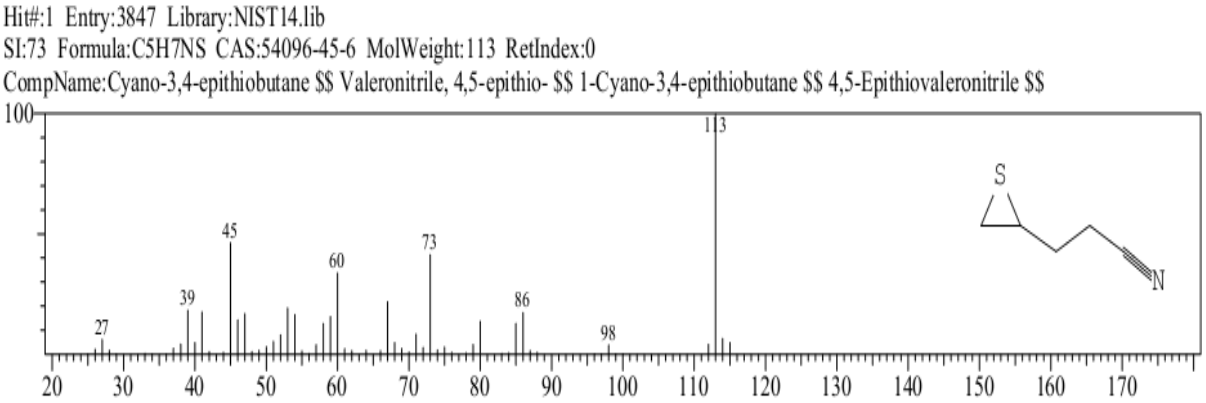
The major mass spectrum (m/z) of Methanol seeds crude extracts of *Sisymbrium irio* were:



Peak no. 27: 2-Furancarboxyaldehyde, 5-methyl

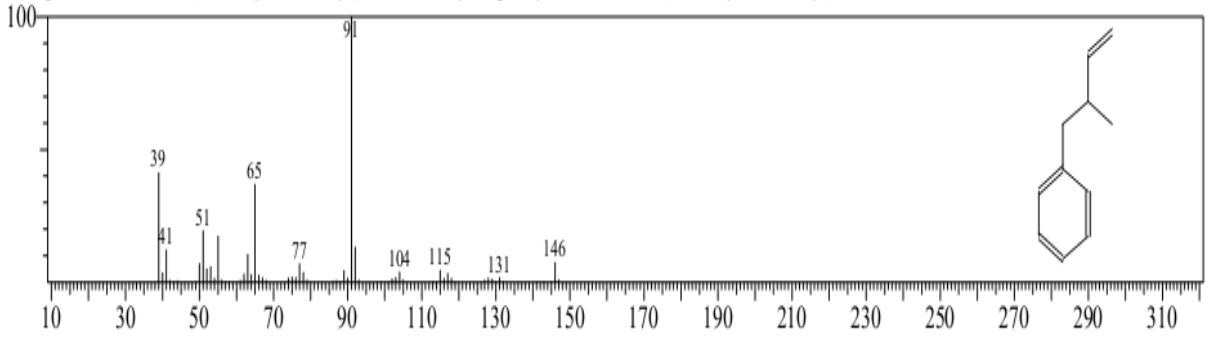


Peak no. 28: 4-isothiocyanto, 1-Butene



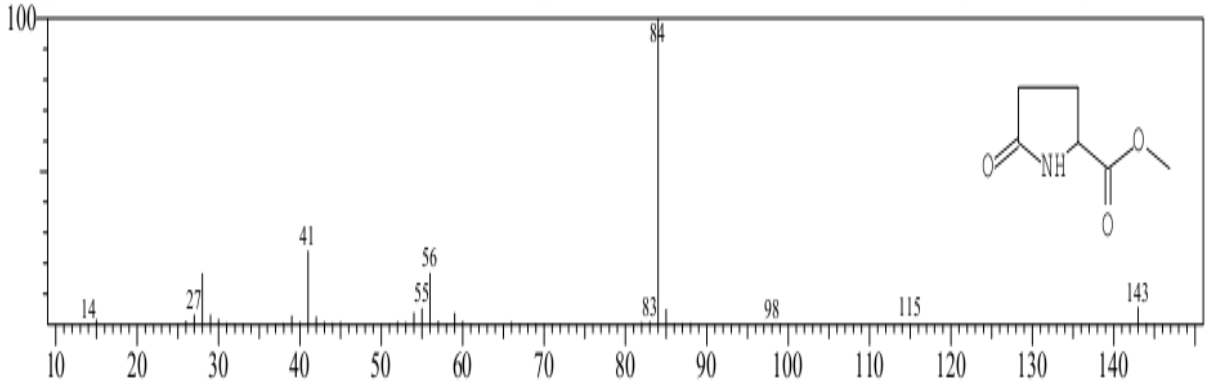
Peak no. 29: Cyano-3, 4-epithiobutane

Hit#:1 Entry:8558 Library:NIST14s.lib
SI:83 Formula:C11H14 CAS:1647-06-9 MolWeight:146 RetIndex:1117
CompName:Benzene, (2-methyl-3-butenyl)- $$ 3-Methyl-4-phenyl-1-butene $$ (2-Methyl-3-butenyl)benzene # $$



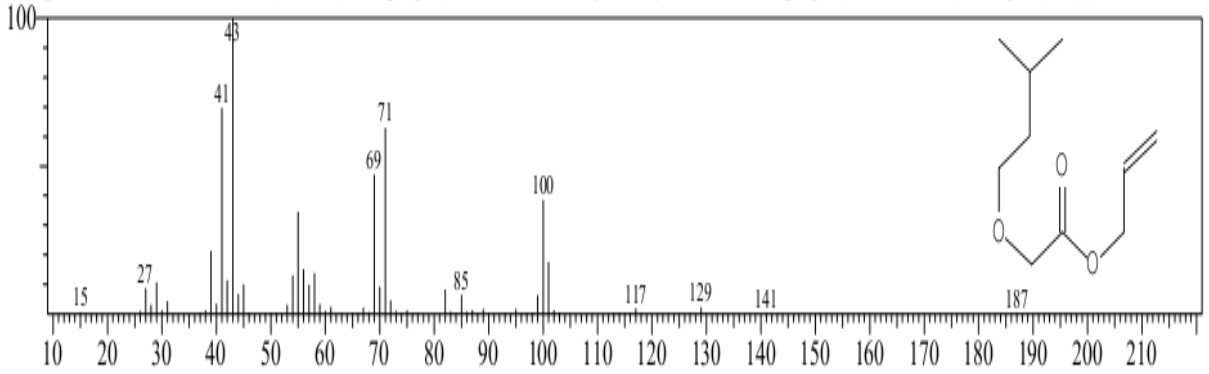
Peak no. 30: Benzene, (2-methyl-3-butenyl)

Hit#:1 Entry:12722 Library:NIST14.lib
SI:95 Formula:C6H9NO3 CAS:54571-66-3 MolWeight:143 RetIndex:1091
CompName:DL-Proline, 5-oxo-, methyl ester $$ Methyl 5-oxo-2-pyrrolidinecarboxylate # $$ 2-Pyrrolidone-5-carboxylic acid, methyl ester $$

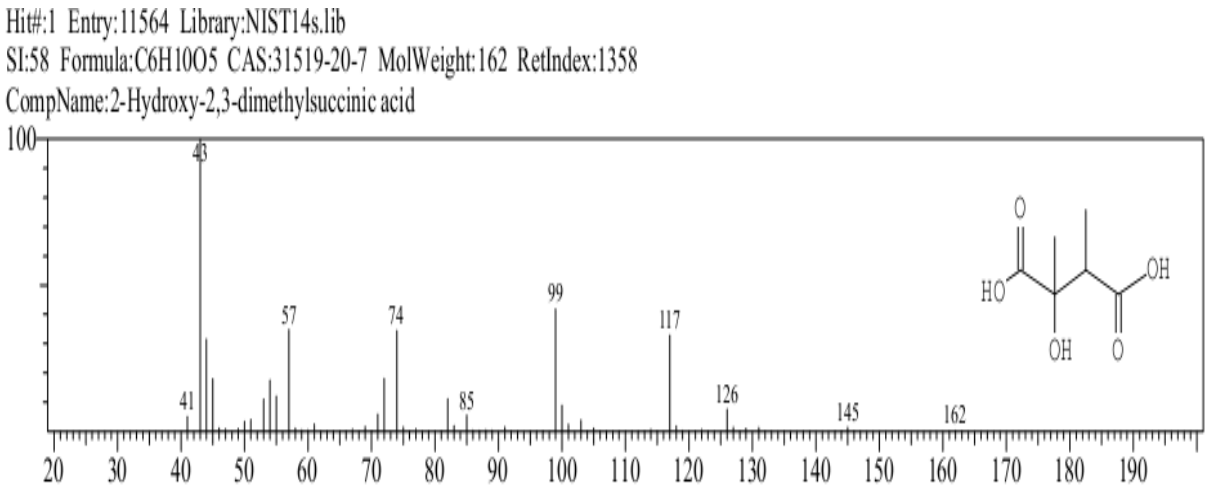


Peak no. 31: DL-proline, 5-oxo-, methyl ester

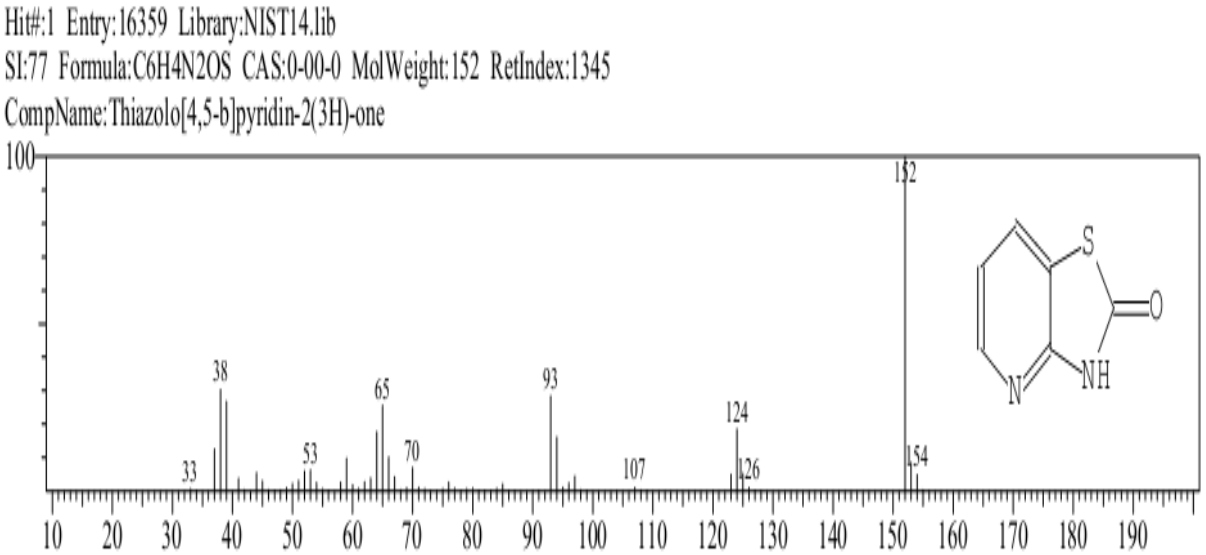
Hit#:1 Entry:36967 Library:NIST14.lib
SI:81 Formula:C10H18O3 CAS:67634-00-8 MolWeight:186 RetIndex:1184
CompName:Acetic acid, (3-methylbutoxy)-, 2-propenyl ester $$ 3-Methylbutoxy-acetic acid, 2-propenyl ester $$ Allyl (isopentyloxy)acetate # $$ (



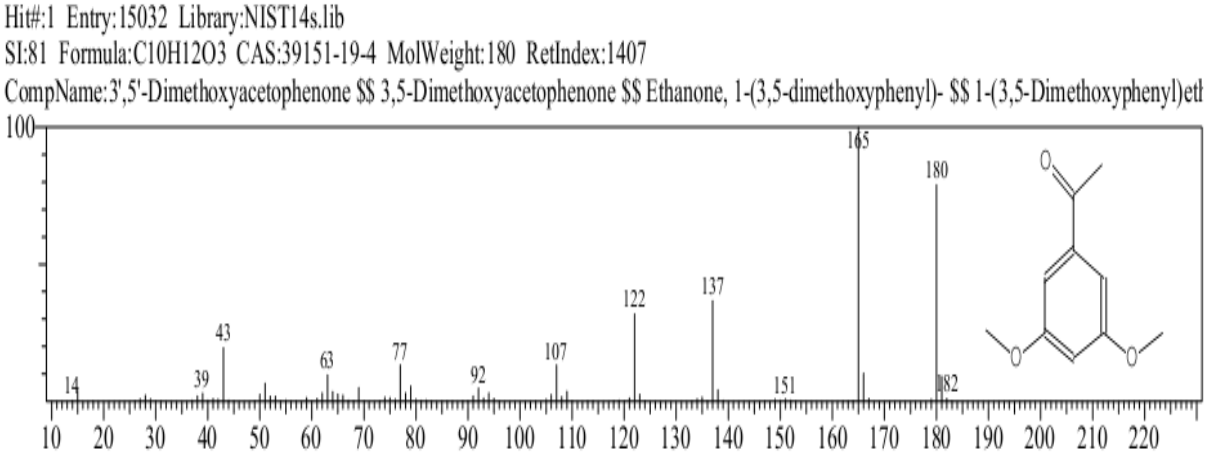
Peak no. 32: Acetic acid, (3-methylbutoxy)-, 2-propenyl ester



Peak no. 33: 2-Hydroxyl-2, 3-dimethylsuccinic acid

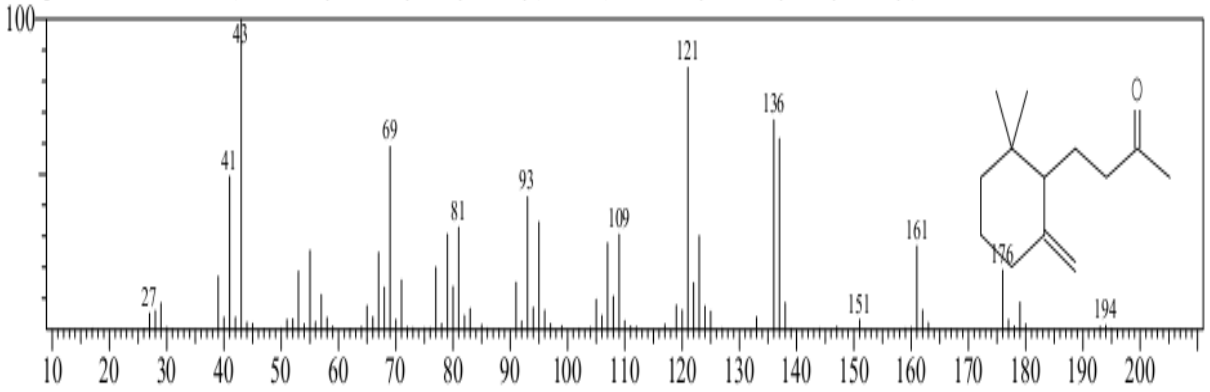


Peak no. 34: Thiazolo[4,5-b] pyridine-2(3H)-one



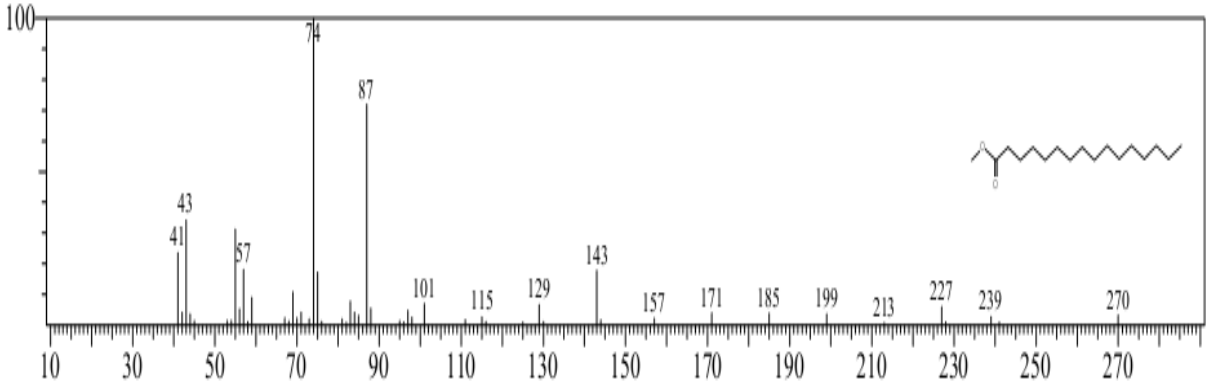
Peak no. 35: 3', 5'-Dimethoxyacetophenone

Hit#:1 Entry:17433 Library:NIST14s.lib
SI:81 Formula:C13H22O CAS:13720-12-2 MolWeight:194 RetIndex:1416
CompName:2-Butanone, 4-(2,2-dimethyl-6-methylenecyclohexyl)- $$ 4-(2,2-Dimethyl-6-methylenecyclohexyl)-2-butanone # $$



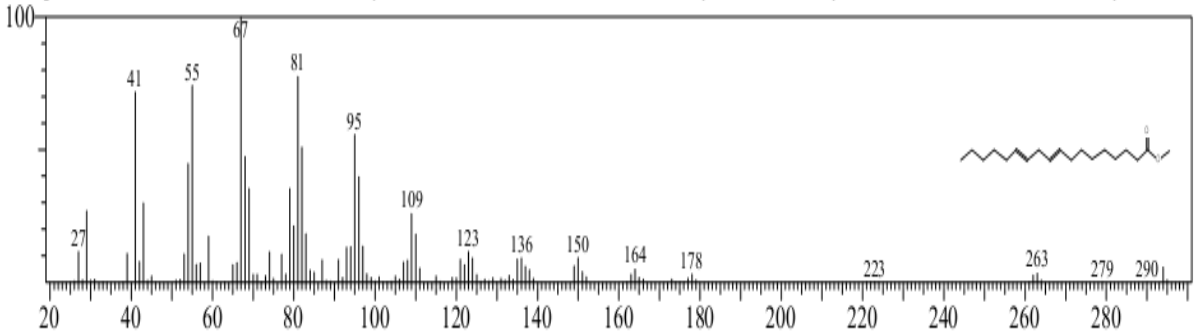
Peak no. 36: 2-Butanone, 4-(2, 2-dimethyl-6-methylenecyclohexyl)-

Hit#:1 Entry:26272 Library:NIST14s.lib
SI:96 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl he:



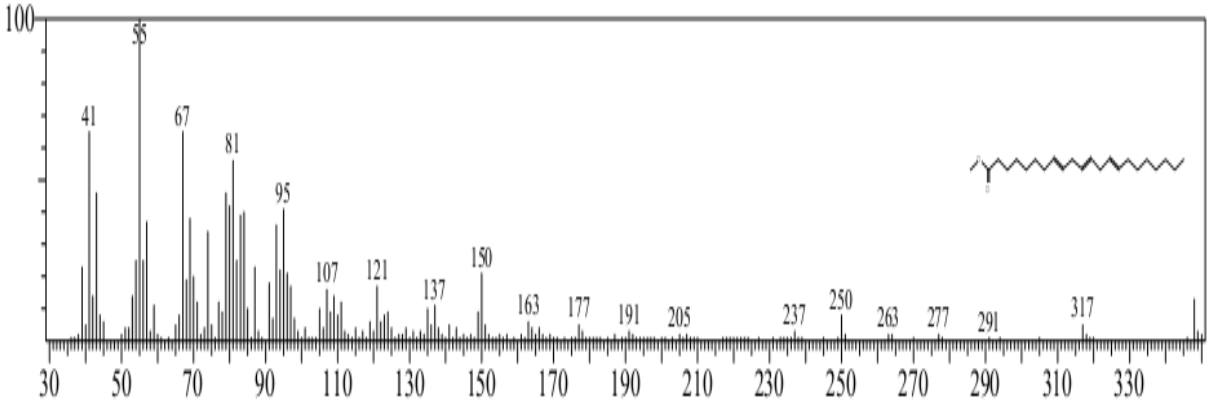
Peak no. 37: Hexadecanoic acid, methyl ester

Hit#:1 Entry:125931 Library:NIST14.lib
SI:94 Formula:C19H34O2 CAS:2462-85-3 MolWeight:294 RetIndex:2093
CompName:9,12-Octadecadienoic acid, methyl ester $$ 9,12-Octadecenoic acid, methyl ester $$ Methyl octadeca-9,12-dienoate $$ Methyl 9,12-oc



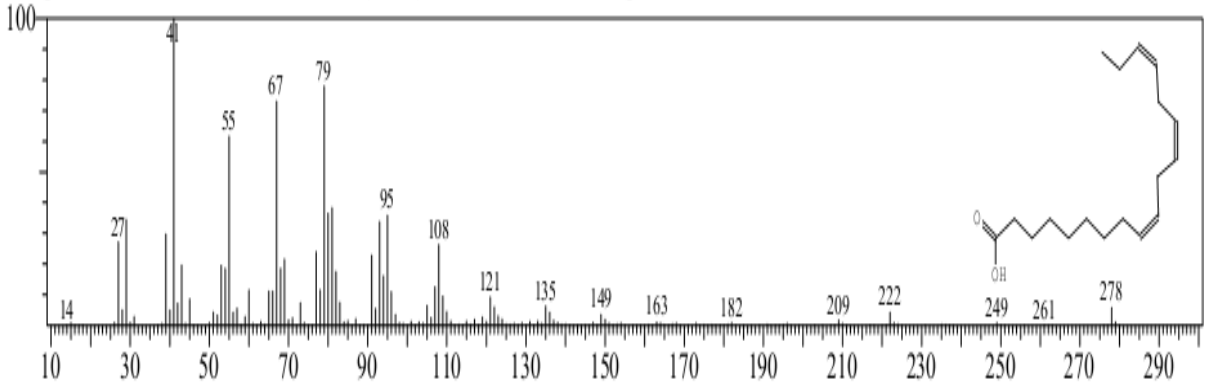
Peak no. 38: 9,12-Octadecadienoic acid, methyl ester

Hit#:1 Entry:173669 Library:NIST14.lib
SI:88 Formula:C23H40O2 CAS:56847-02-0 MolWeight:348 RetIndex:2499
CompName:8,11,14-Docosatrienoic acid, methyl ester $$ Methyl (8E,11E,14E)-8,11,14-docosatrienoate # $$



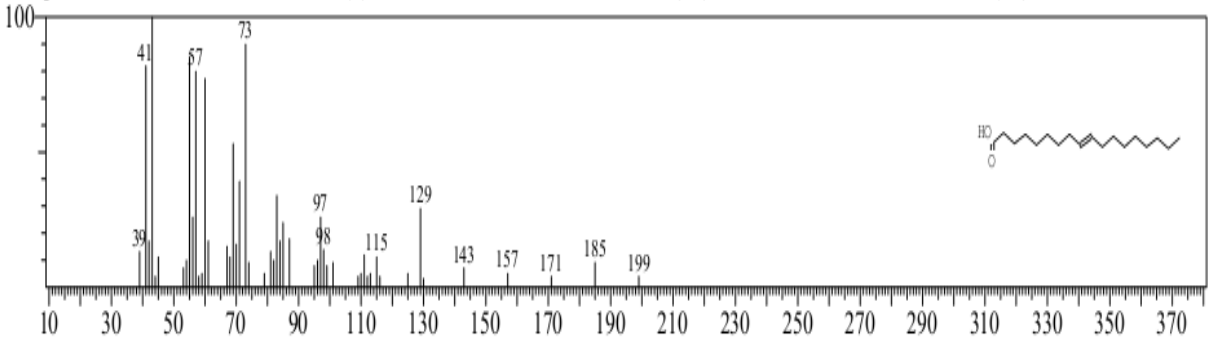
Peak no. 39: 8,11,14-Docosatrienoic acid, methyl ester

Hit#:1 Entry:26858 Library:NIST14s.lib
SI:89 Formula:C18H30O2 CAS:463-40-1 MolWeight:278 RetIndex:2191
CompName:9,12,15-Octadecatrienoic acid, (Z,Z,Z)- $$ Linolenic acid $$ .alpha.-Linolenic acid $$ All-cis-9,12,15-Octadecatrienoic acid $$ cis,cis



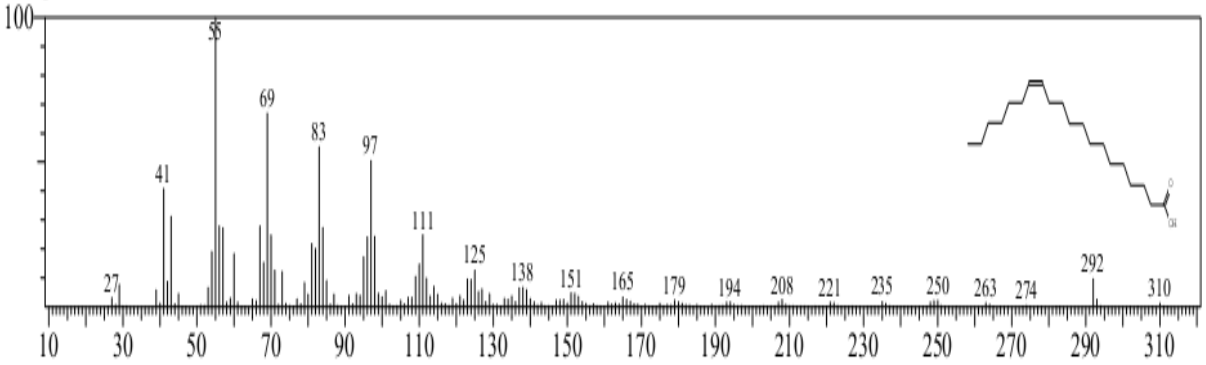
Peak no. 40: 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-

Hit#:1 Entry:217532 Library:WILEY8.LIB
SI:92 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:0
CompName:9-OCTADECENOIC ACID (Z)- $$ OCTADEC-9-ENOIC ACID $$ (9E)-9-OCTADECENOIC ACID # $$ (9E)-9-OCTADECENOIC



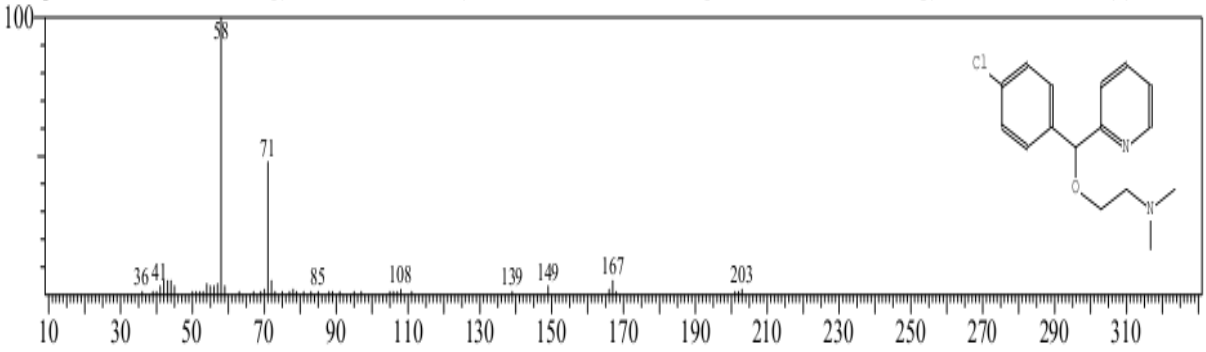
Peak no. 41: 9-Octadecenoic acid (Z)-

Hit#:3 Entry:140357 Library:NIST14.lib
SI:91 Formula:C20H38O2 CAS:17735-94-3 MolWeight:310 RetIndex:2374
CompName:cis-13-Eicosenoic acid



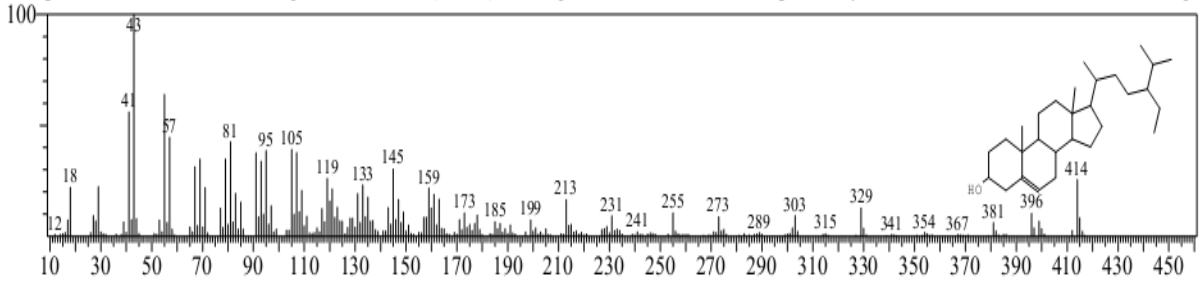
Peak no. 42: Cis-13-Eicosenoic acid

Hit#:1 Entry:227283 Library:WILEY8.LIB
SI:84 Formula:C16H19ClN2O CAS:486-16-8 MolWeight:290 RetIndex:0
CompName:ETHANAMINE, 2-[(4-CHLOROPHENYL)-2-PYRIDINYLMETHOXY]-N,N-DIMETHYL- $$ 2-[(4-CHLOROPHENYL)(2-PYRID



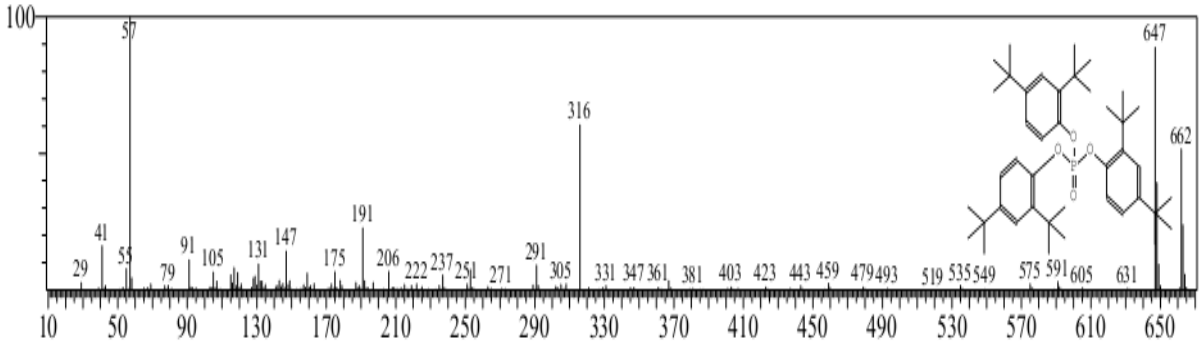
Peak no. 43: Ethanamine, 2-[(4-chlorophenyl)-2-pyridinylmethoxy]-N, N-dimethyl

Hit#:1 Entry:32669 Library:NIST14s.lib
SI:87 Formula:C29H50O CAS:83-46-5 MolWeight:414 RetIndex:2731
CompName:.beta.-Sitosterol $$ Stigmast-5-en-3-ol, (3.beta.)- $$ Stigmast-5-en-3.beta.-ol $$ .alpha.-Dihydrofucosterol $$ .beta.-Sitosterin $$ Ange



Peak no. 44: beta. -Sitosterol

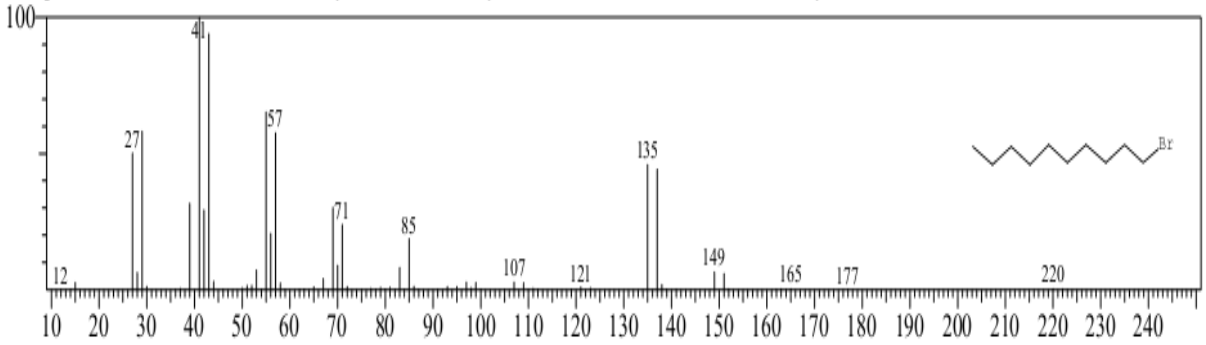
Hit#:1 Entry:240877 Library:NIST14.lit
SI:77 Formula:C42H63O4P CAS:95906-11-9 MolWeight:662 RetIndex:0
CompName:Tris(2,4-di-tert-butylphenyl) phosphate $$ Phenol, 2,4-bis(1,1-dimethylethyl)-, 1,1',1''-phosphate $$ Phenol, 2,4-bis(1,1-dimethylethyl



Peak no. 45: Tris (2, 4-tert-butylphenol) phosphate

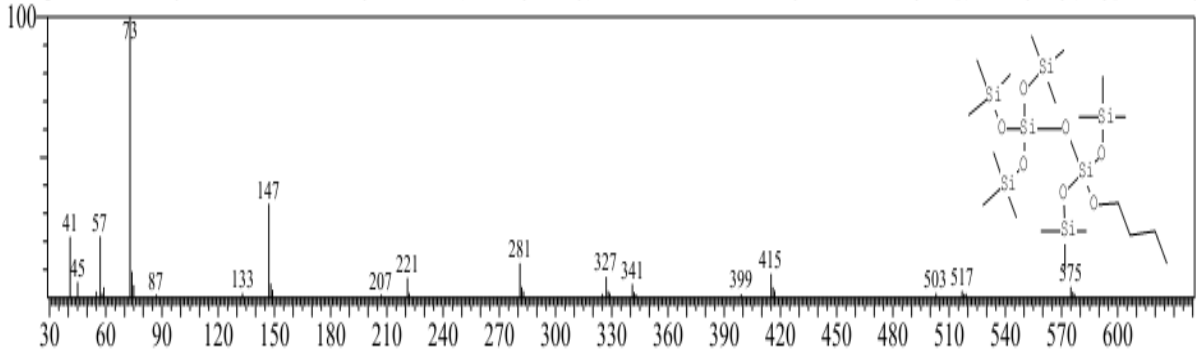
The major mass spectrum (m/z) of n-Hexane seed crude extracts of *Colchicum autumnale* were:

Hit#:1 Entry:21249 Library:NIST14s.lib
SI:96 Formula:C10H21Br CAS:112-29-8 MolWeight:220 RetIndex:1311
CompName:Decane, 1-bromo- $$ n-Decyl bromide $$ Decyl bromide $$ 1-Bromodecane $$ 1-Decyl bromide $$



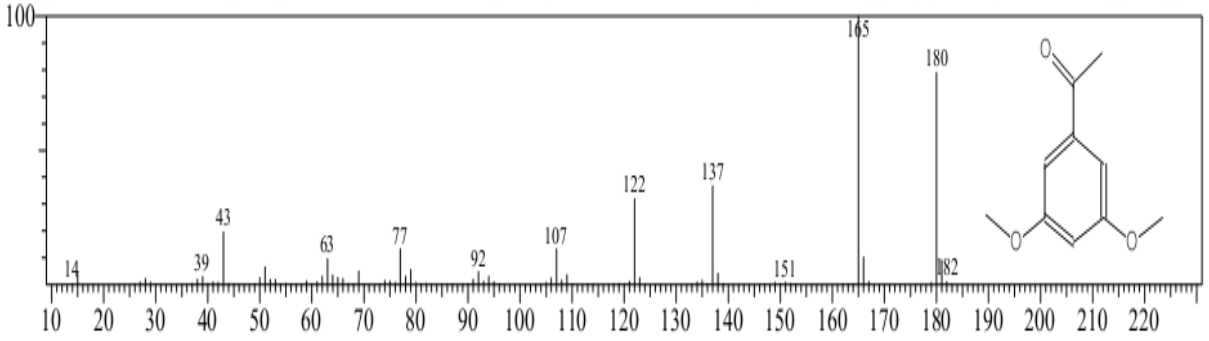
Peak no. 46: 1-bromo- Decane

Hit#:1 Entry:239105 Library:NIST14.lib
SI:83 Formula:C19H54O7Si7 CAS:72439-84-0 MolWeight:590 RetIndex:1811
CompName:3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane $$ 1-Butoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy]disiloxan



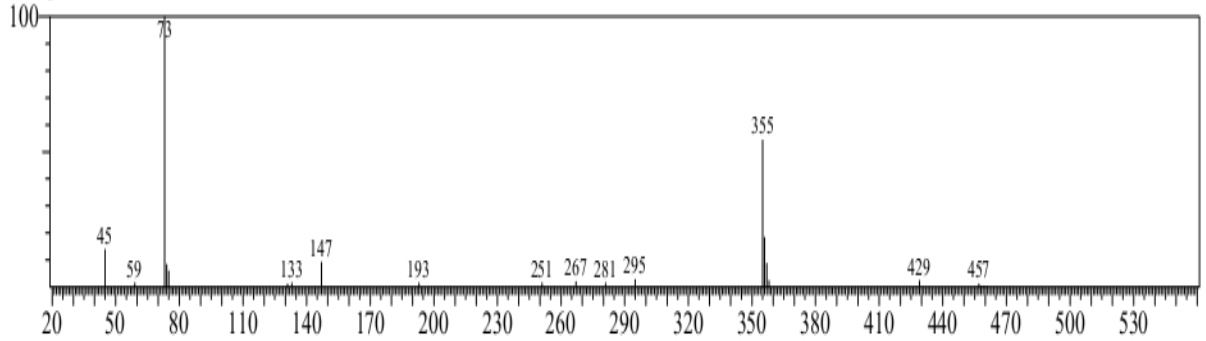
Peak no. 47: 3-Butoxy-1,1,1,7,7,7, -hexamethyl-3,5,5-tris(trimethylsiloy)tetrasixone

Hit#:1 Entry:15032 Library:NIST14s.lib
SI:70 Formula:C10H12O3 CAS:39151-19-4 MolWeight:180 RetIndex:1407
CompName:3',5'-Dimethoxyacetophenone $$ 3,5-Dimethoxyacetophenone $$ Ethanone, 1-(3,5-dimethoxyphenyl)- $$ 1-(3,5-Dimethoxyphenyl)etl



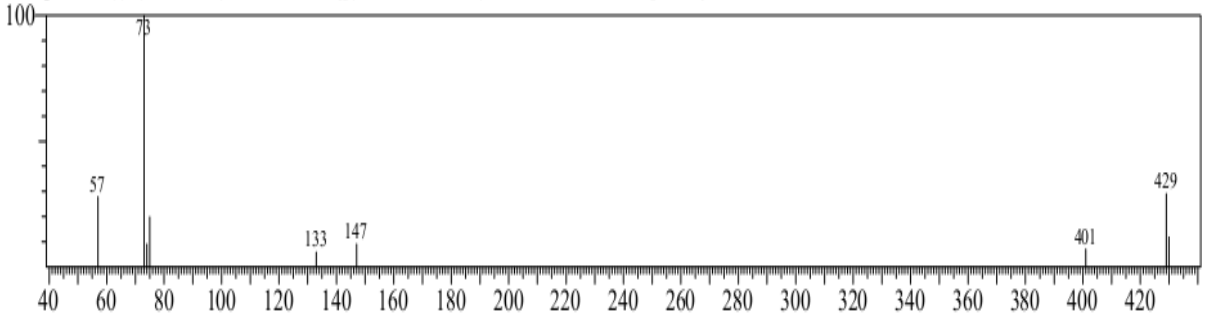
Peak no. 48: 3', 5'-Dimethoxyacetophenone

Hit#:1 Entry:368328 Library:WILEY8.LIB
SI:83 Formula:C20H40O5Si4 CAS:0-00-0 MolWeight:472 RetIndex:0
CompName:3,4-DIHYDROXYMANDELIC ACID-TETRATMS



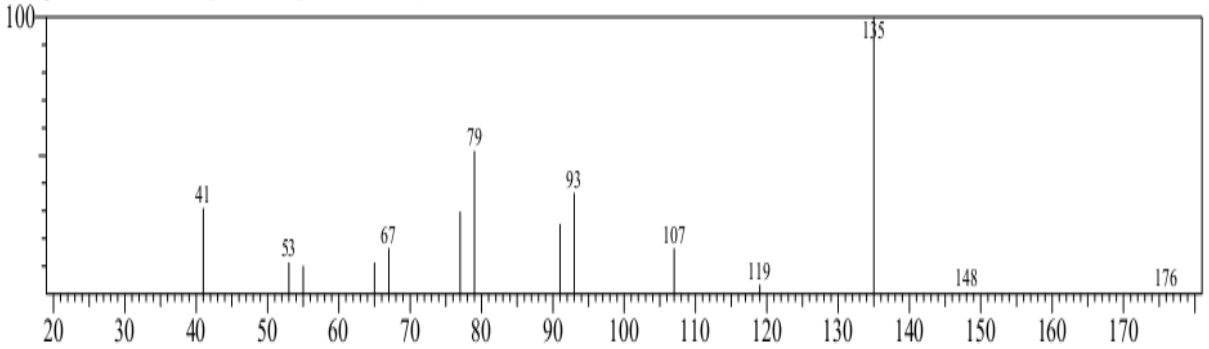
Peak no. 49: 3, 4-Dihdroxymandelic acid tetratms

Hit#:1 Entry:372678 Library:WILEY8.LIB
SI:78 Formula:C24H50O4Si3 CAS:129745-62-6 MolWeight:486 RetIndex:0
CompName:(-)-(4R,5S,6R)-4,5,6-TRIS-{[(TERT-BUTYL)DIMETHYLSILYL]OXY}CYCLOHEX-2-EN-1-ONE



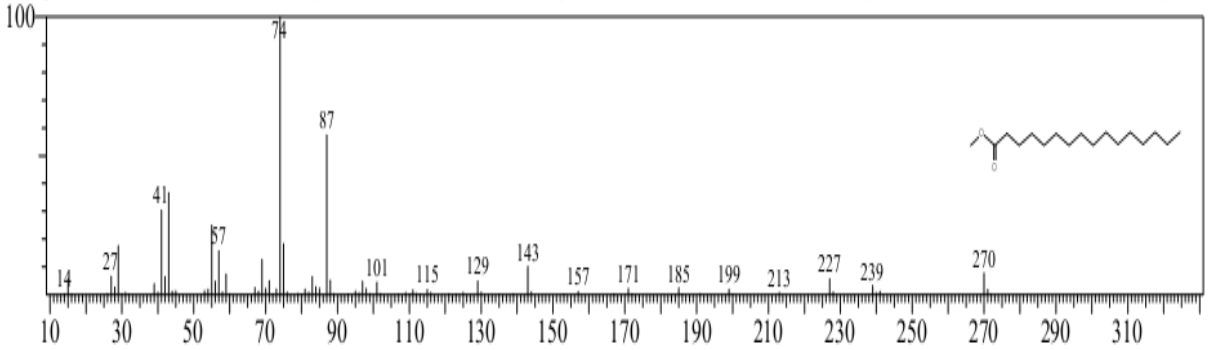
Peak no. 50: (-) -(4R,5S,6R)-4,5,6-tris-{[tert-butyl) dimethyl silyl} oxy} cyclohex-2-en-1-one

Hit#:1 Entry:72622 Library:WILEY8.LIB
SI:87 Formula:C12H16O CAS:113279-41-7 MolWeight:176 RetIndex:0
CompName:TRICYCLO[3.3.1.13,7]DECANE, 1-(ETHYNYLOXY)- $$ (1-ADAMANTYLOXY)ETHYNE



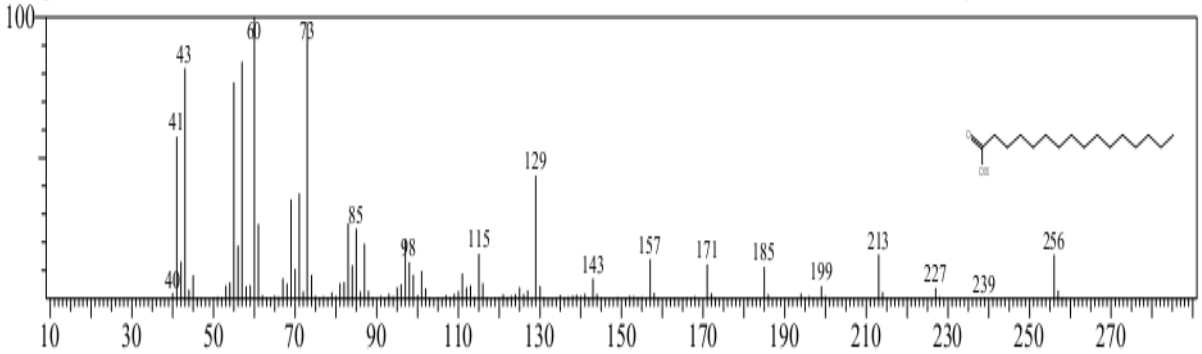
Peak no. 51: Tricyclo [3,3.1.13,7] decane, 1-(Ethynyloxy)-

Hit#:1 Entry:26269 Library:NIST14s.lib
SI:95 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl he:



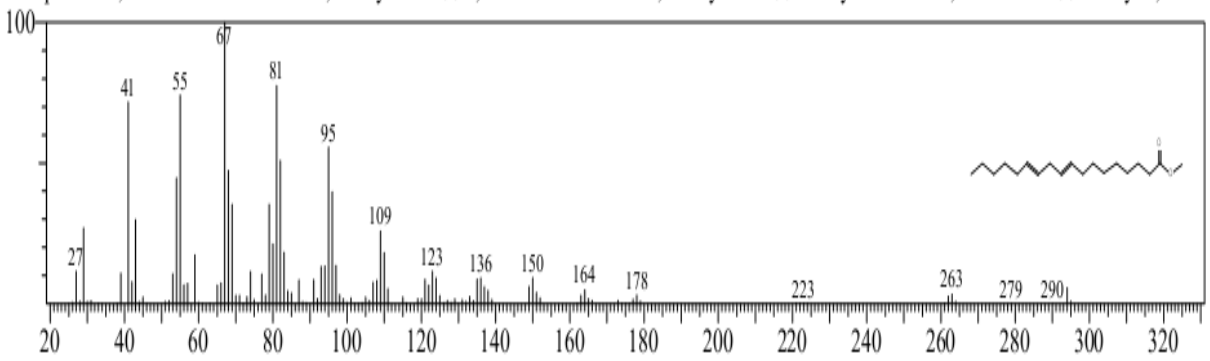
Peak no. 52: Hexadecanoic acid, methyl ester

Hit#:1 Entry:25118 Library:NIST14s.lib
SI:95 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecaneca



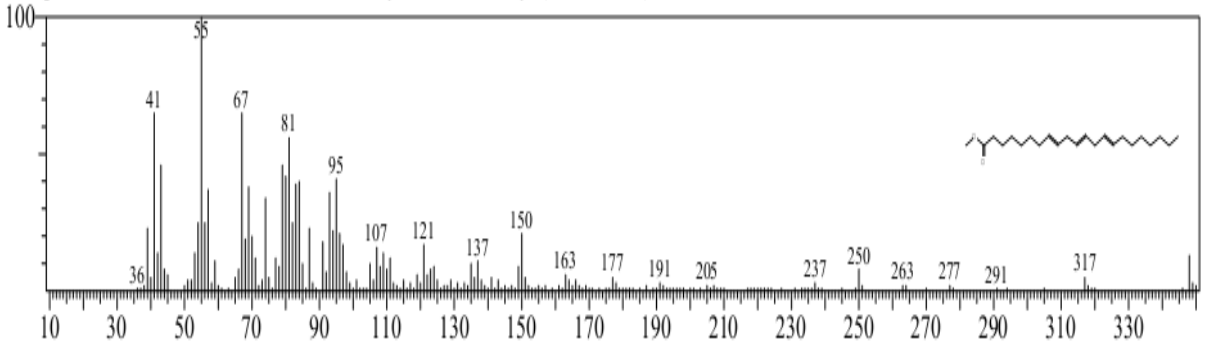
Peak no. 53: n-Hexadecanoic acid

Hit#:1 Entry:125931 Library:NIST14.lib
SI:95 Formula:C19H34O2 CAS:2462-85-3 MolWeight:294 RetIndex:2093
CompName:9,12-Octadecadienoic acid, methyl ester $$ 9,12-Octadecenoic acid, methyl ester $$ Methyl octadeca-9,12-dienoate $$ Methyl 9,12-oc



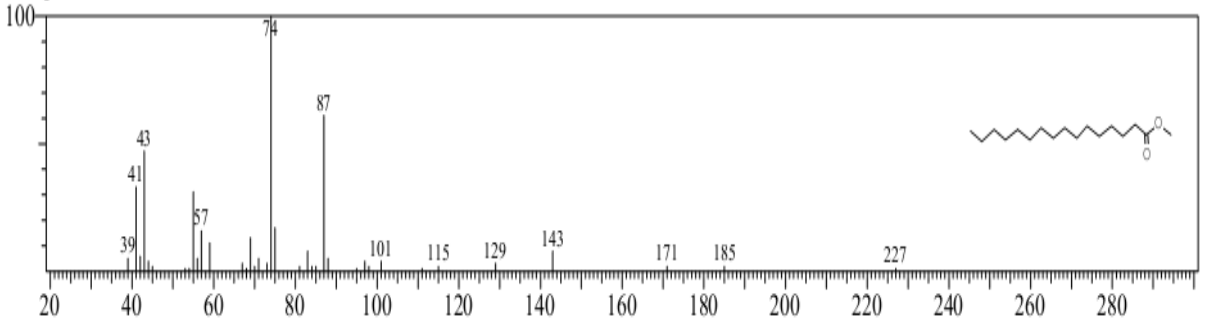
Peak no. 54: 9, 12-Octadecadienoic acid, methyl ester

Hit#:1 Entry:173669 Library:NIST14.lib
SI:89 Formula:C23H40O2 CAS:56847-02-0 MolWeight:348 RetIndex:2499
CompName:8,11,14-Docosatrienoic acid, methyl ester $$ Methyl (8E,11E,14E)-8,11,14-docosatrienoate # $$



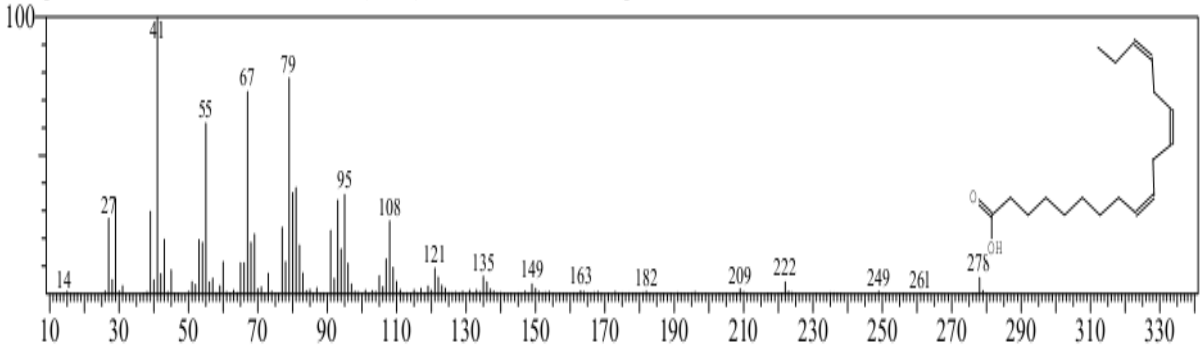
Peak no. 55: 8,11,14-Docosatrienoic acid, methyl ester

Hit#:1 Entry:201937 Library:WILEY8.LIB
SI:88 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:0
CompName:HEXADECANOIC ACID, METHYL ESTER $$ METHYL HEXADECANOATE $$ PALMITIC ACID METHYL ESTER $$ AI3-0



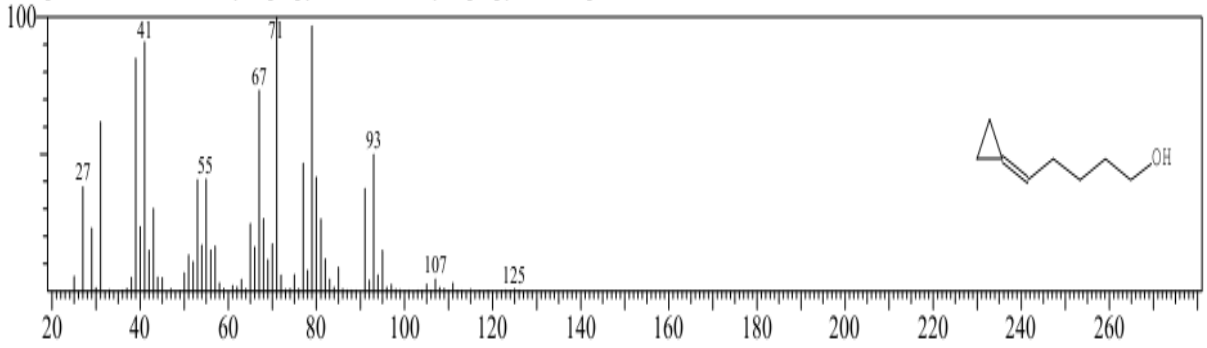
Peak no. 56: Hexadecanoic acid, methyl ester

Hit#:1 Entry:26858 Library:NIST14s.lib
SI:88 Formula:C18H30O2 CAS:463-40-1 MolWeight:278 RetIndex:2191
CompName:9,12,15-Octadecatrienoic acid, (Z,Z,Z)- $$ Linolenic acid $$ .alpha.-Linolenic acid $$ All-cis-9,12,15-Octadecatrienoic acid $$ cis,cis



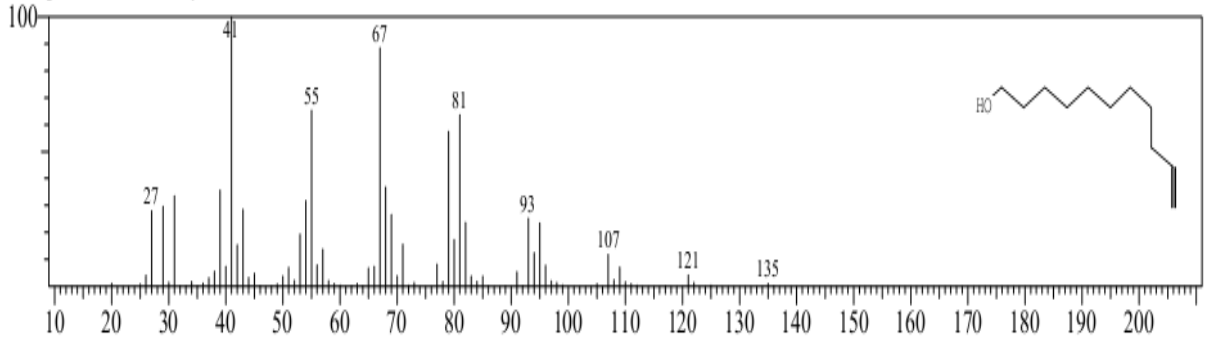
Peak no. 57: 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-

Hit#:1 Entry:6723 Library:NIST14.lib
SI:76 Formula:C8H14O CAS:162377-97-1 MolWeight:126 RetIndex:1085
CompName:1-Pentanol, 5-cyclopropylidene- $$ 5-Cyclopropylidene-1-pentanol # $$



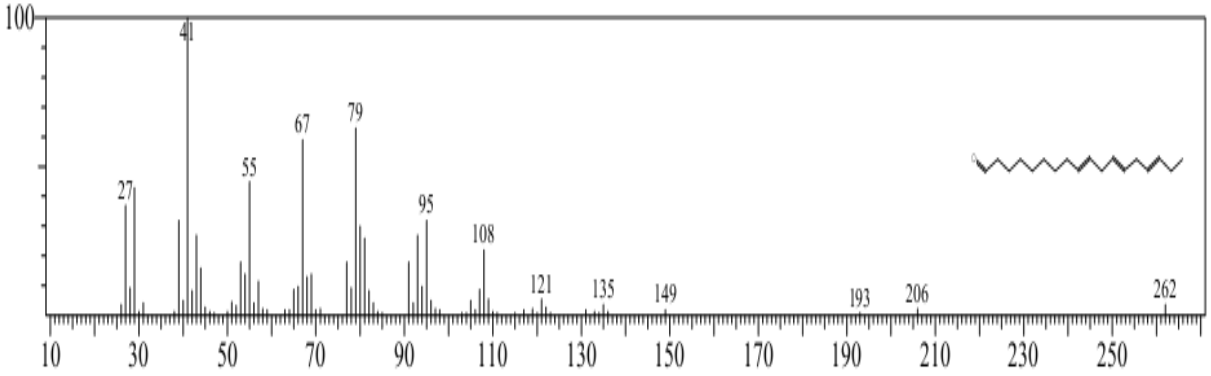
Peak no. 58: 5-cyclopropylidene-1-pentanol

Hit#:1 Entry:12964 Library:NIST14s.lib
SI:84 Formula:C11H20O CAS:2774-84-7 MolWeight:168 RetIndex:1355
CompName:10-Undecyn-1-ol



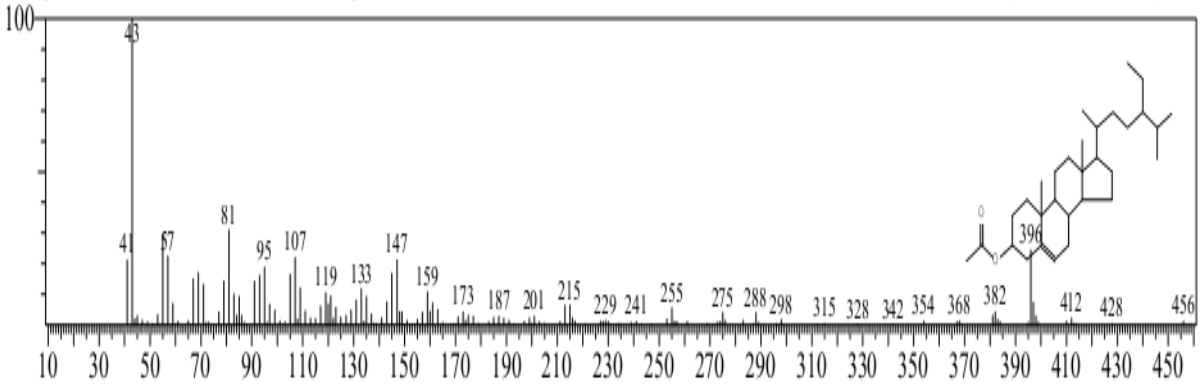
Peak no. 59: 10-undecyn-1-ol

Hit#:1 Entry:97667 Library:NIST14.lib
SI:82 Formula:C18H30O CAS:26537-71-3 MolWeight:262 RetIndex:2023
CompName:9,12,15-Octadecatrienal $$ (9E,12E,15E)-9,12,15-Octadecatrienal # $$



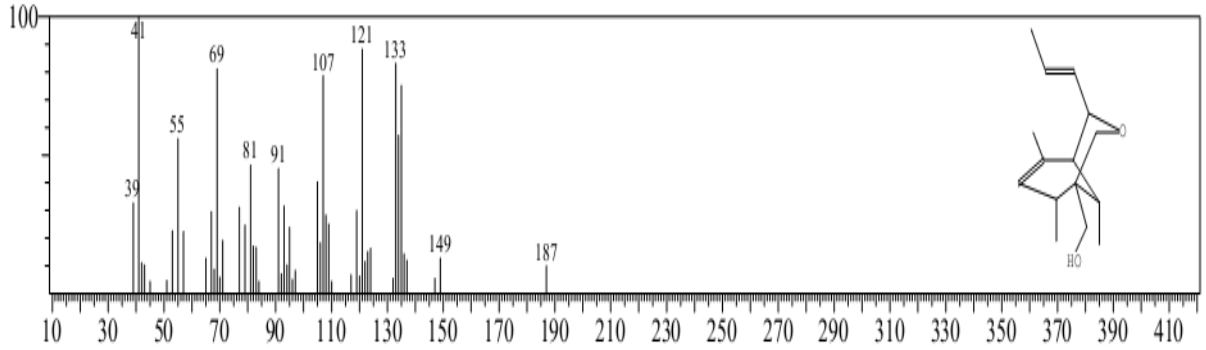
Peak no. 60: 9, 12, 15-Octadecatrienal

Hit#:1 Entry:33121 Library:NIST14s.lib
SI:78 Formula:C31H52O2 CAS:915-05-9 MolWeight:456 RetIndex:2871
CompName:.beta.-Sitosterol acetate $$ Stigmast-5-en-3-ol, acetate, (3.beta.)- $$ .beta.-Sitosterol 3-acetate $$ .beta.-Sitosteryl acetate $$ Acetyl-.be



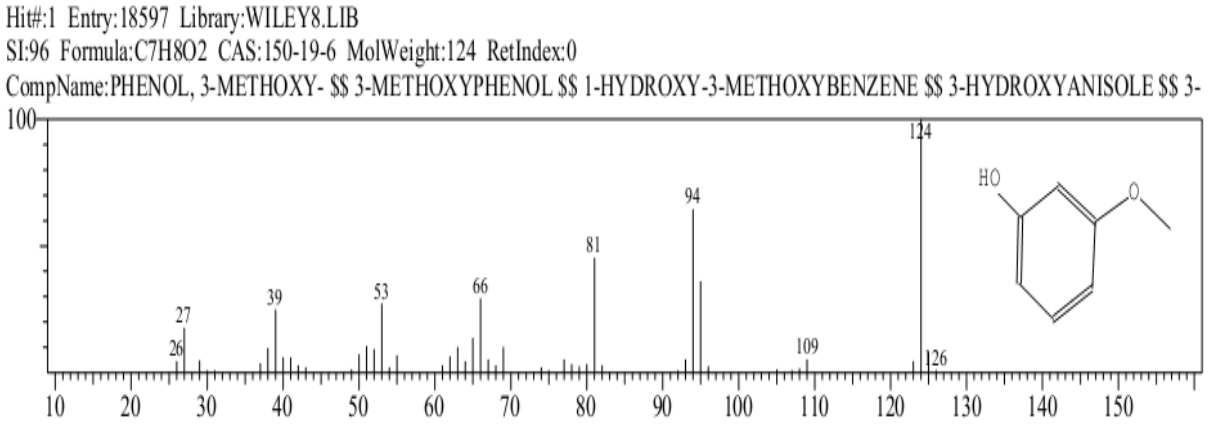
Peak no. 61: beta. -Sitosterol acetate

Hit#:1 Entry:155727 Library:WILEY8.LIB
SI:77 Formula:C15H24O2 CAS:0-00-0 MolWeight:236 RetIndex:0
CompName:METHANOL, [5,7,9-TRIMETHYL-4-(1-PROPENYL)-3-OXABICYCLO[3.3.1]NON-6-EN-1-YL]- $$ {6,8,9-TRIMETHYL-4-[1-PI

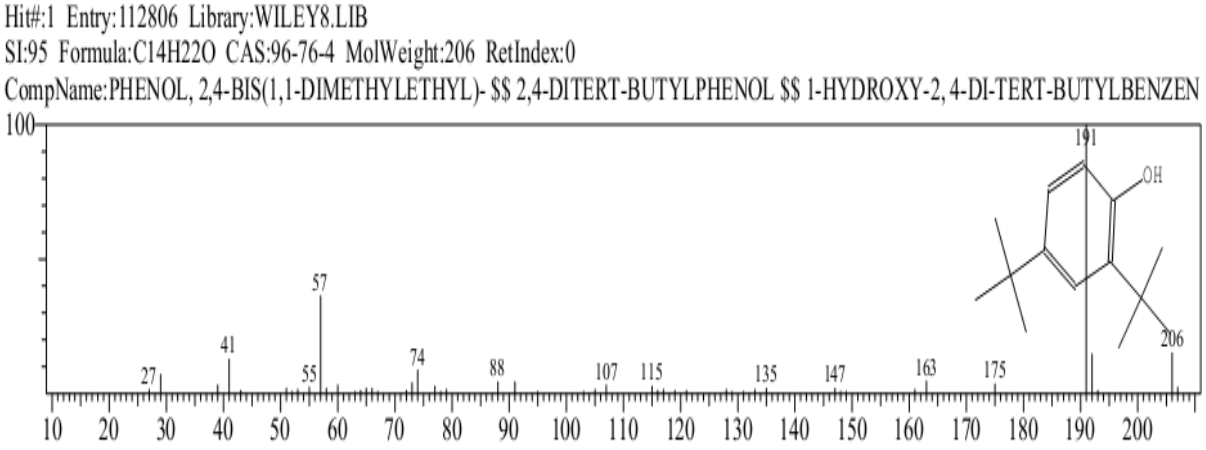


Peak no. 62: Methanol, [5,7,9-trimethyl-4-(1-propenyl-3-oxabicylo [3,3.1] non-6-en-1-yl

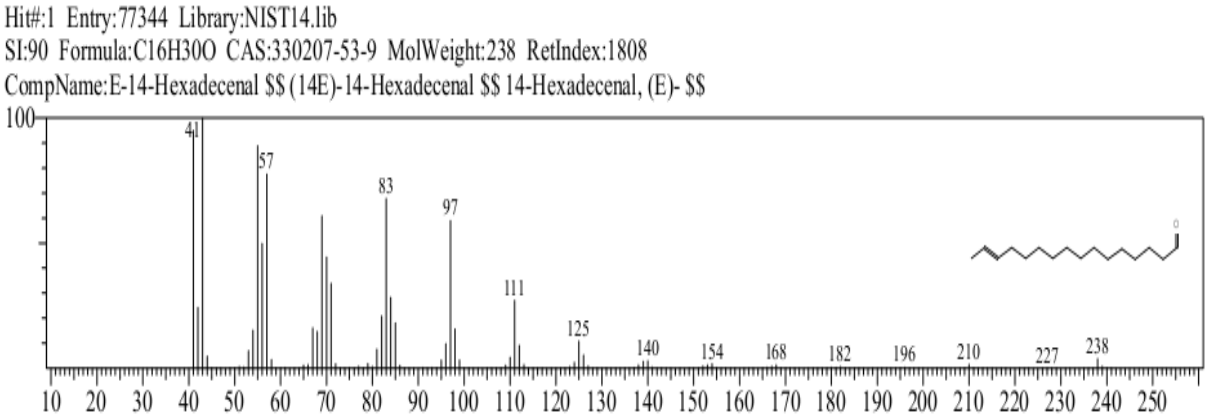
The major mass spectrum (m/z) of Dichloromethane crude extracts of *Colchicum autumnale* were:



Peak no. 63: 3-methoxy, phenol

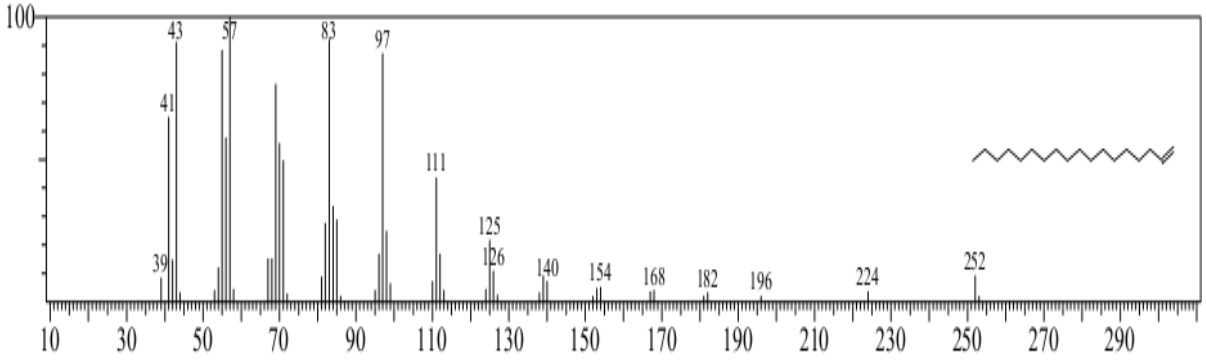


Peak no. 64: 2, 4-bis (1, 1-dimethylethyl), Phenol



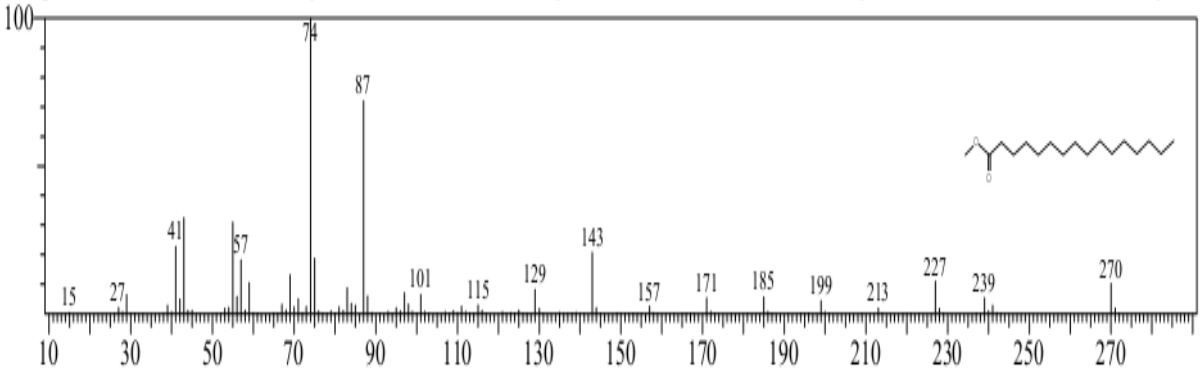
Peak no. 65: E-14-Hexadecenal

Hit#:1 Entry:178032 Library:WILEY8.LIB
SI:97 Formula:C18H36 CAS:112-88-9 MolWeight:252 RetIndex:0
CompName:1-OCTADECENE $$ OCTADEC-1-ENE $$ .ALPHA.-OCTADECENE $$ AI3-06521 $$ ALPHA-OCTADECENE $$ EINECS 204-



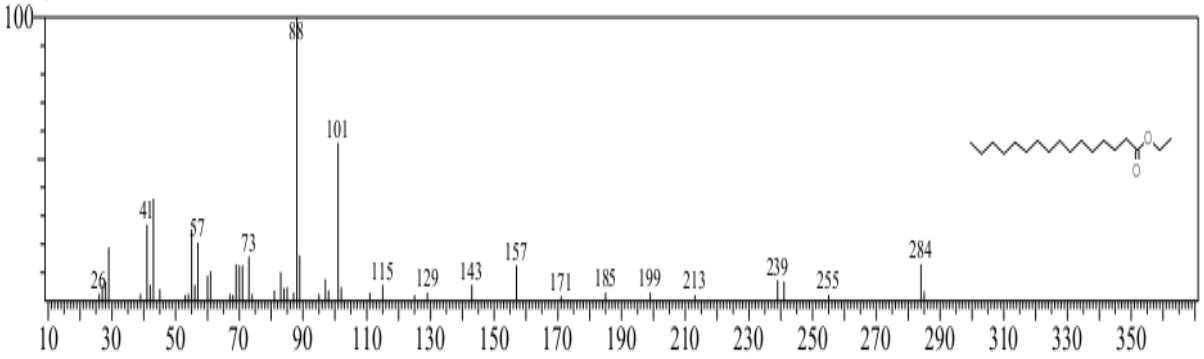
Peak no. 66: 1-Octadecene

Hit#:1 Entry:104648 Library:NIST14.lib
SI:97 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl he:



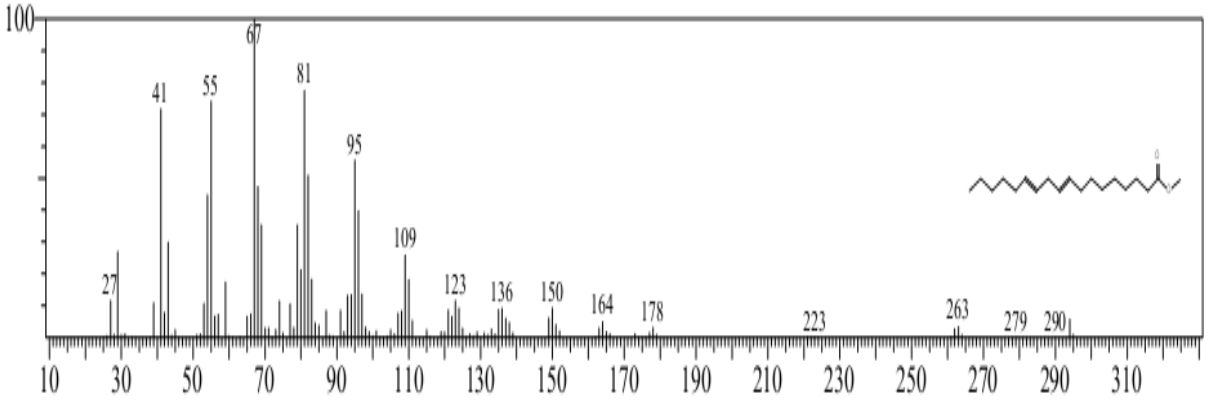
Peak no. 67: Hexadecanoic acid, methyl ester

Hit#:1 Entry:220263 Library:WILEY8.LIB
SI:83 Formula:C18H36O2 CAS:628-97-7 MolWeight:284 RetIndex:0
CompName:HEXADECANOIC ACID, ETHYL ESTER $$ ETHYL HEXADECANOATE $$ PALMITIC ACID ETHYL ESTER $$ AI3-06331 $



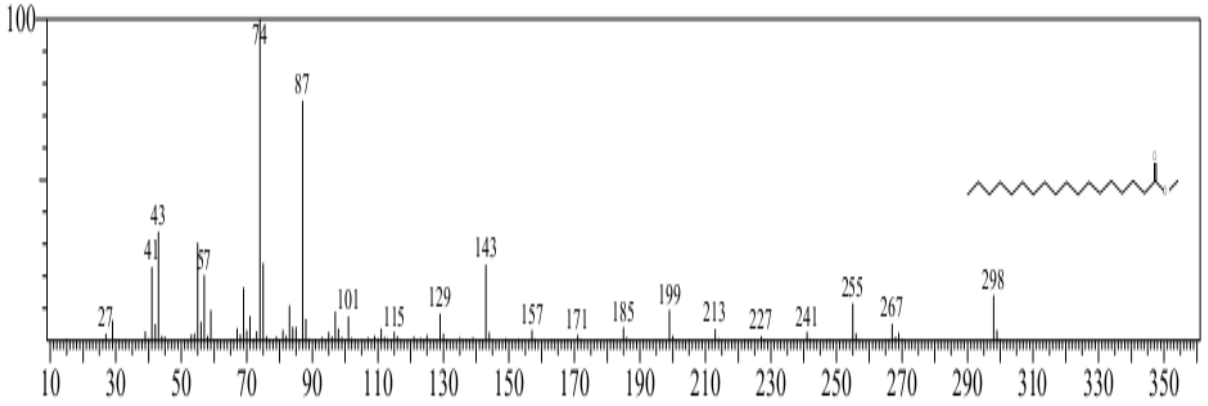
Peak no. 68: Hexadecanoic acid, ethyl ester

Hit#:1 Entry:125931 Library:NIST14.lib
SI:96 Formula:C19H34O2 CAS:2462-85-3 MolWeight:294 RetIndex:2093
CompName:9,12-Octadecadienoic acid, methyl ester $$ 9,12-Octadecenoic acid, methyl ester $$ Methyl octadeca-9,12-dienoate $$ Methyl 9,12-oc



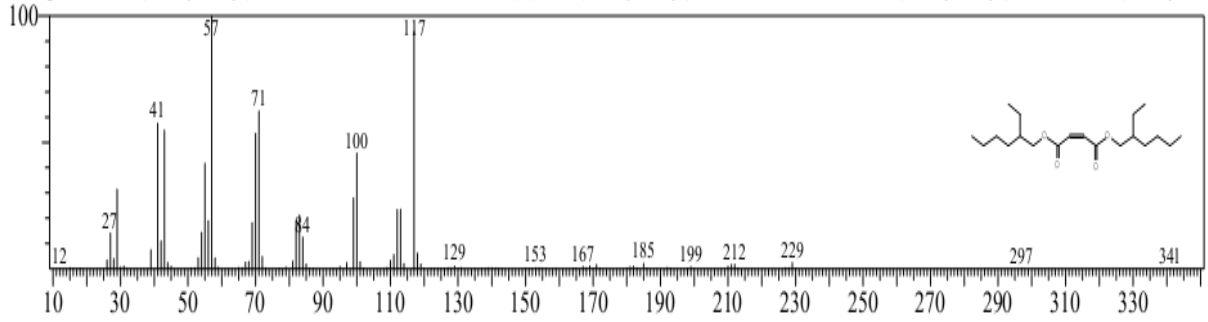
Peak no. 69: 9, 12-Octadecadienoic acid, methyl ester

Hit#:1 Entry:129694 Library:NIST14.lib
SI:96 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077
CompName:Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemester 971



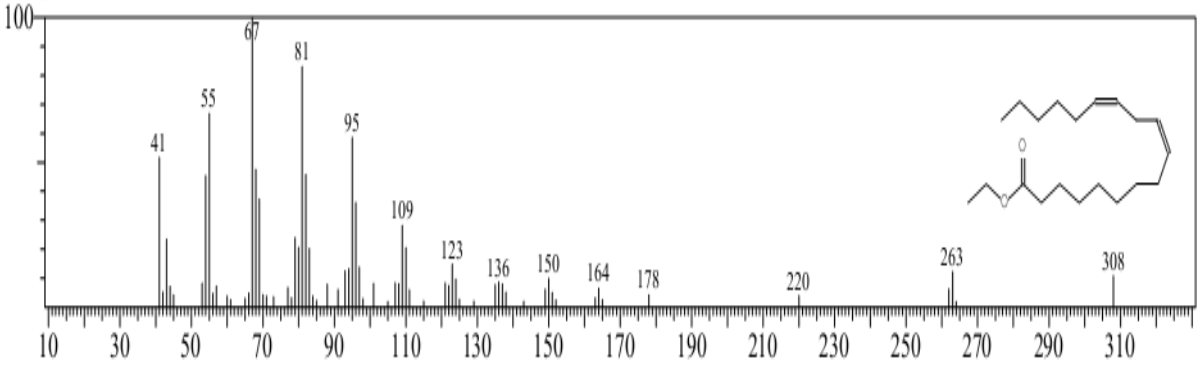
Peak no. 70: Methyl stearate

Hit#:1 Entry:166628 Library:NIST14.lib
SI:95 Formula:C20H36O4 CAS:142-16-5 MolWeight:340 RetIndex:2224
CompName:Bis(2-ethylhexyl) maleate $$ 2-Butenedioic acid (Z)-, bis(2-ethylhexyl) ester $$ Maleic acid, bis(2-ethylhexyl) ester $$ Bis-(2-ethylhe:



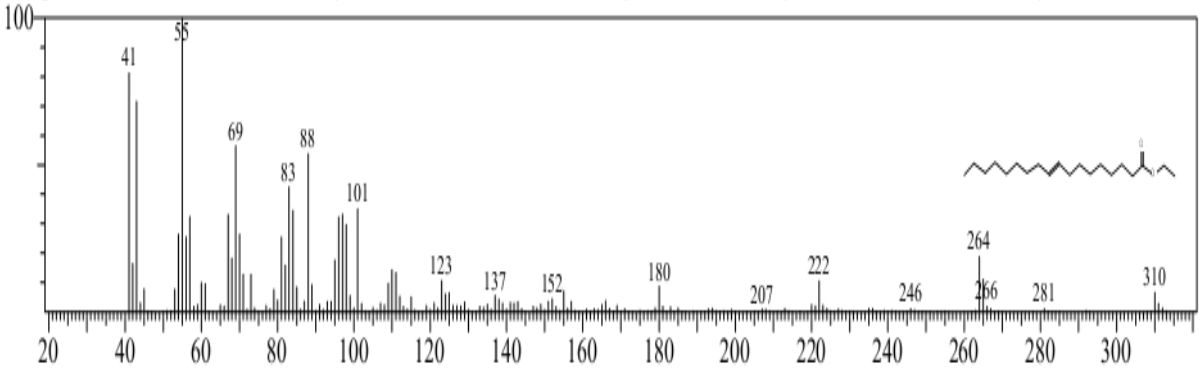
Peak no. 71: bis(2-ethylhexyl) maleate

Hit#:1 Entry:28869 Library:NIST14s.lib
SI:91 Formula:C20H36O2 CAS:544-35-4 MolWeight:308 RetIndex:2193
CompName:Linoleic acid ethyl ester $$ Ethyl linoleate $$ 9,12-Octadecadienoic acid (Z,Z)-, ethyl ester $$ Ethyl cis,cis-9,12-octadecadienoate $$ E



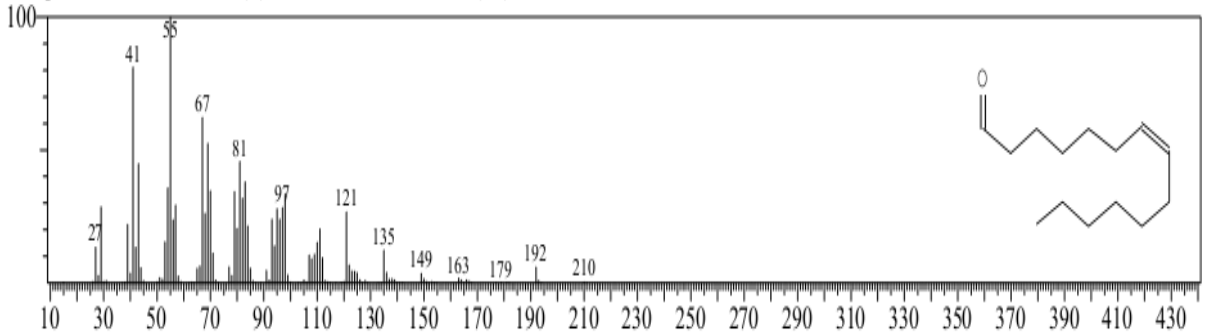
Peak no. 72: Linoleic acid ethyl ester

Hit#:1 Entry:29007 Library:NIST14s.lib
SI:80 Formula:C20H38O2 CAS:6114-18-7 MolWeight:310 RetIndex:2185
CompName:(E)-9-Octadecenoic acid ethyl ester $$ 9-Octadecenoic acid, ethyl ester, (E)- $$ Ethyl 9-octadecenoate, (E)- $$ Ethyl (9E)-9-octadecen



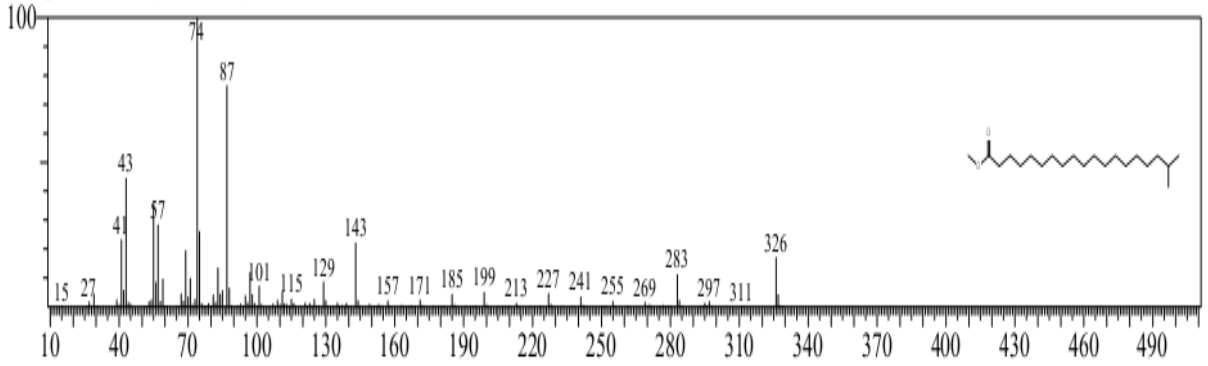
Peak no. 73: (E)-9-Octadecenoic acid ethyl ester

Hit#:1 Entry:54829 Library:NIST14.lib
SI:89 Formula:C14H26O CAS:65128-96-3 MolWeight:210 RetIndex:1609
CompName:7-Tetradecenal, (Z)- $$ Z-7-Tetradecenal $$ (7Z)-7-Tetradecenal # $$



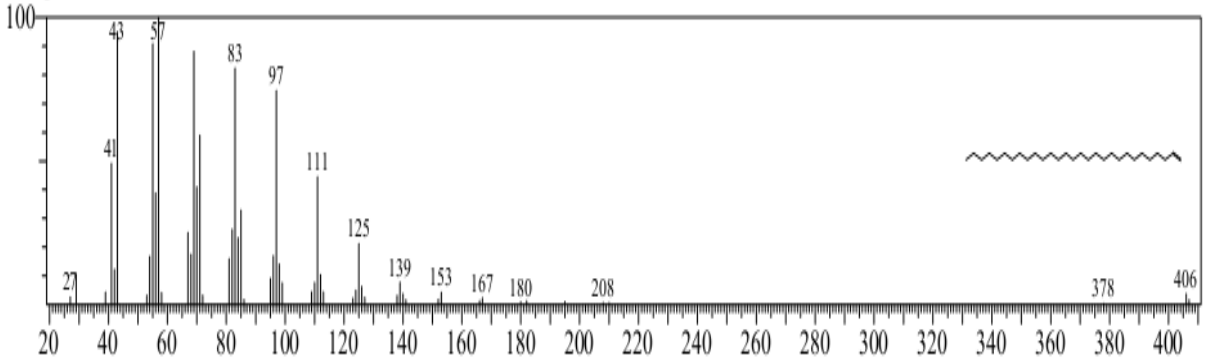
Peak no. 74: 7-Tetradecenal

Hit#:1 Entry:154704 Library:NIST14.lib
SI:92 Formula:C21H42O2 CAS:0-00-0 MolWeight:326 RetIndex:2212
CompName:Methyl 18-methylnonadecanoate



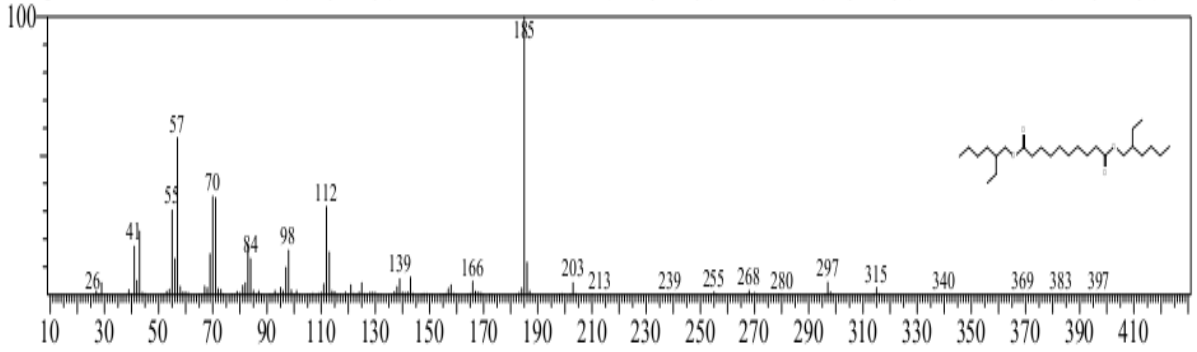
Peak no. 75: Methyl-18-methylnonadecanoate

Hit#:1 Entry:209016 Library:NIST14.lib
SI:90 Formula:C29H58 CAS:18835-35-3 MolWeight:406 RetIndex:0
CompName:Nonacos-1-ene $$ 1-Nonacosene $$

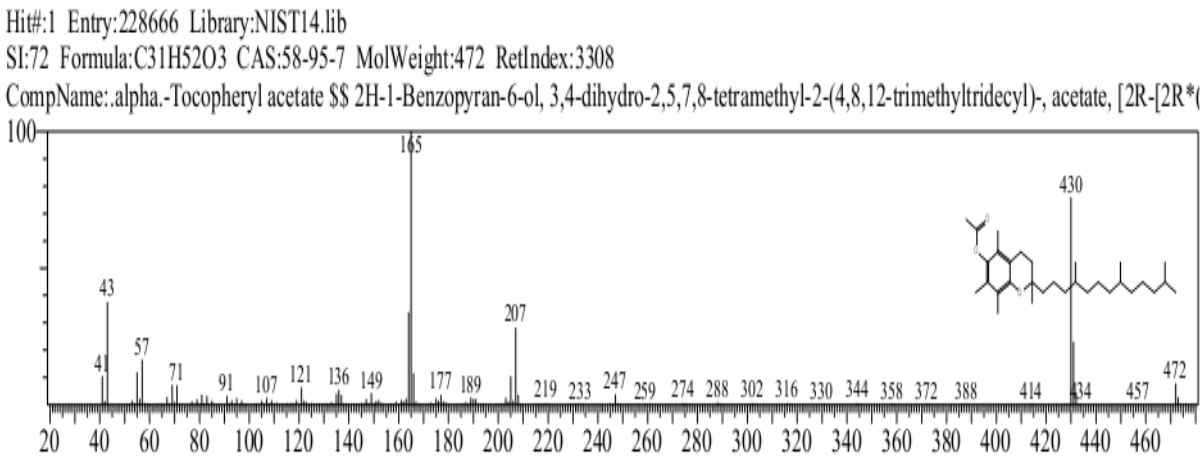


Peak no. 76: Nonacos-1-ene

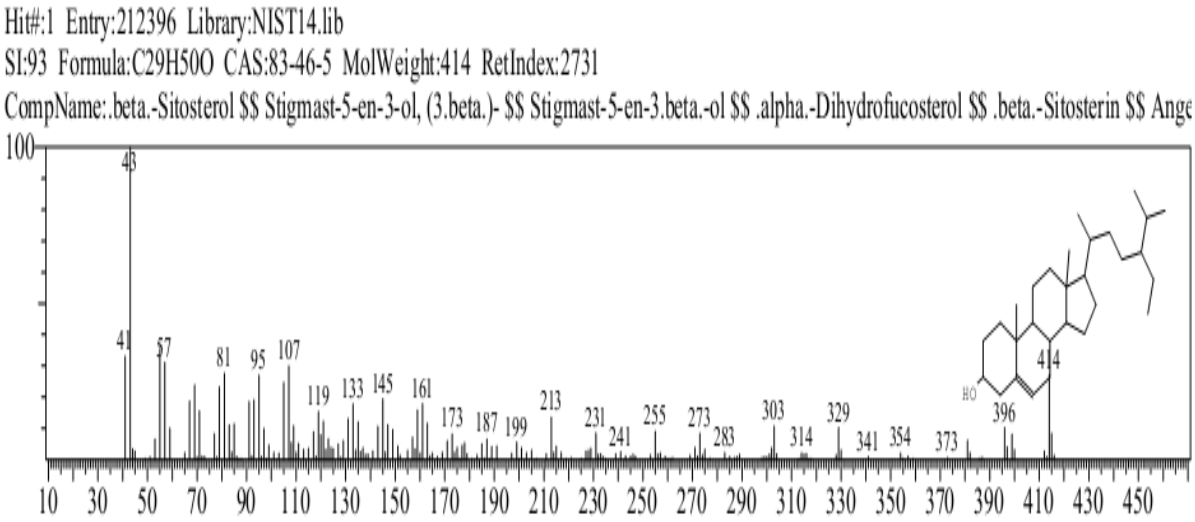
Hit#:1 Entry:216569 Library:NIST14.lib
SI:86 Formula:C26H50O4 CAS:122-62-3 MolWeight:426 RetIndex:2812
CompName:Decanedioic acid, bis(2-ethylhexyl) ester $$ Sebacic acid, bis(2-ethylhexyl) ester $$ Bis(ethylhexyl) sebacate $$ Bis(2-ethylhexyl) dec



Peak no. 77: Decanedioic acid, bis(2-ethylhexyl) ester

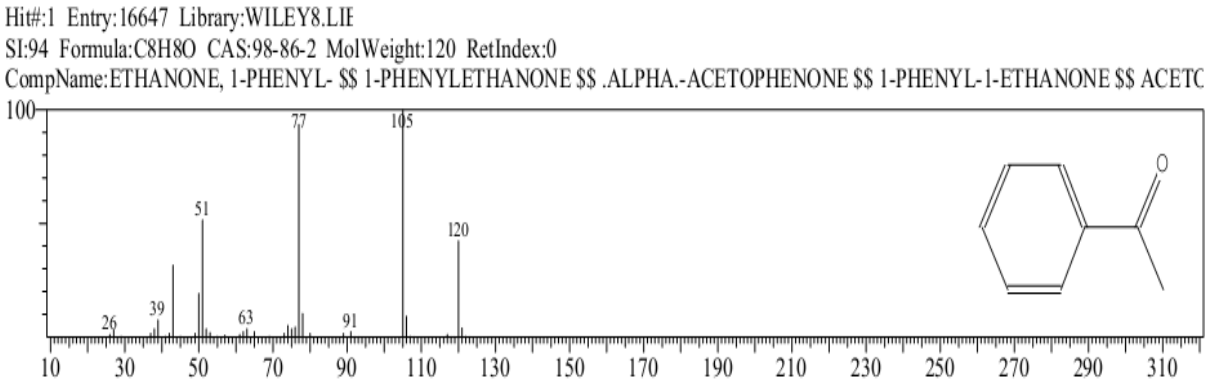


Peak no. 78: alpha, -Tocopheryl acetate



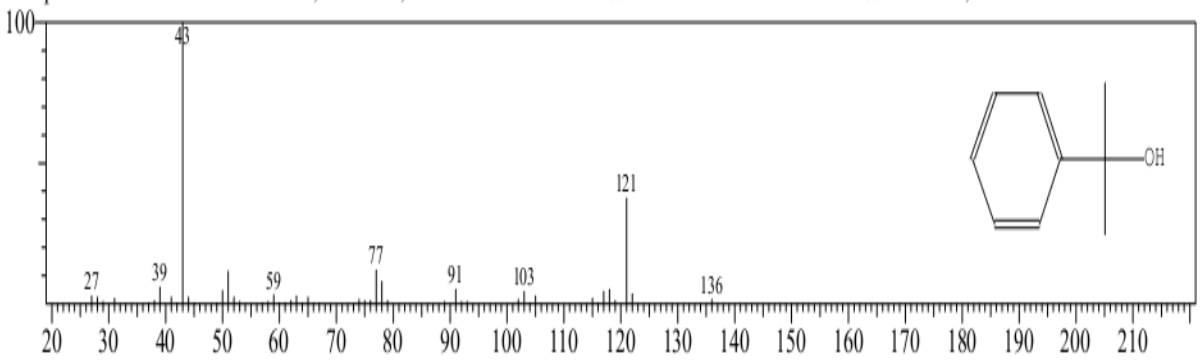
Peak no. 79: beta, -Sitosterol

The major mass spectrum (m/z) of methanol seeds crude extracts of *Colchicum autumnale* were:



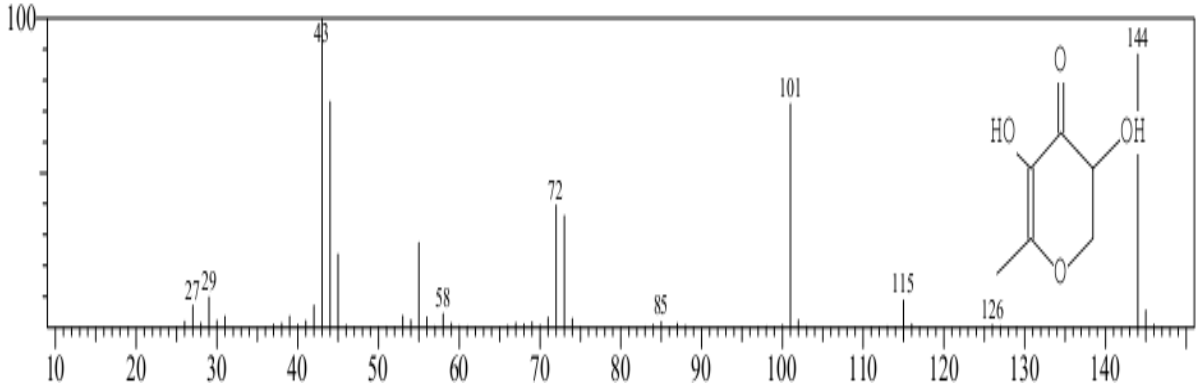
Peak no. 80: 1-phenyl, Ethanone

Hit#:1 Entry:27769 Library:WILEY8.LIB
SI:83 Formula:C9H12O CAS:617-94-7 MolWeight:136 RetIndex:0
CompName:BENZENEMETHANOL, .ALPHA.,.ALPHA.-DIMETHYL- $$ 2-PHENYLPROPAN-2-OL $$ .ALPHA.,.ALPHA.-DIMETHYLBEN



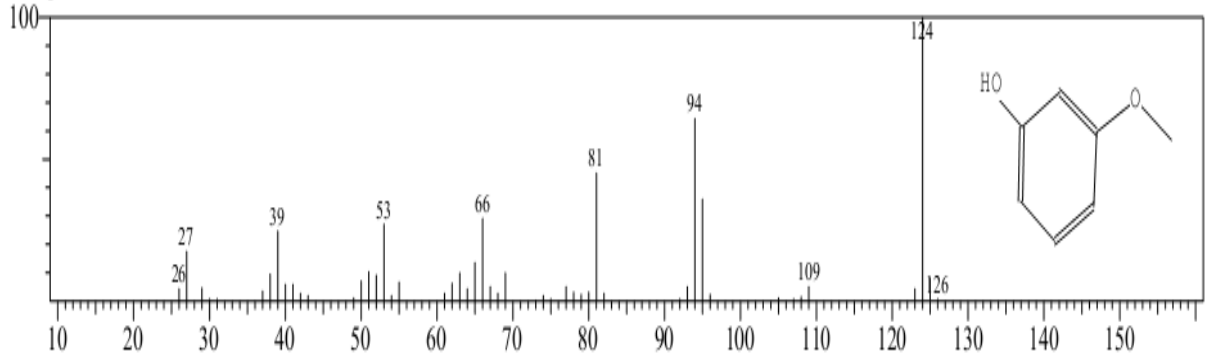
Peak no. 81: alpha, alpha, dimethyl benzene methanol

Hit#:1 Entry:8053 Library:NIST14s.lib
SI:84 Formula:C6H8O4 CAS:28564-83-2 MolWeight:144 RetIndex:1269
CompName:4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- $$ 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one $$ 2,3-dihydro-3,5-di



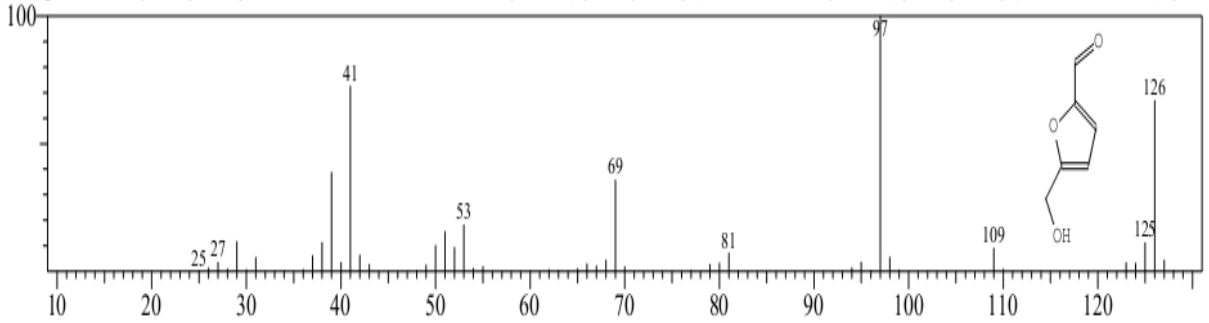
Peak no. 82: 2, 3-dihydro-6-methyl, 4H-Pyran-4-one

Hit#:1 Entry:18597 Library:WILEY8.LIB
SI:94 Formula:C7H8O2 CAS:150-19-6 MolWeight:124 RetIndex:0
CompName:PHENOL, 3-METHOXY- $$ 3-METHOXYPHENOL $$ 1-HYDROXY-3-METHOXYBENZENE $$ 3-HYDROXYANISOLE $$ 3-



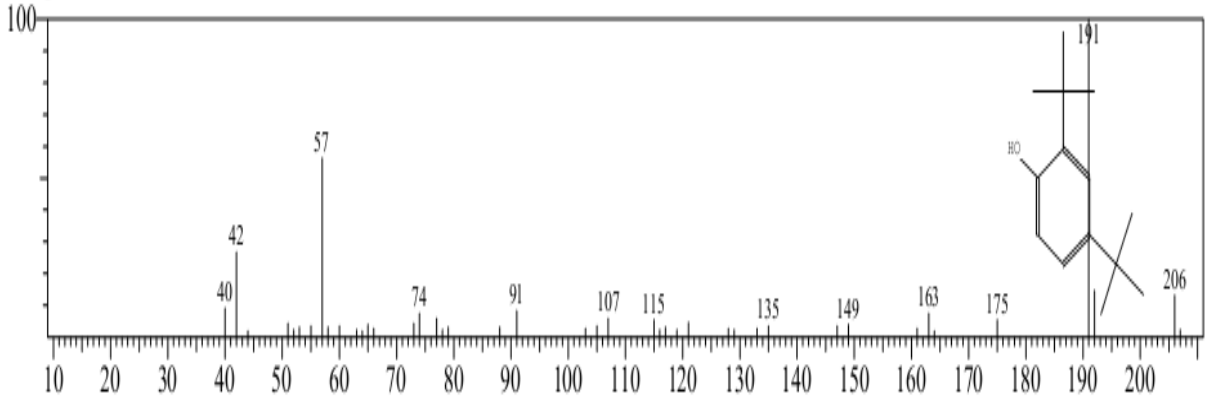
Peak no. 83: 3-Methoxy, Phenol

Hit#:1 Entry:4840 Library:NIST14s.lib
SI:92 Formula:C6H6O3 CAS:67-47-0 MolWeight:126 RetIndex:1163
CompName:5-Hydroxymethylfurfural $$ 2-Furancarboxaldehyde, 5-(hydroxymethyl)- $$ 2-Furaldehyde, 5-(hydroxymethyl)- $$ HMF $$ 5-(Hydd



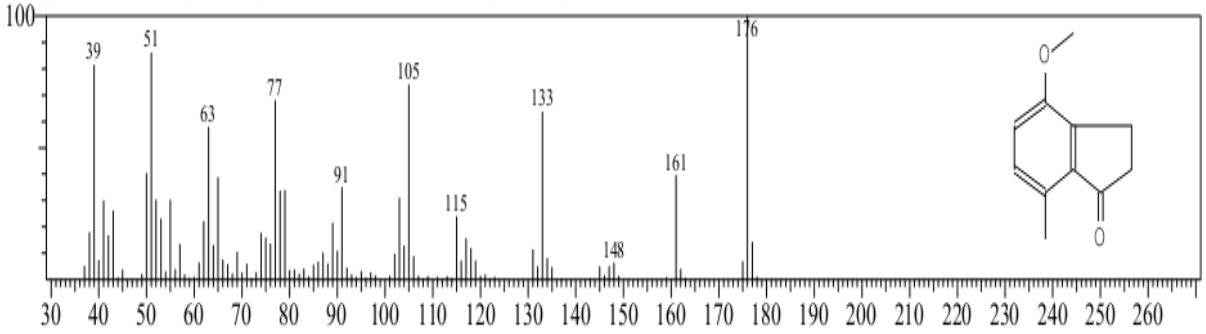
Peak no. 84: 5-Hydroxymethylfurfural

Hit#:1 Entry:113154 Library:WILEY8.LIB
SI:94 Formula:C14H22O CAS:0-00-0 MolWeight:206 RetIndex:0
CompName:2,4-DITERT-BUTYLPHENOL $$ 2,4-DI-TERT-BUTYLPHENOL



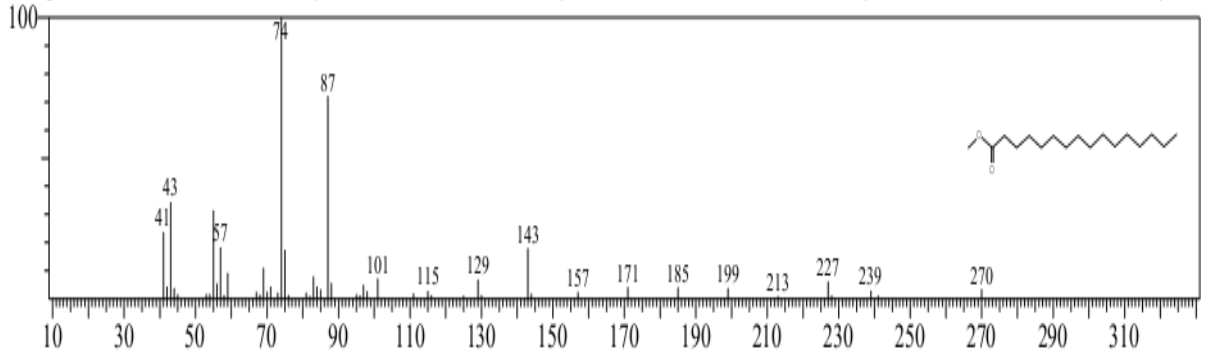
Peak no. 85: 2, 4-Ditert-butyl phenol

Hit#:1 Entry:30578 Library:NIST14.lib
SI:74 Formula:C11H12O2 CAS:103988-25-6 MolWeight:176 RetIndex:1520
CompName:4-Methoxy-7-methylindan-1-one $$ 4-Methoxy-7-methyl-1-indanone # $$



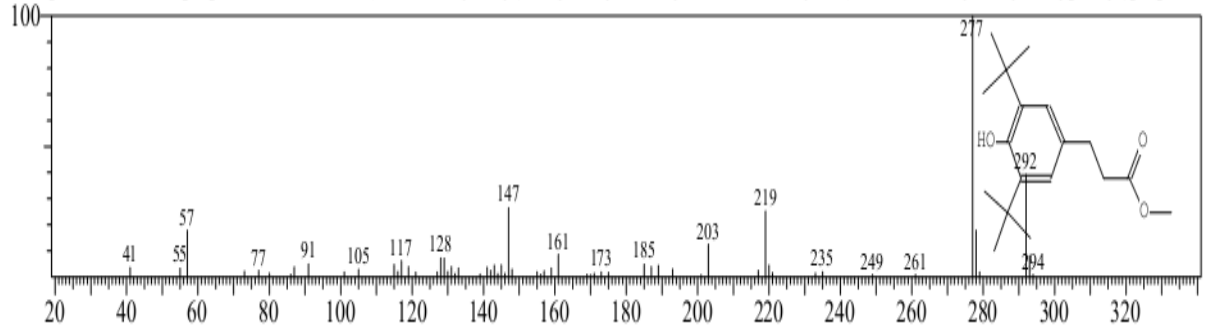
Peak no. 86: 4-Methoxy-7-methylindan-1-one

Hit#:1 Entry:26272 Library:NIST14s.lib
SI:97 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl he:



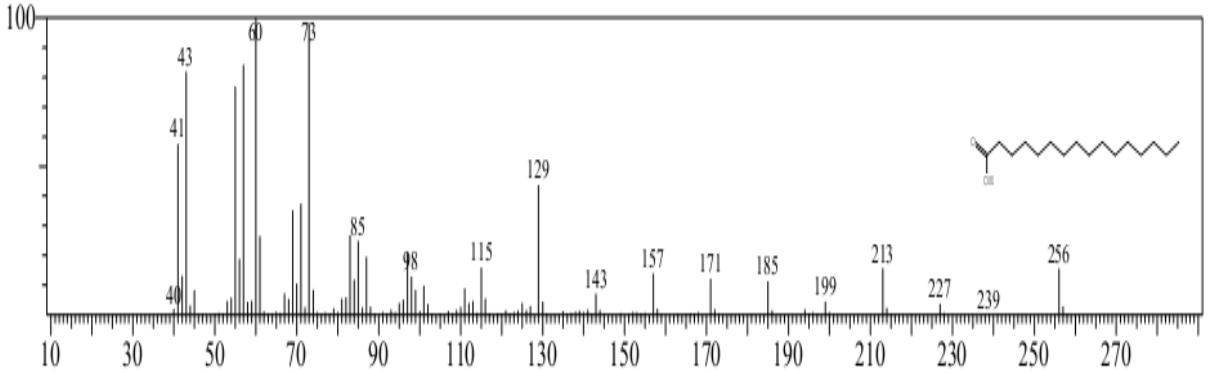
Peak no. 87: Hexadecanoic acid, methyl ester

Hit#:1 Entry:27863 Library:NIST14s.lib
SI:84 Formula:C18H28O3 CAS:6386-38-5 MolWeight:292 RetIndex:2134
CompName:Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester $$ Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propiona



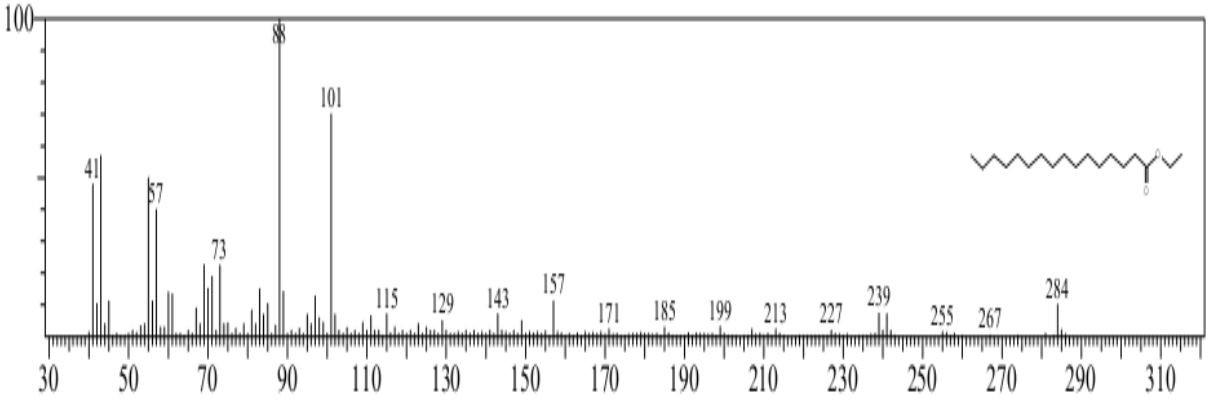
Peak no. 88: Benzene propanoic acid, 3, 5-bis (1, 1-dimethylethyl)-4-hydroxy-, methyl ester

Hit#:1 Entry:25118 Library:NIST14s.lib
SI:97 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecaneca



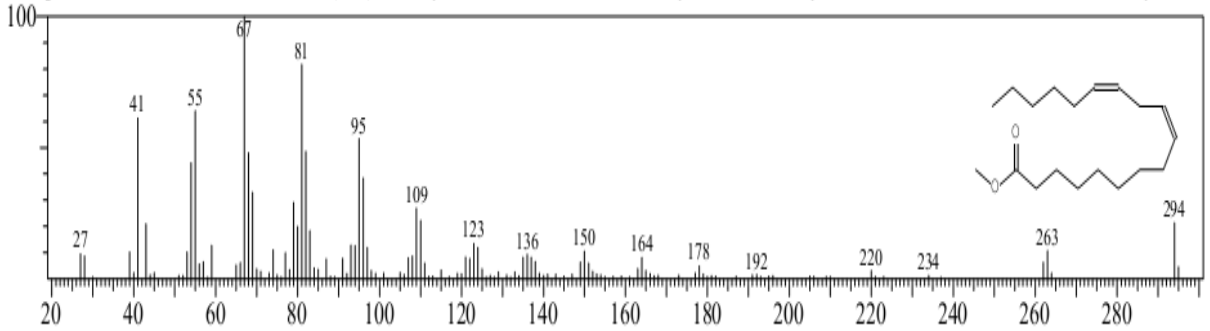
Peak no. 89: n-Hexadecanoic acid

Hit#:1 Entry:27283 Library:NIST14s.lib
SI:93 Formula:C18H36O2 CAS:628-97-7 MolWeight:284 RetIndex:1978
CompName:Hexadecanoic acid, ethyl ester $$ Palmitic acid, ethyl ester $$ Ethyl hexadecanoate $$ Ethyl palmitate $$ Ethyl n-hexadecanoate $$



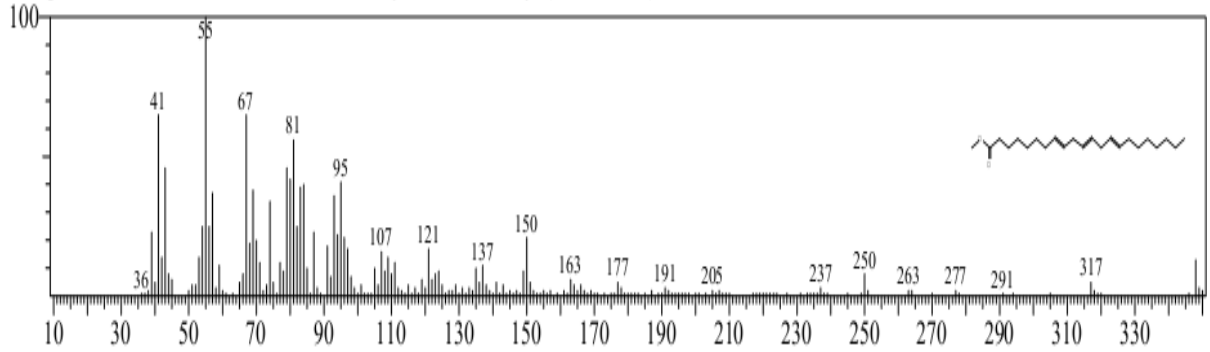
Peak no. 90: Hexadecanoic acid, ethyl ester

Hit#:1 Entry:27999 Library:NIST14s.lib
SI:96 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:2093
CompName:9,12-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester $$ Methyl cis,cis-9,12-octadecadienoate $$ Methyl linol

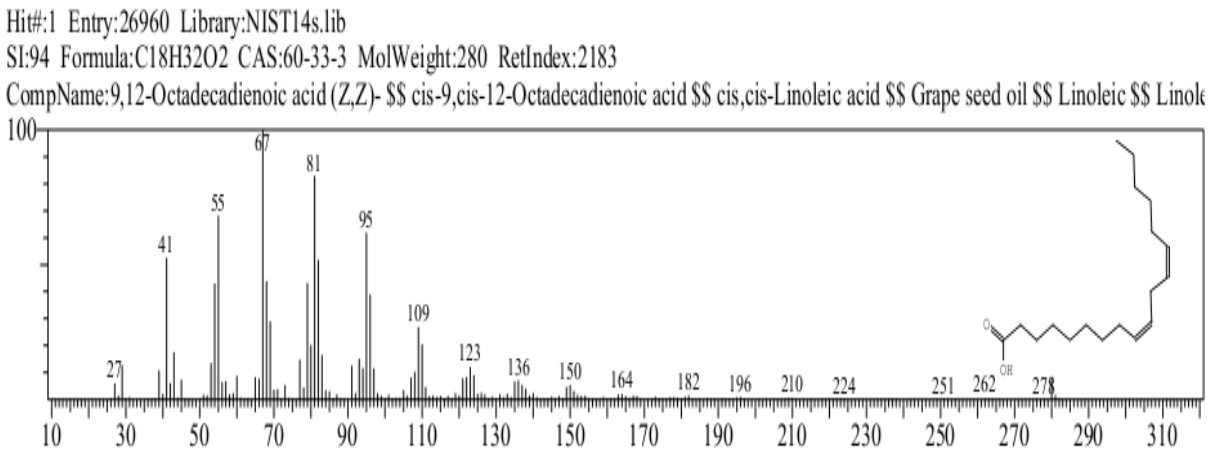


Peak no. 91: 9,12-Octadecadienoic acid (Z, Z)-, methyl ester

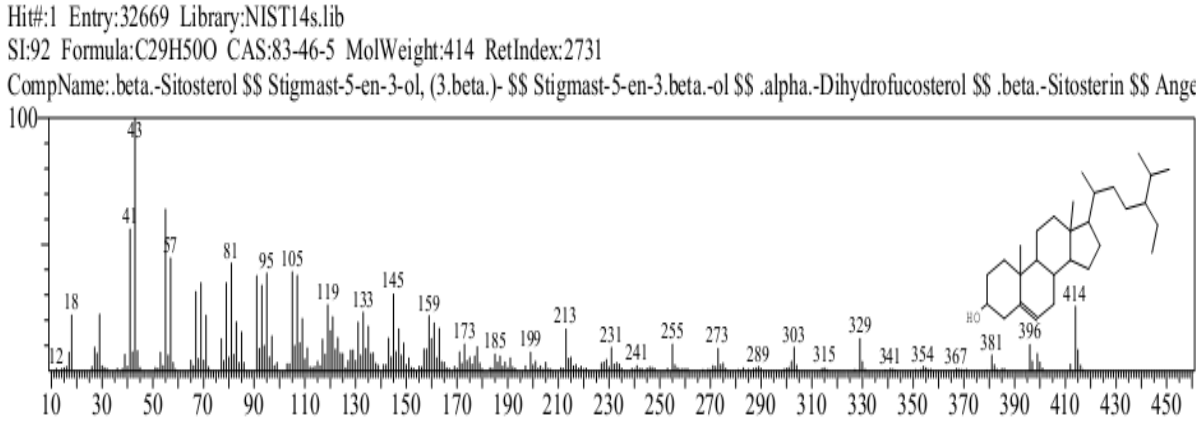
Hit#:1 Entry:173669 Library:NIST14.lib
SI:90 Formula:C23H40O2 CAS:56847-02-0 MolWeight:348 RetIndex:2499
CompName:8,11,14-Docosatrienoic acid, methyl ester $$ Methyl (8E,11E,14E)-8,11,14-docosatrienoate # $$



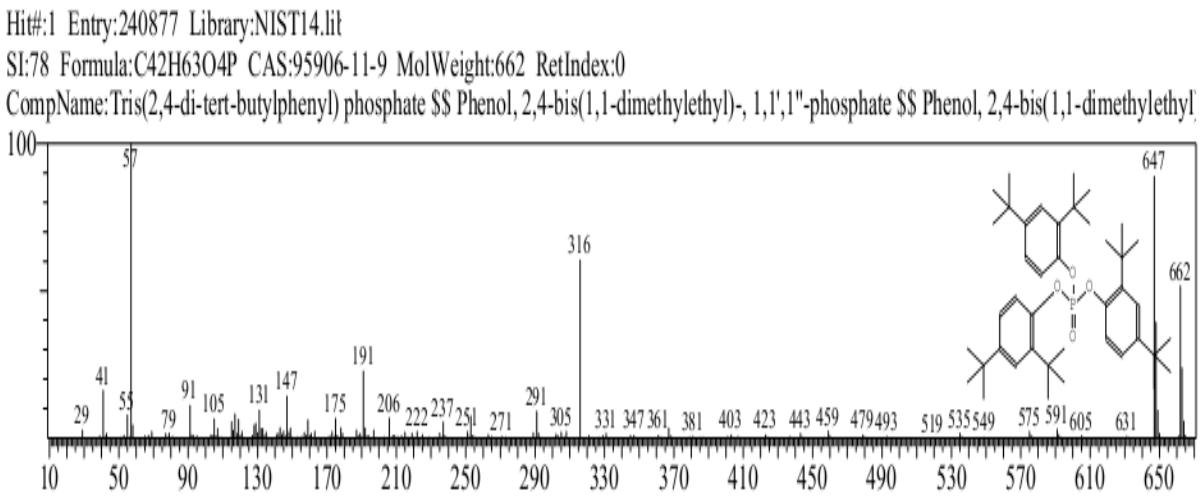
Peak no. 92: 8, 11, 14-Docosatrienoic acid, methyl ester



Peak no. 93: 9, 12-Octadecadienoic acid (Z, Z)



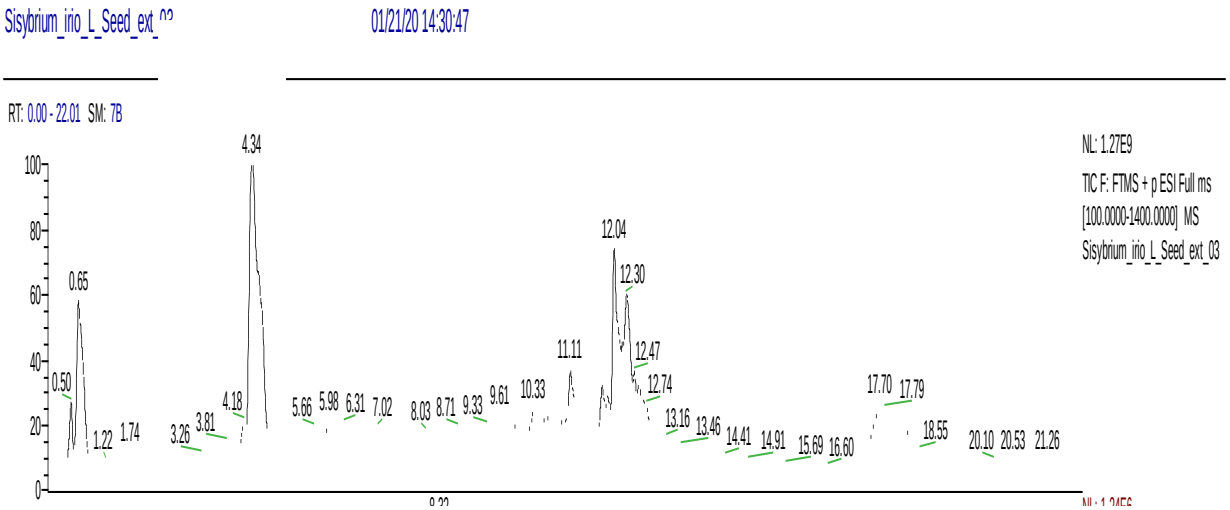
Peak no. 94: beta. -Sitosterol



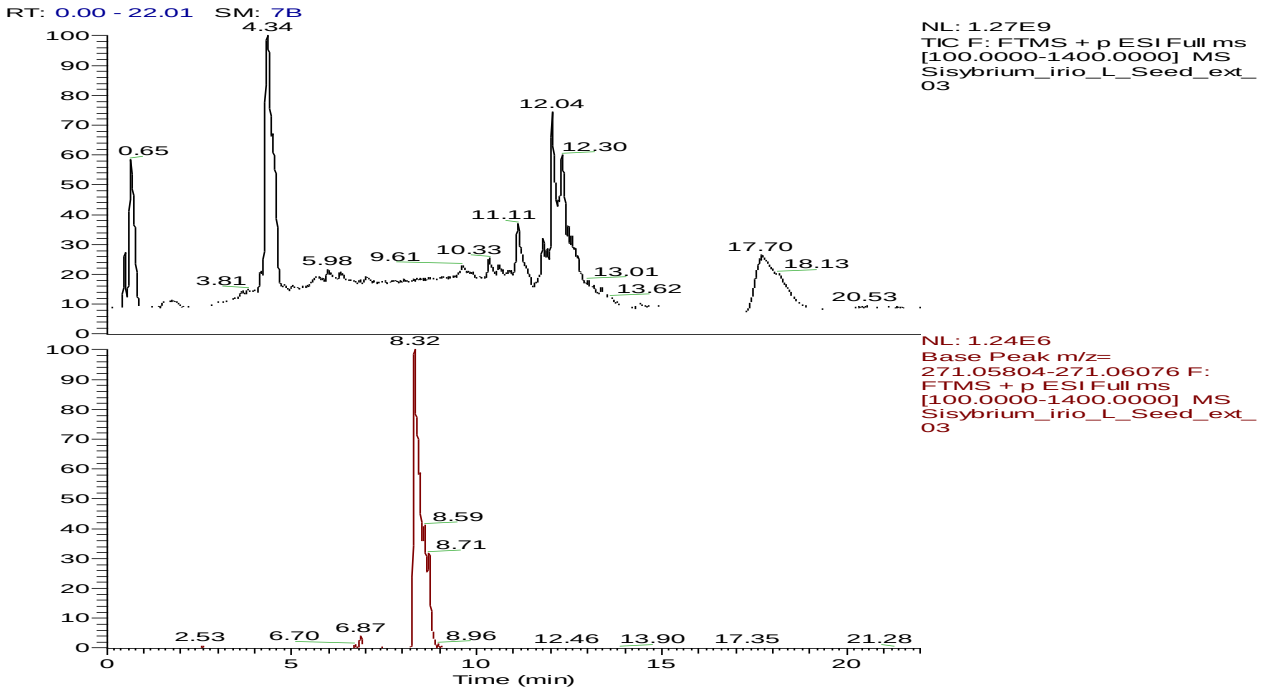
Peak no. 95: Tris (2, 4-di-tert-butylphenyl) phosphate

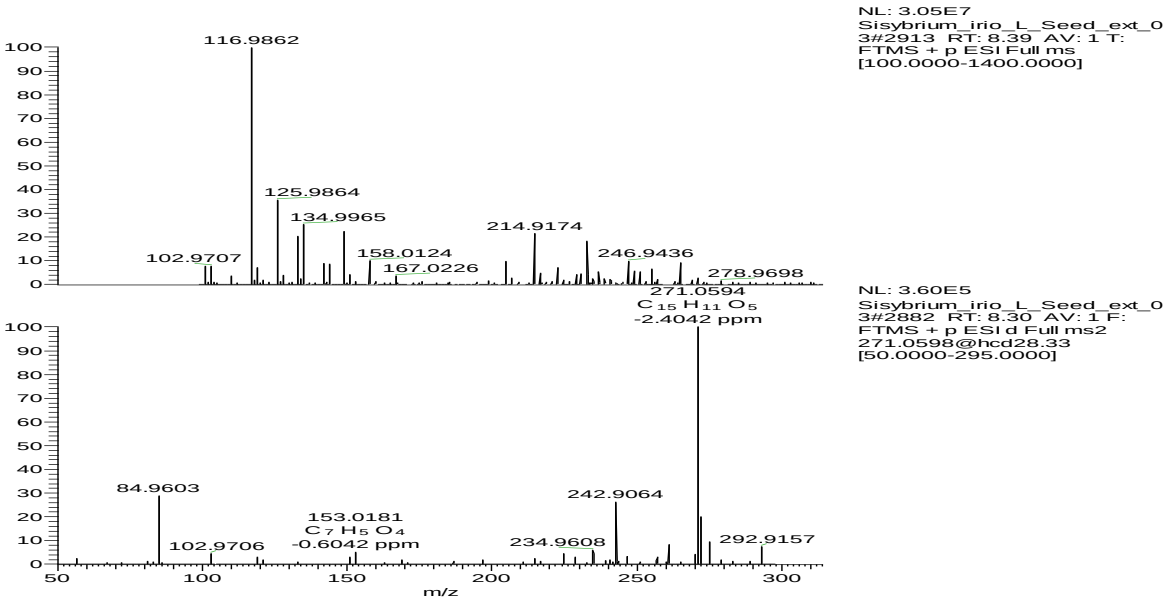
Appendix figure 2: Mass spectrum (m/z) of n-Hexane, dichloromethane, and methanol extracts of *Colchicum autumnale* L

***Sisymbrium irio* L seed extract 03**

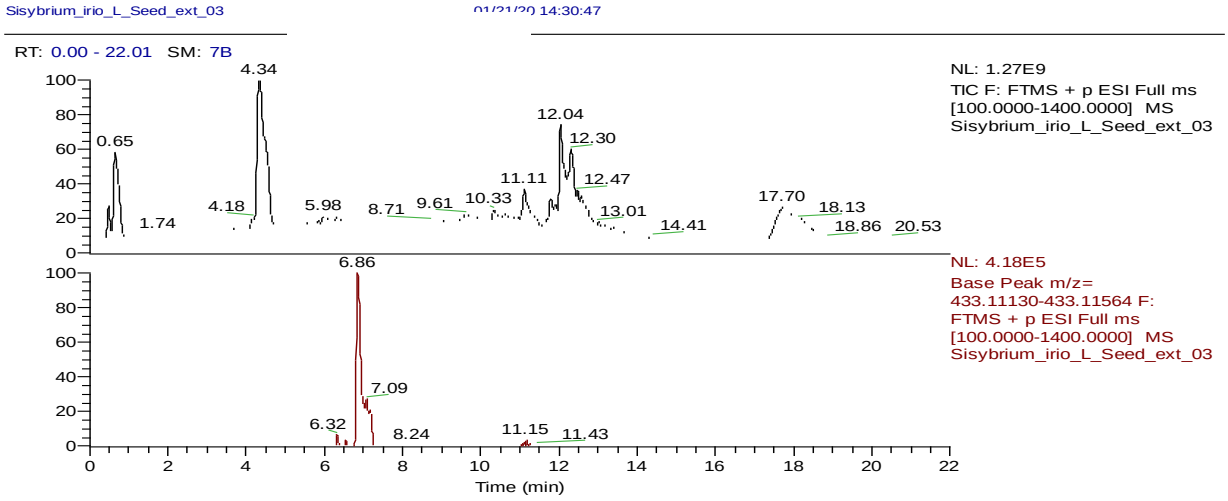


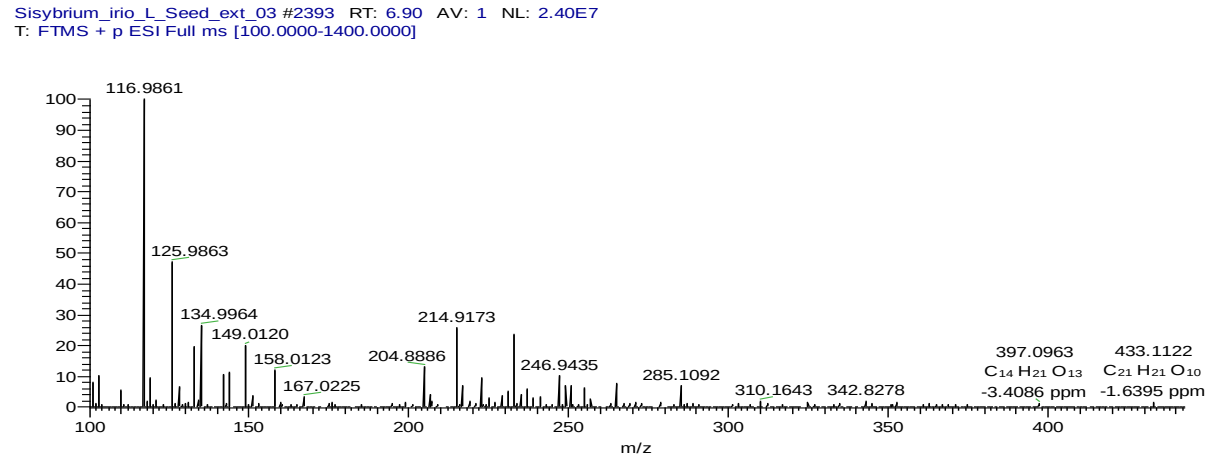
Appendix figure 3. A total ion chromatogram of *Sisymbrium irio* L seed extract acquired in full scan ddMS2 mode.



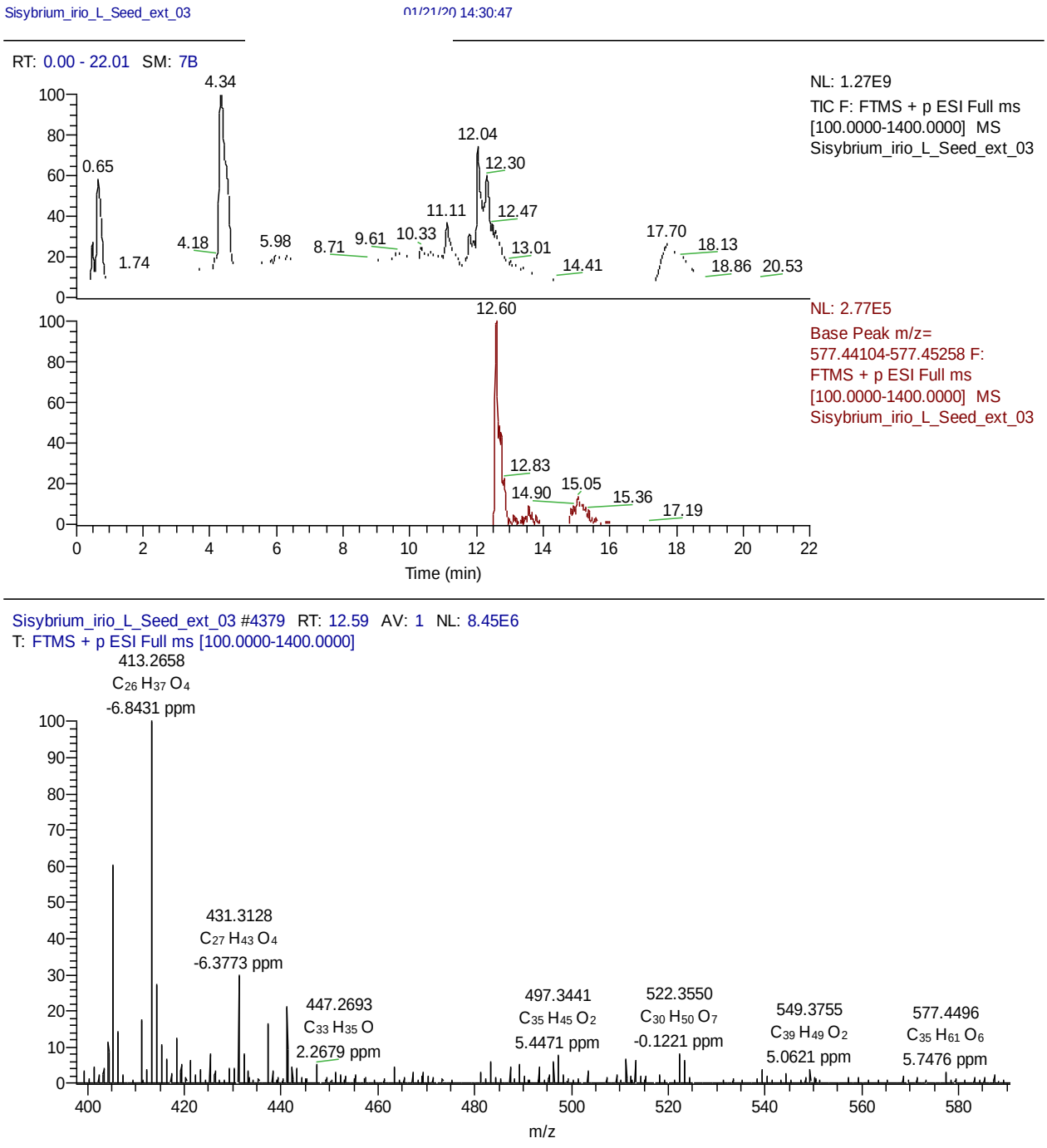


Peak no. 96: Apigenin

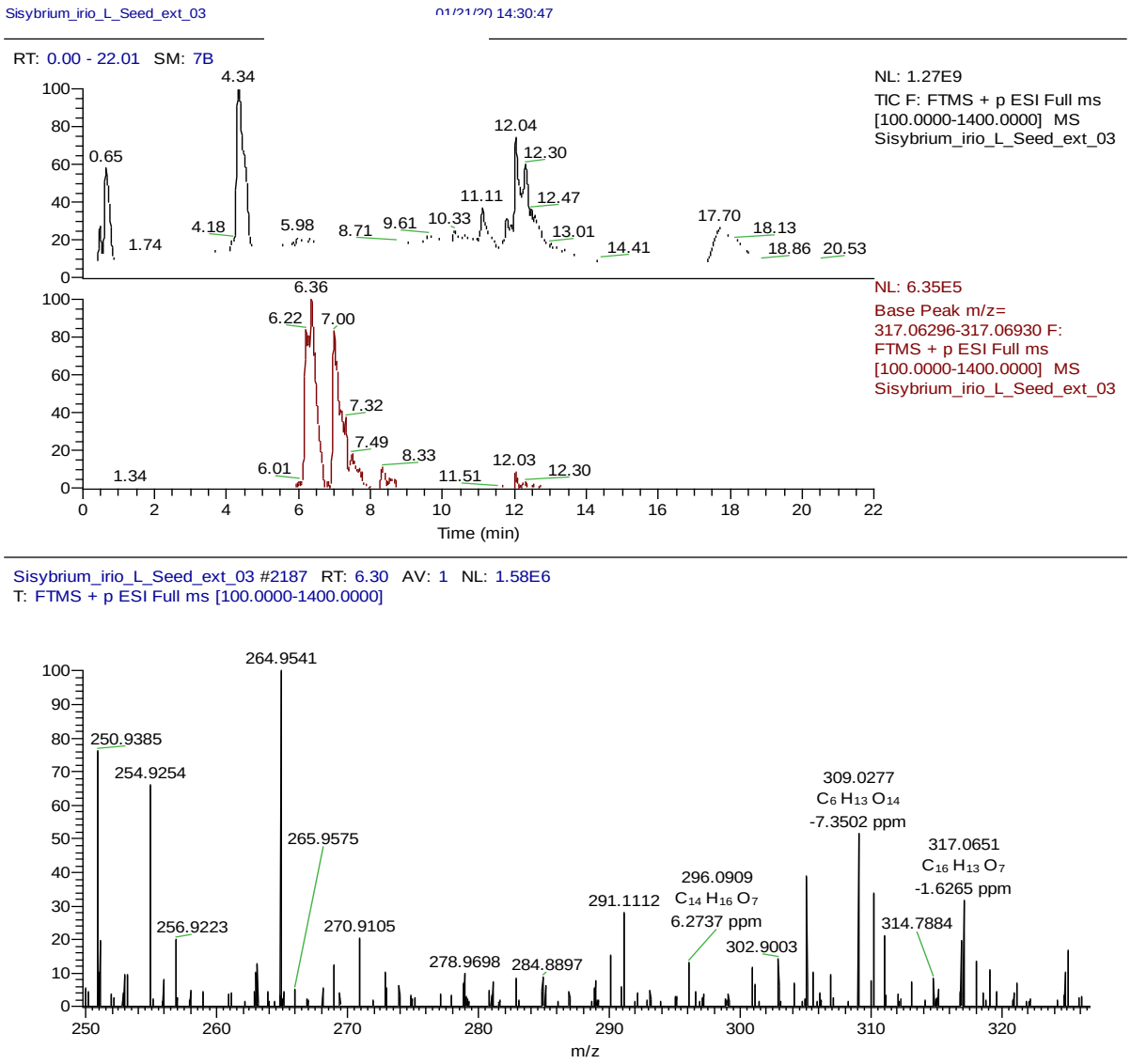




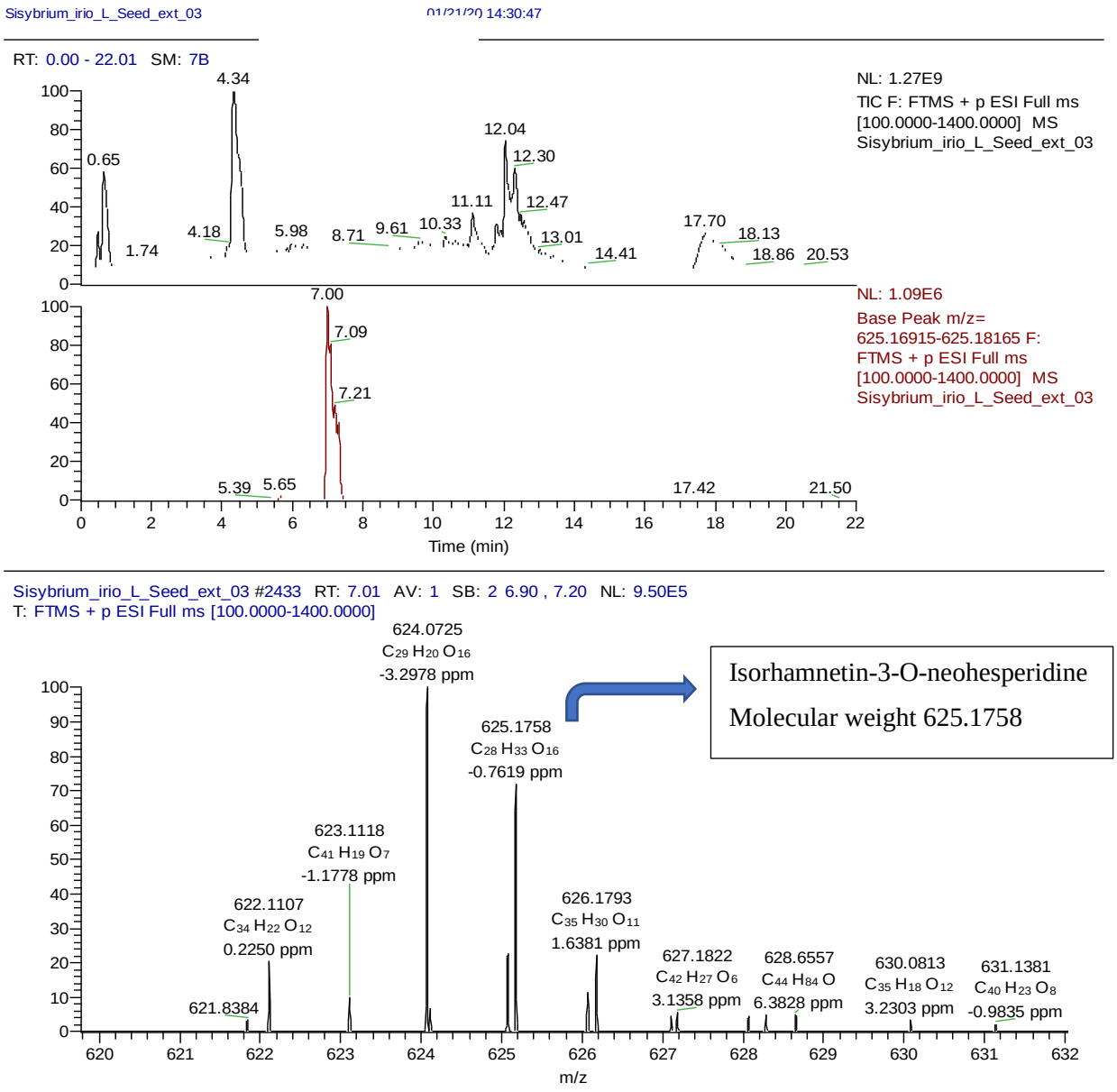
Peak no. 97: Apigenin-7-O-glucoside



Peak no. 98: (17ξ)-Stigmast-5-en-3-yl β-D-glucopyranoside



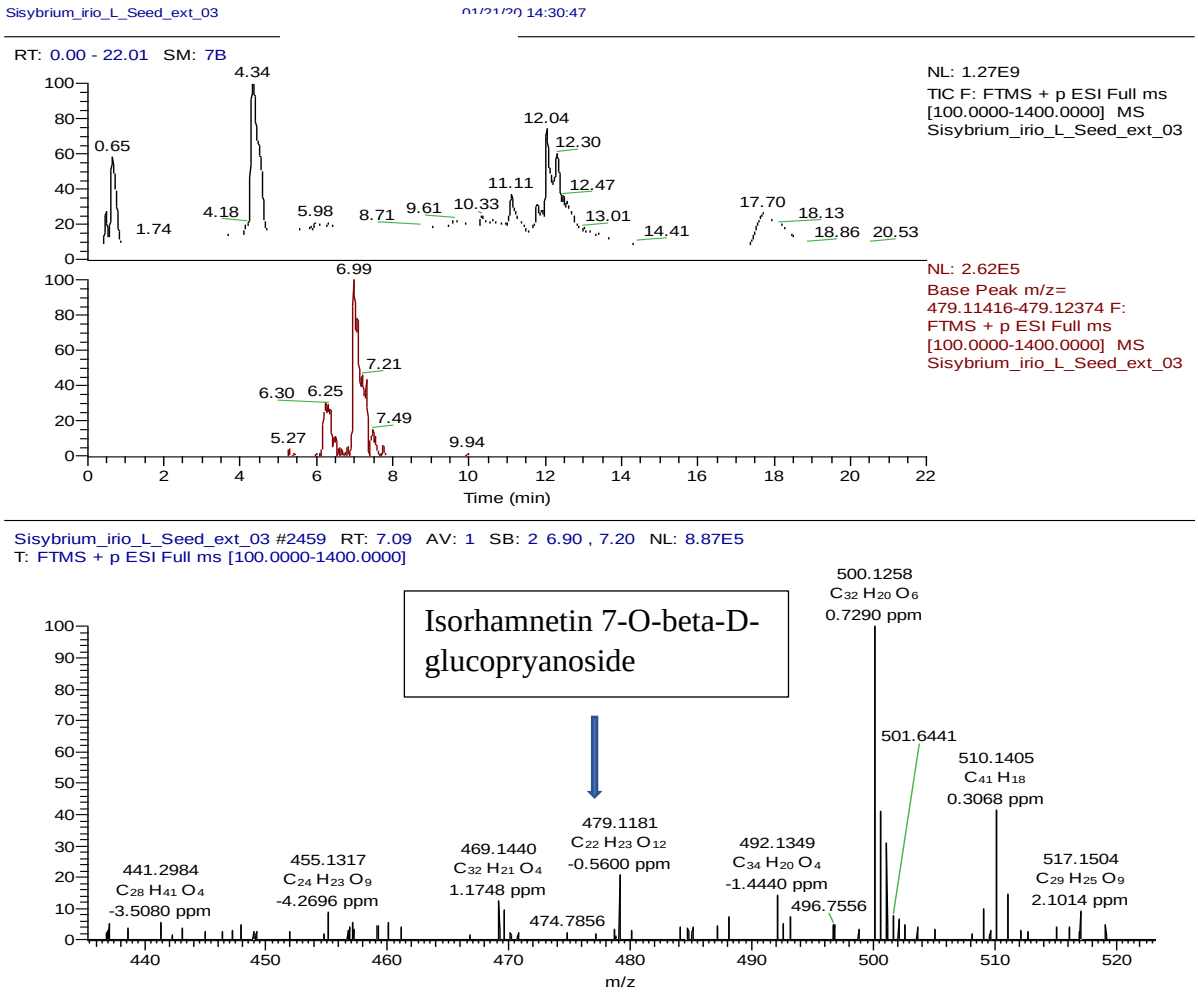
Peak no. 99: Isorhamnetin



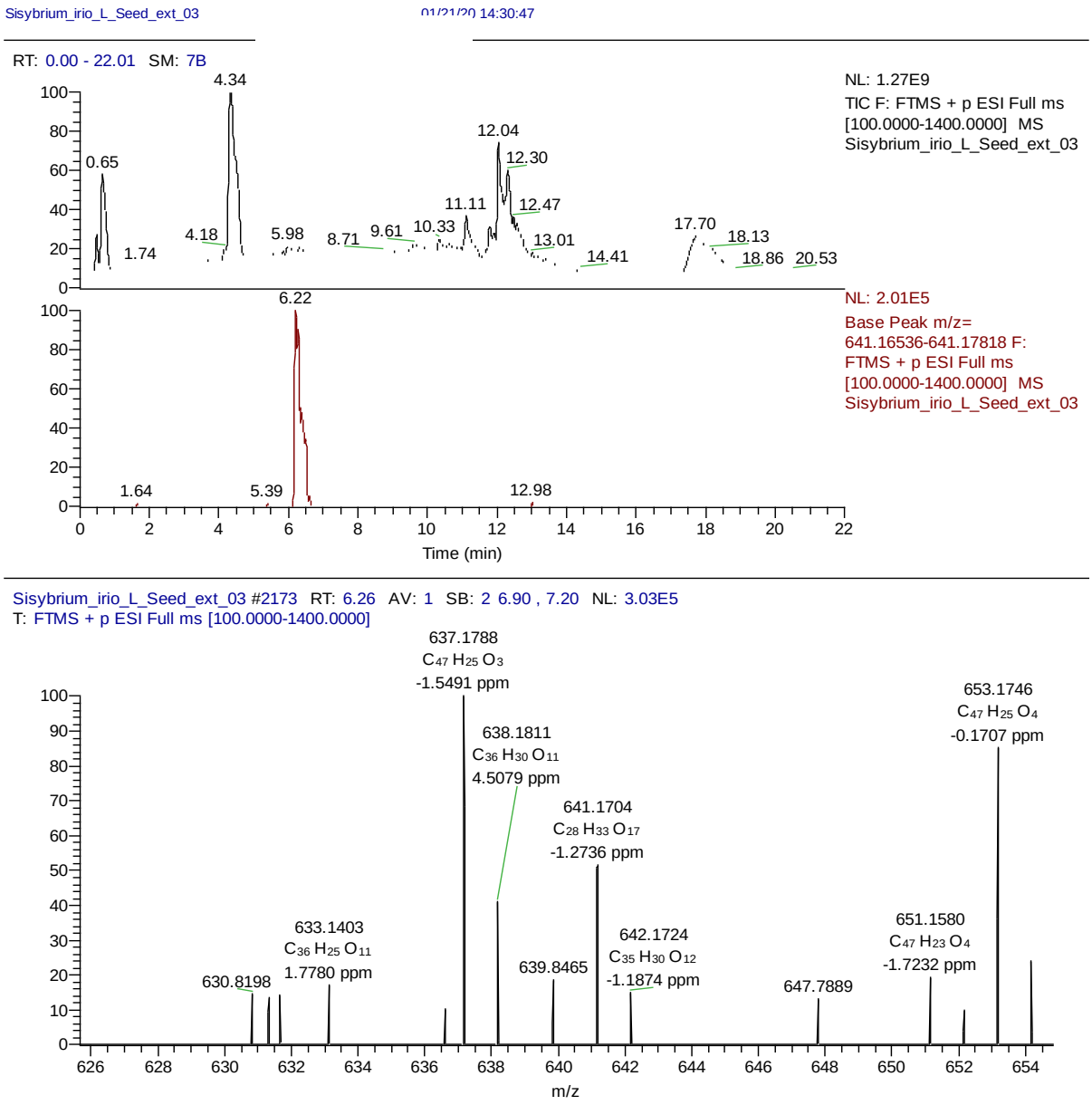
Isorhamnetin-3-O-neohesperidine

Molecular weight 625.1758

Peak no. 100: Isorhamnetin-3-O-neohesperidine

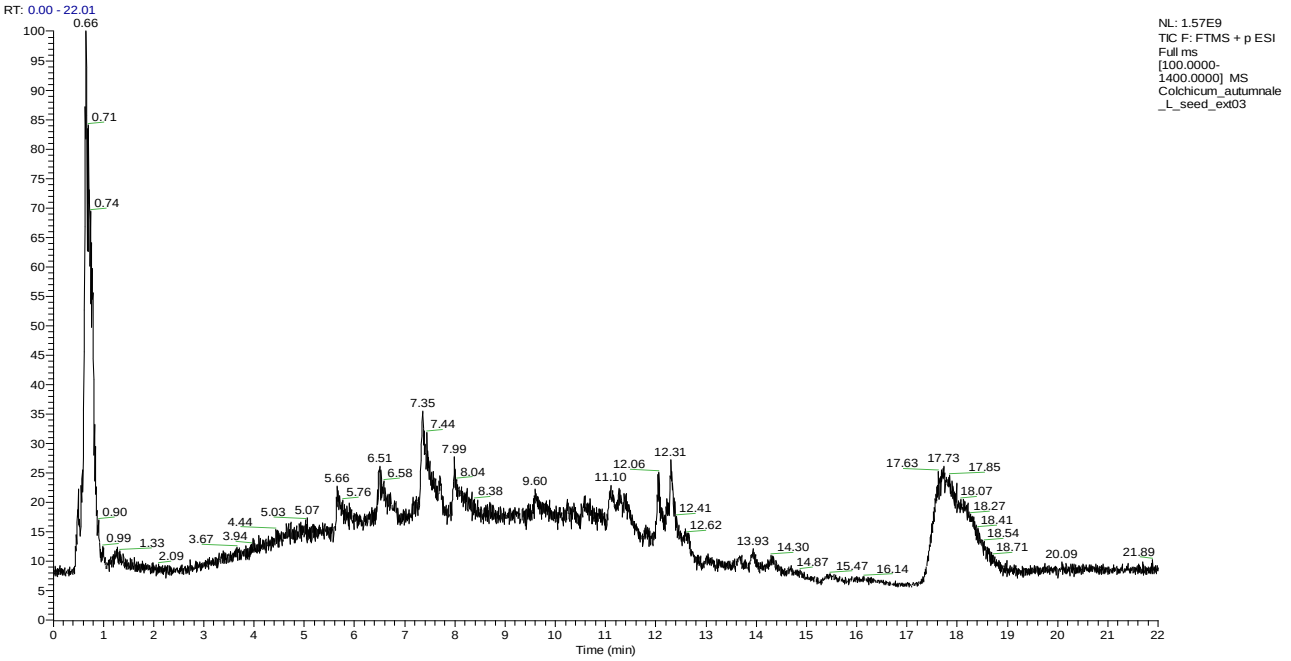


Peak no. 101: Isorhamnetin 7-O-beta-D-glucopryanoside, Isorhamnetin 7-glucoside

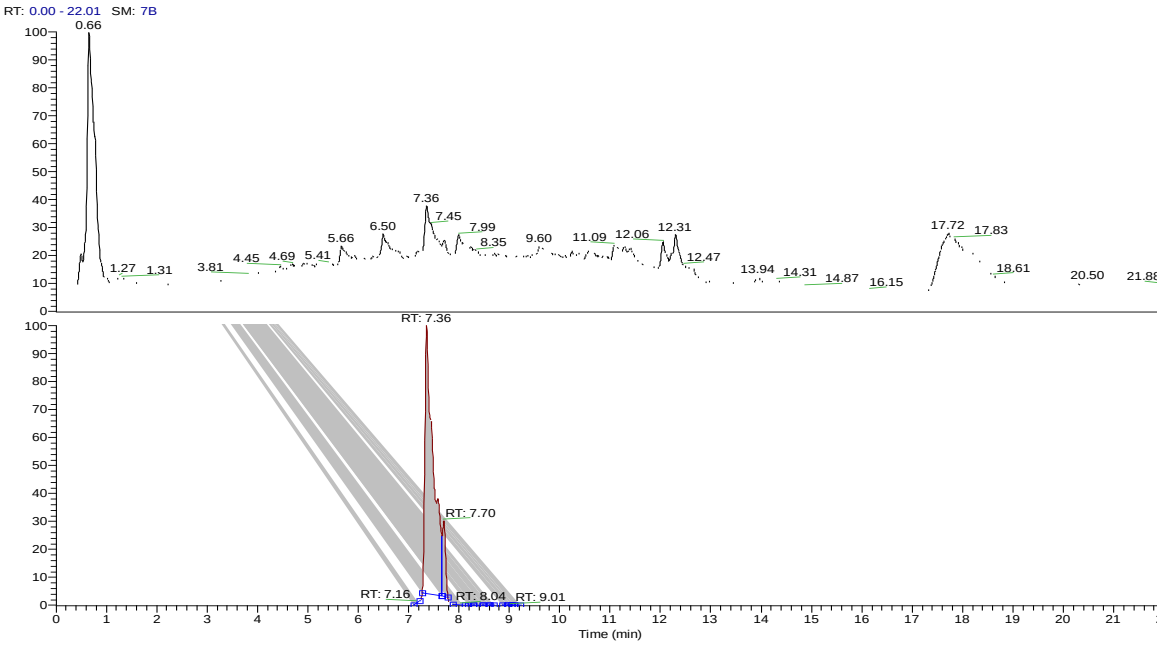


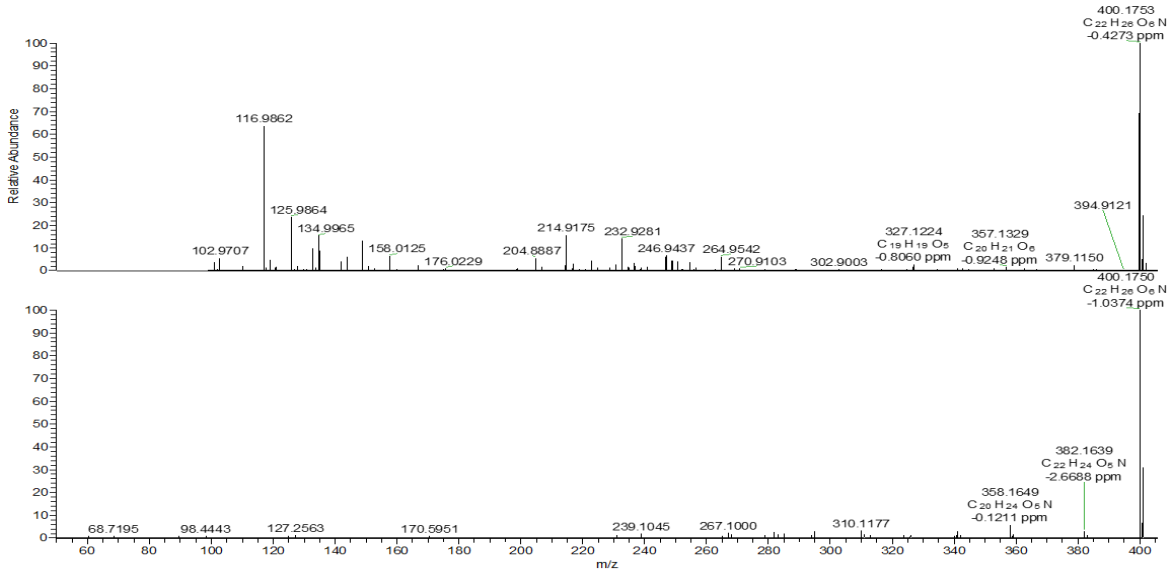
Peak no. 102: Isorhamnetin-3-Laminaribioside

Colchicum autumnale L ext.03

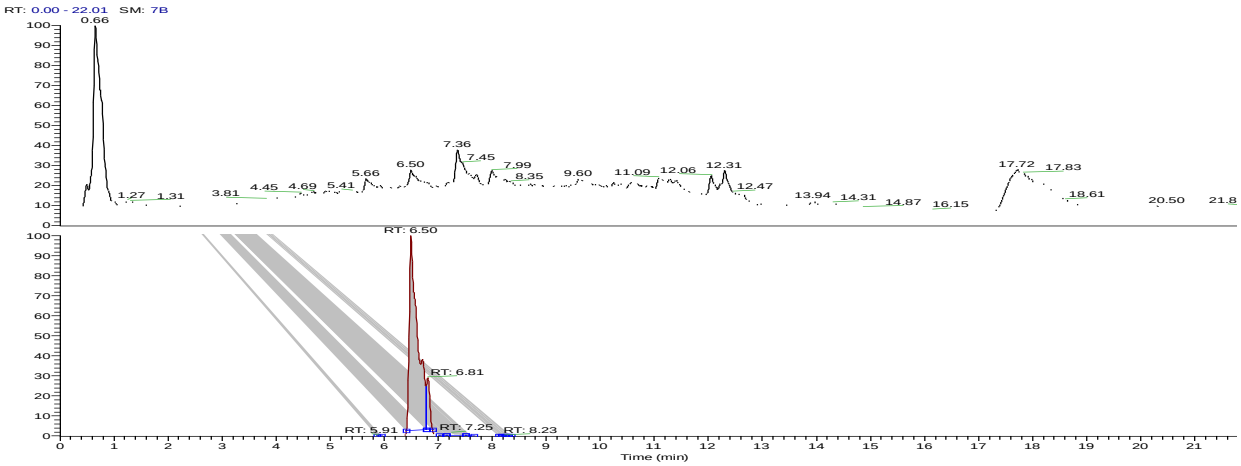


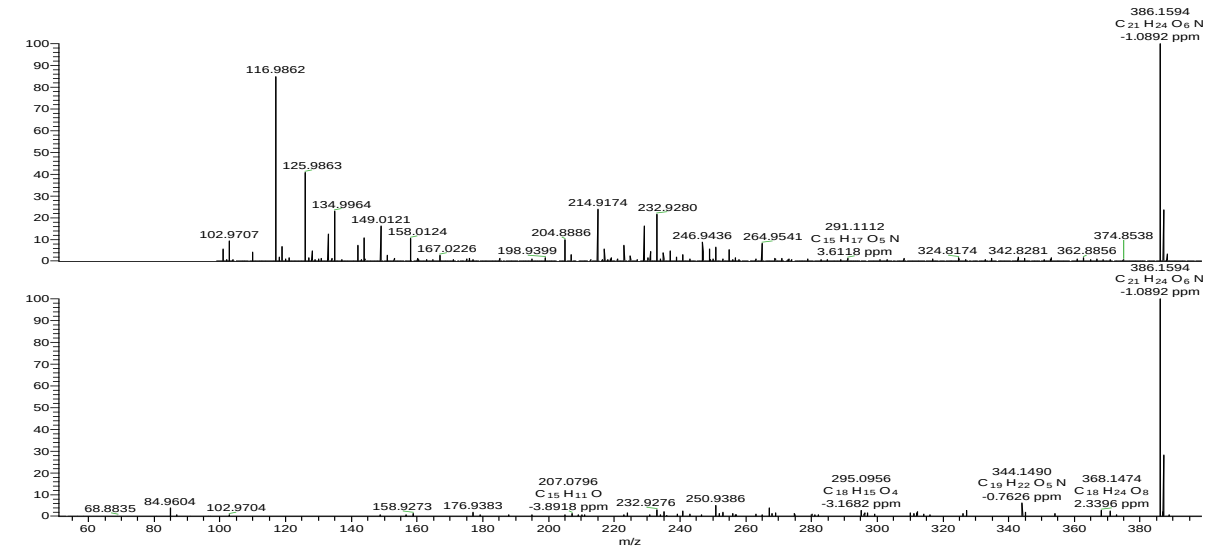
Appendix figure 4. A total ion chromatogram or *Colchicum autumnale* L ext03 sample acquired in full scan ddMS2 mode.



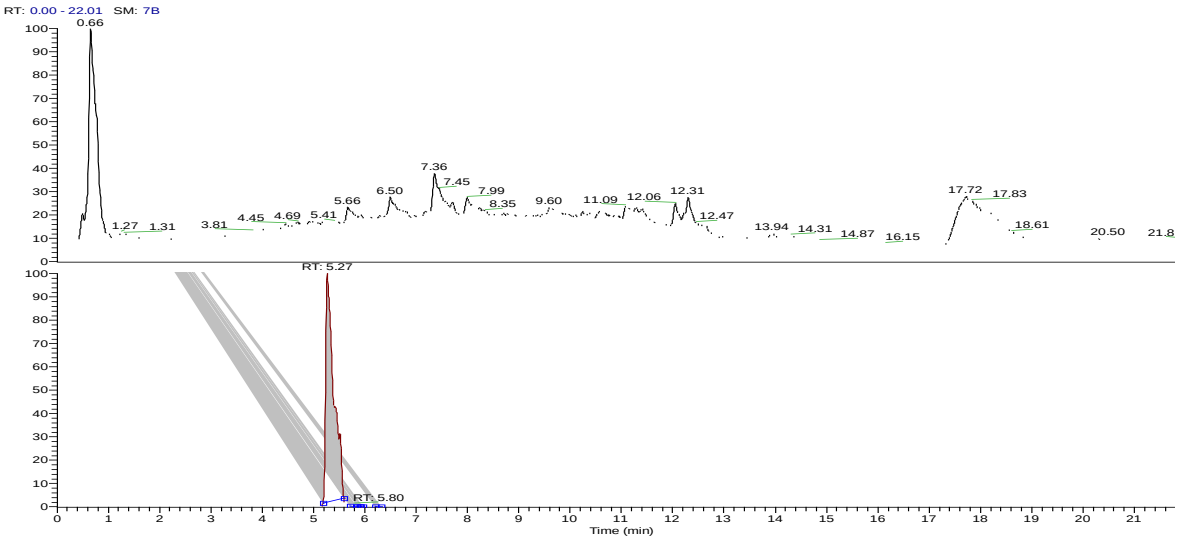


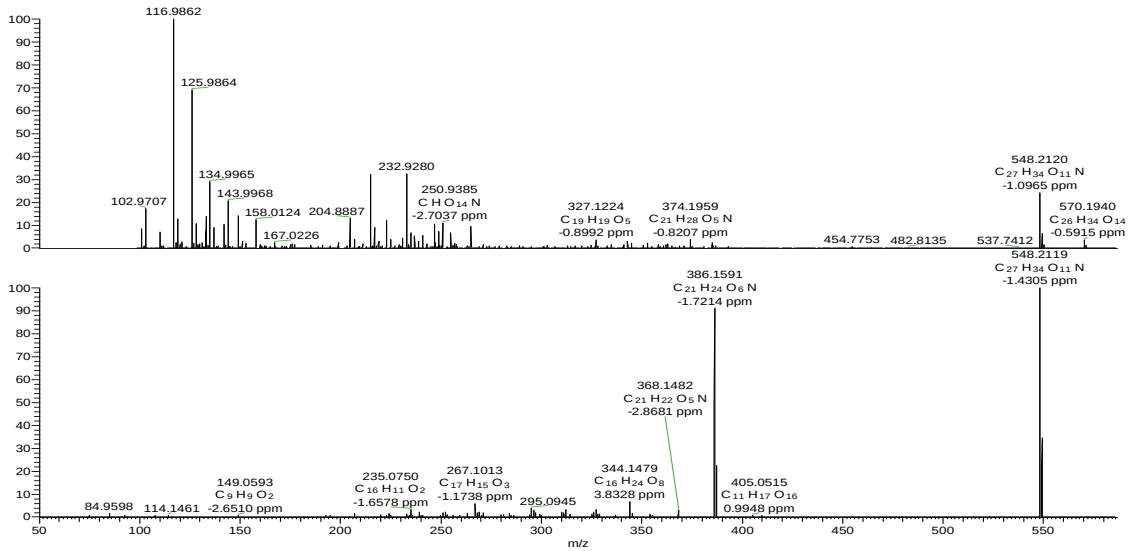
Peak no. 103: Colchicine



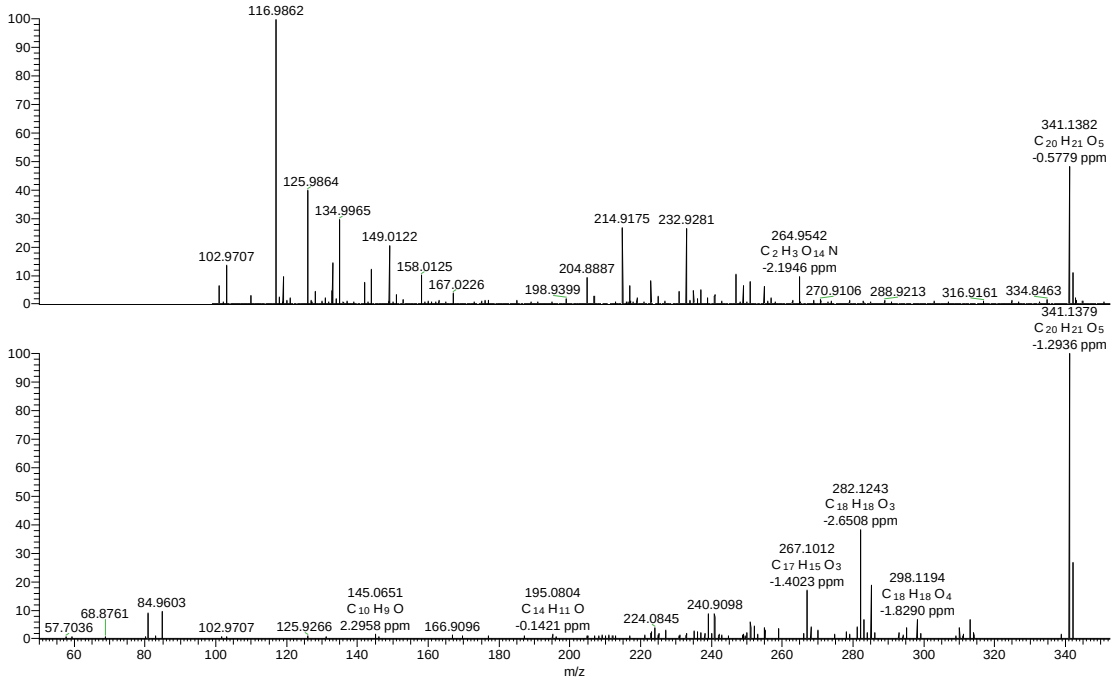


Peak no. 104: 3-demethylcoclchicine

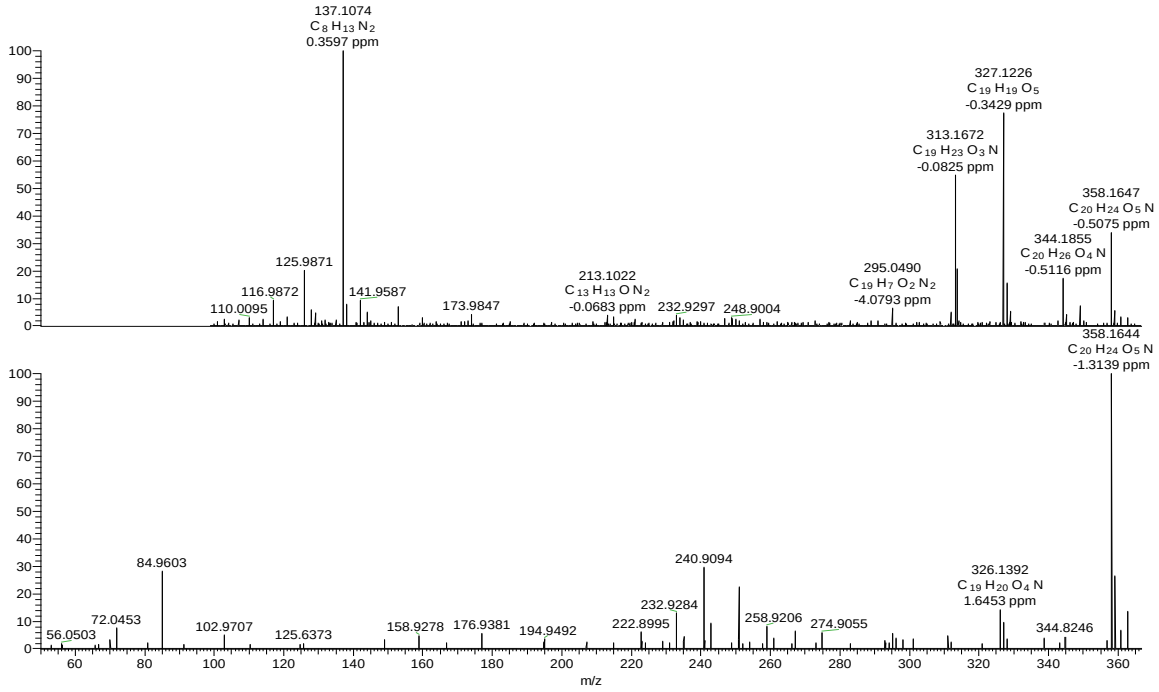




Peak no. 105: 3-demethylcolchicine glucoside

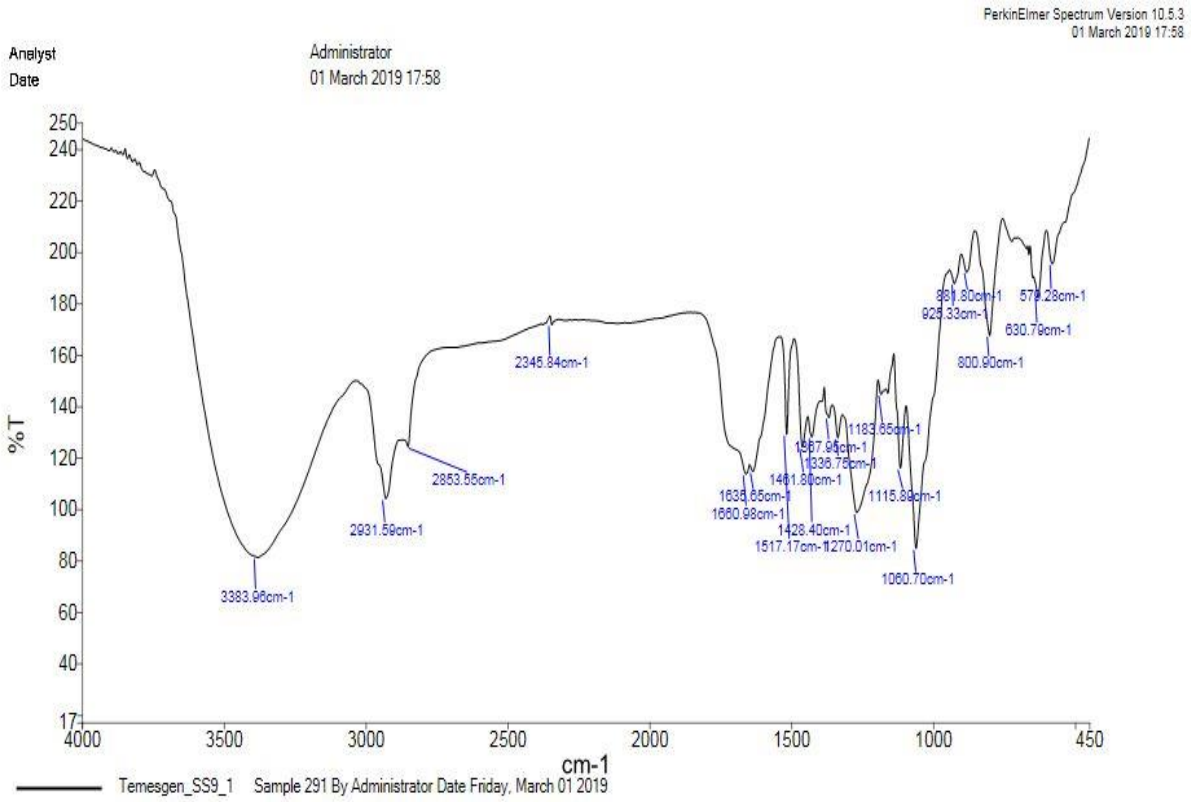


Peak no. 106: Deacetamido-5, 6-Dihydrocolchicine

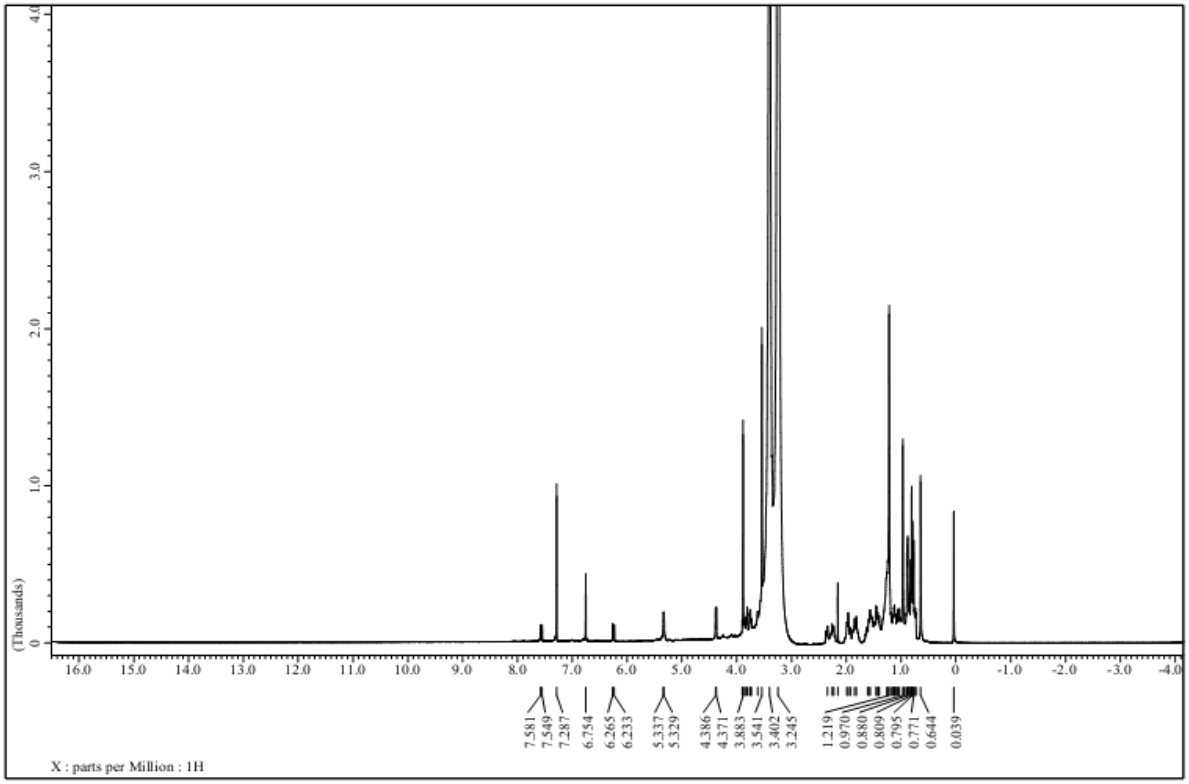


Peak no. 107: (R/S)- Deacetyl Colchicine

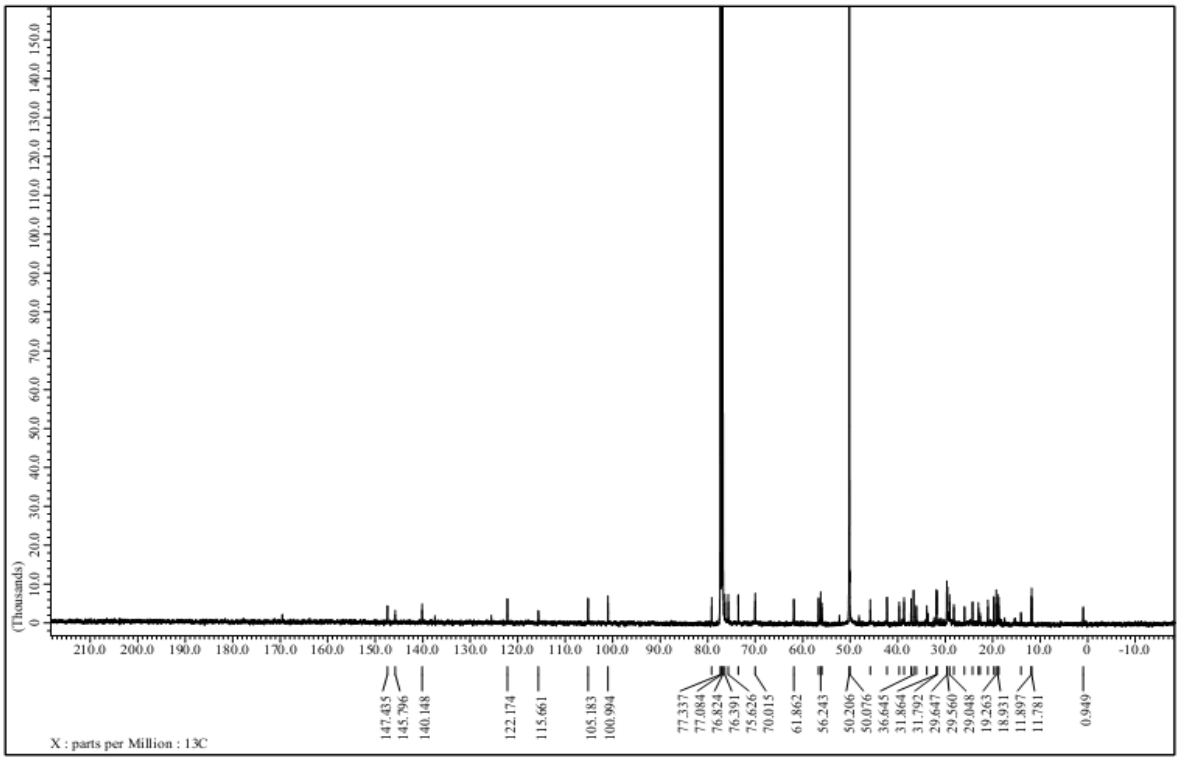
Appendix figure 5: Mass spectra of identified compounds from *Colchicum autumnale* L extract 03



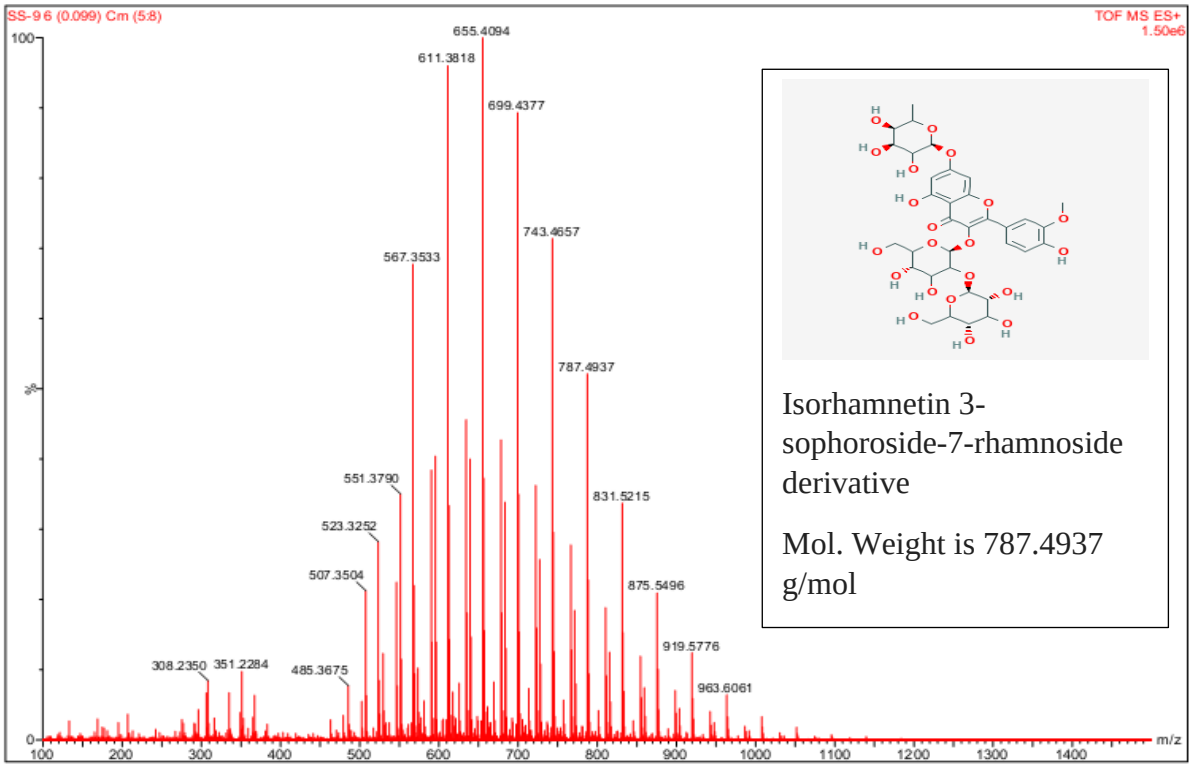
Appendix figure: 6. FT-IR spectrum of the isolated compound of SM-9



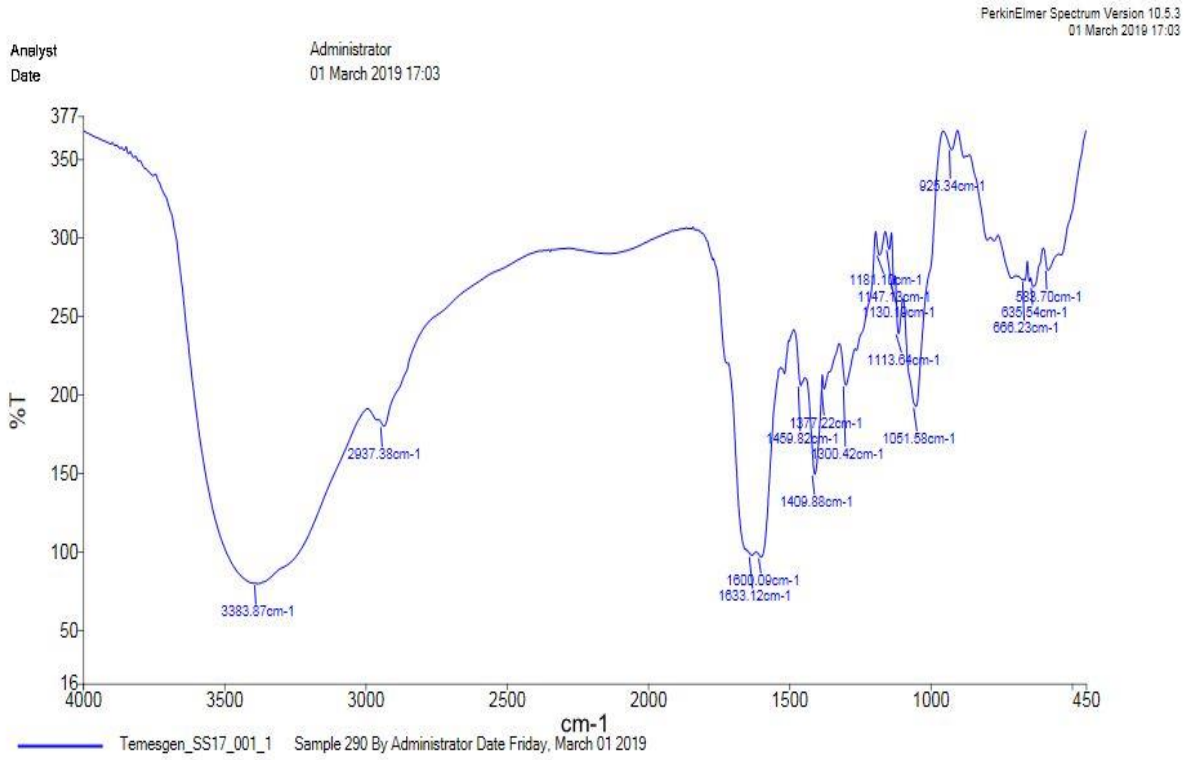
Appendix figure 7: ^{1}H-NMR result of SM-9



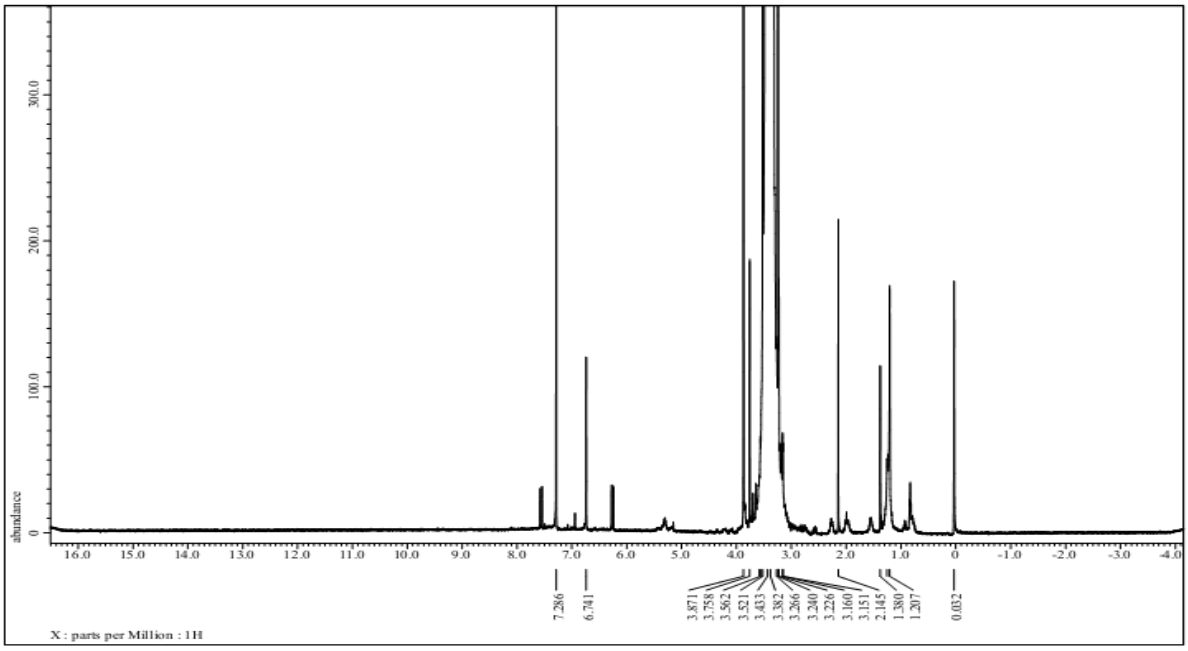
Appendix figure 8: ^{13}C-NMR result of SM-9



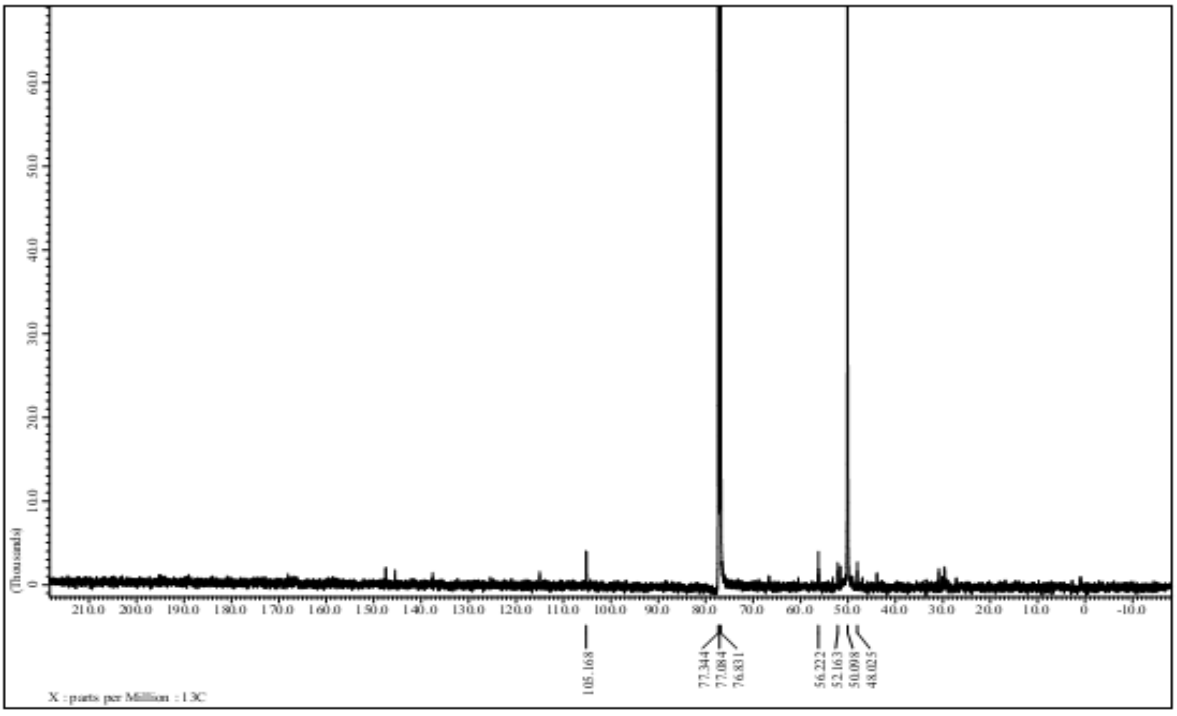
Appendix figure 9: Mass spectrum of SM-9



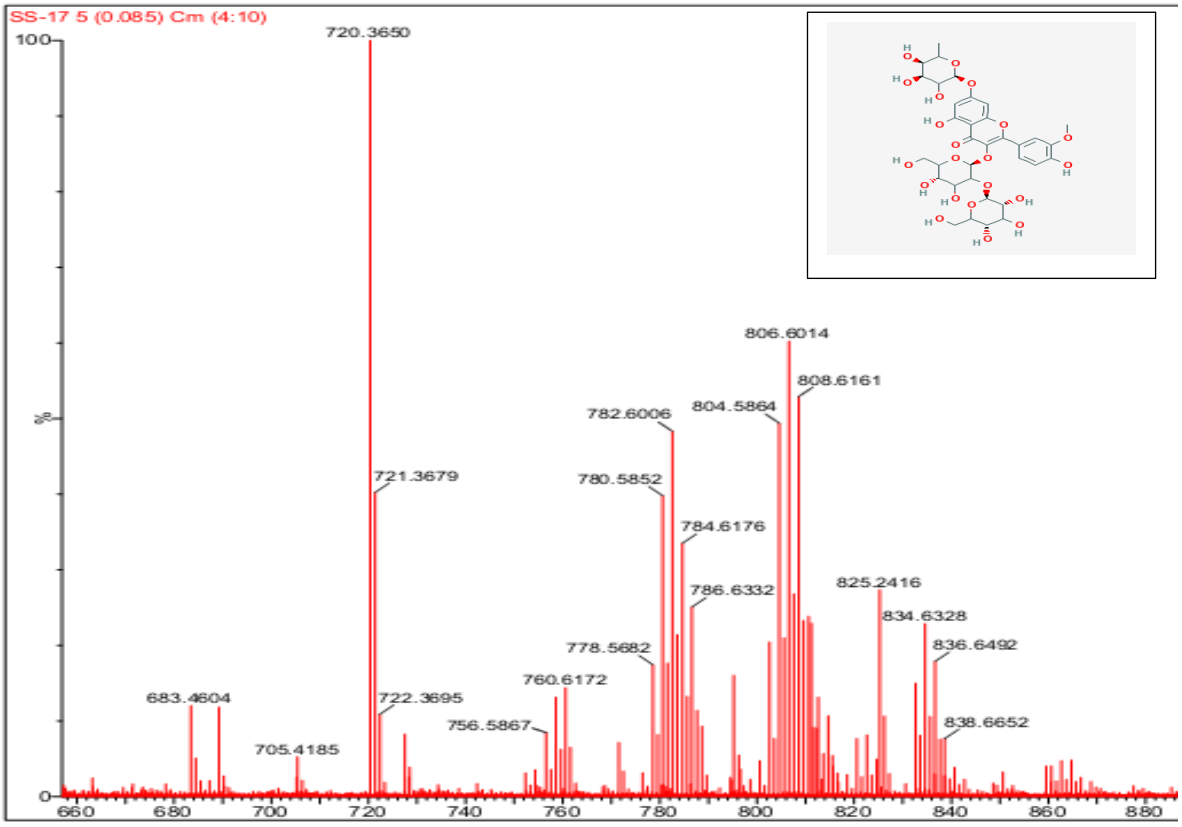
Appendix figure 10: FT-IR spectrum of the isolated compound of SM-17



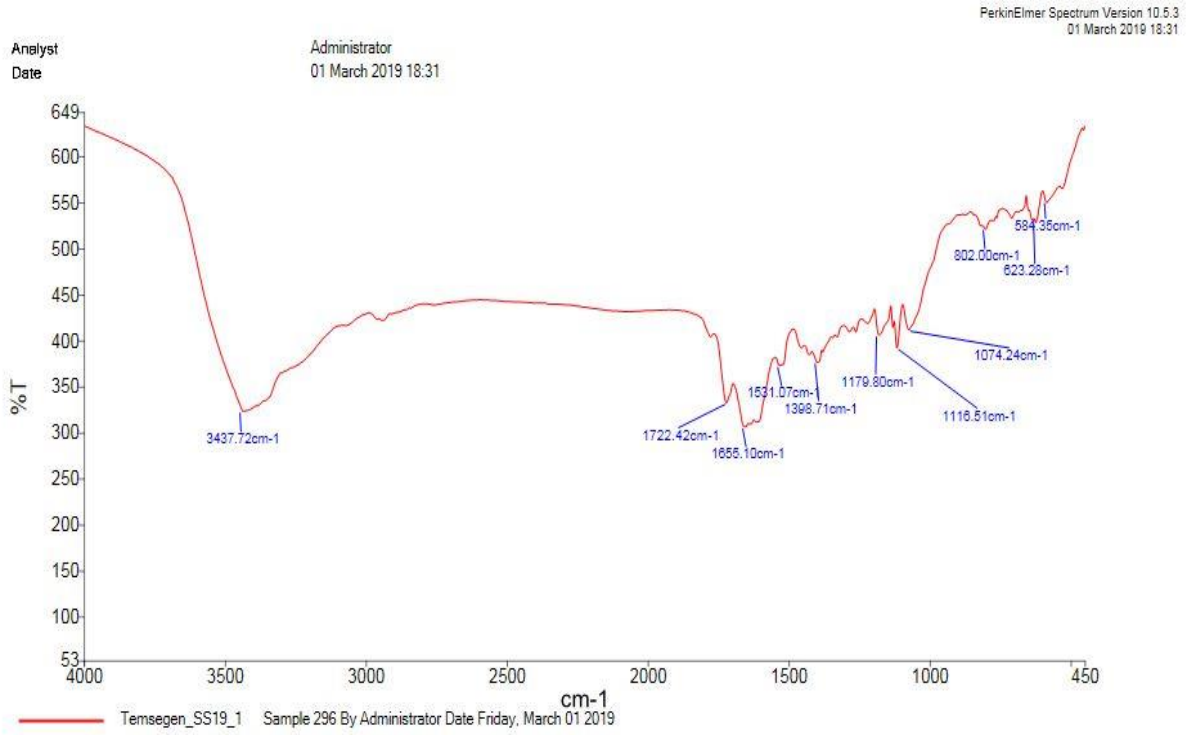
Appendix figure 11: ^{1}H-NMR of isolated compound of SM-17



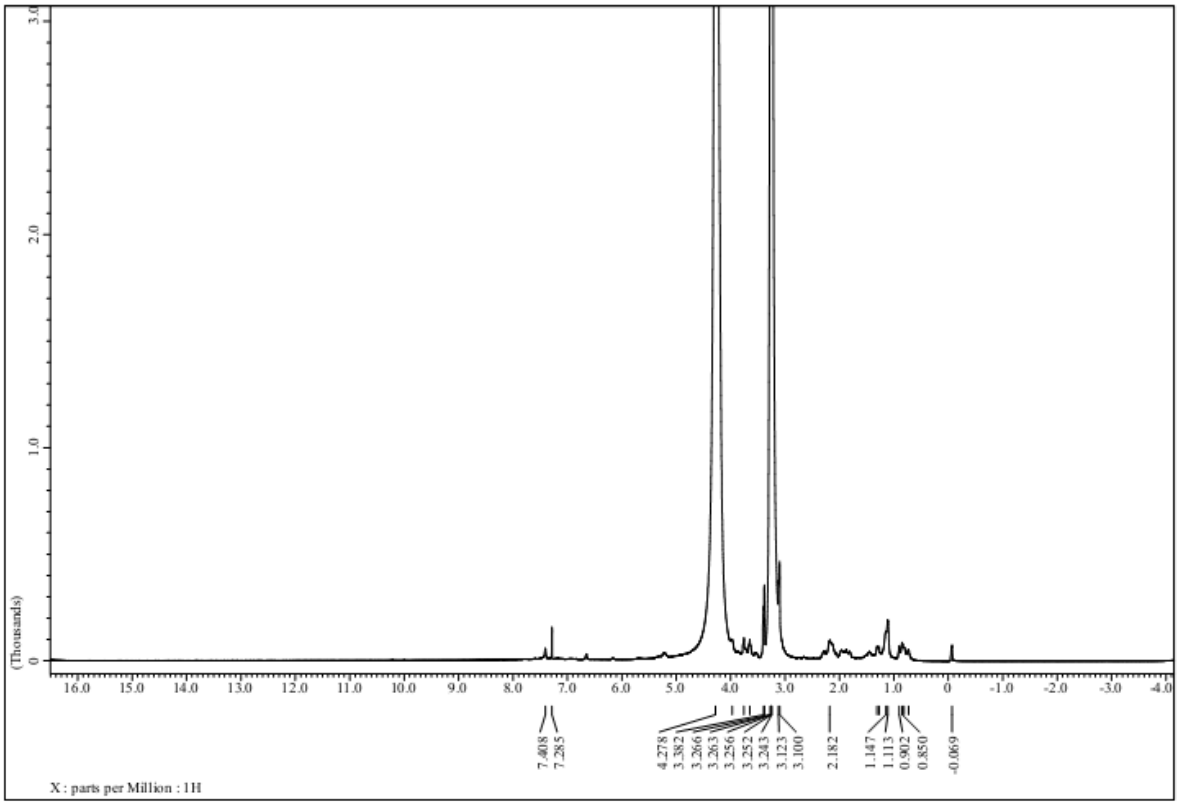
Appendix figure 12: ^{13}C-NMR result of isolated compound SM-17



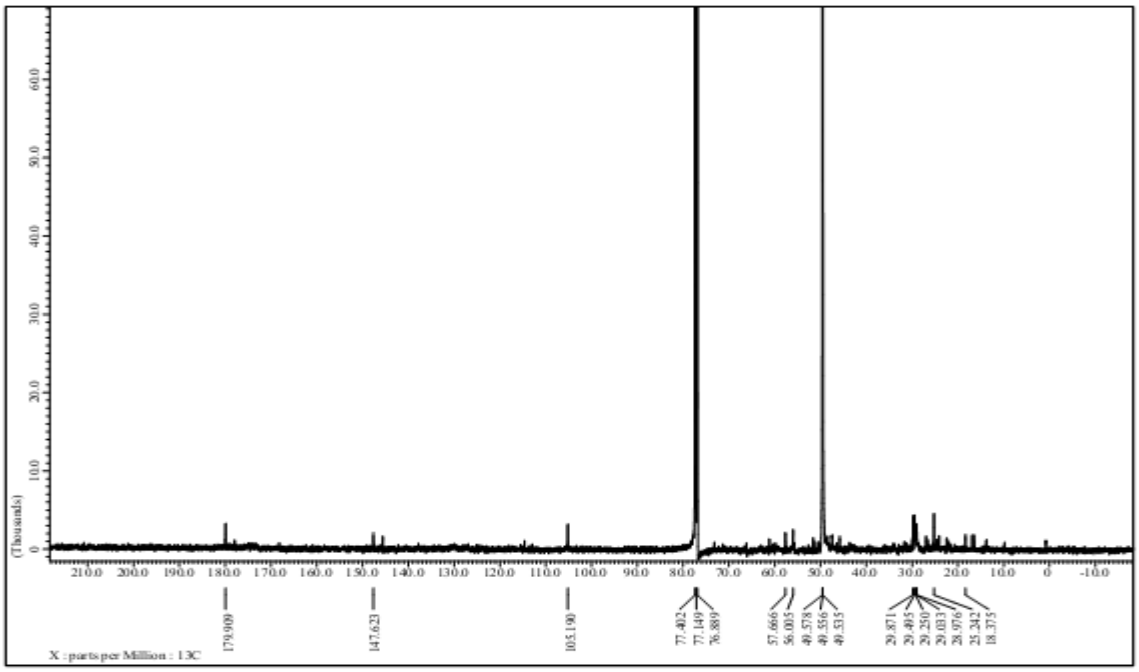
Appendix figure 13: Mass spectrum of Isorhamnetin 3-sophoroside-7-rhamnoside derivative of SM-17



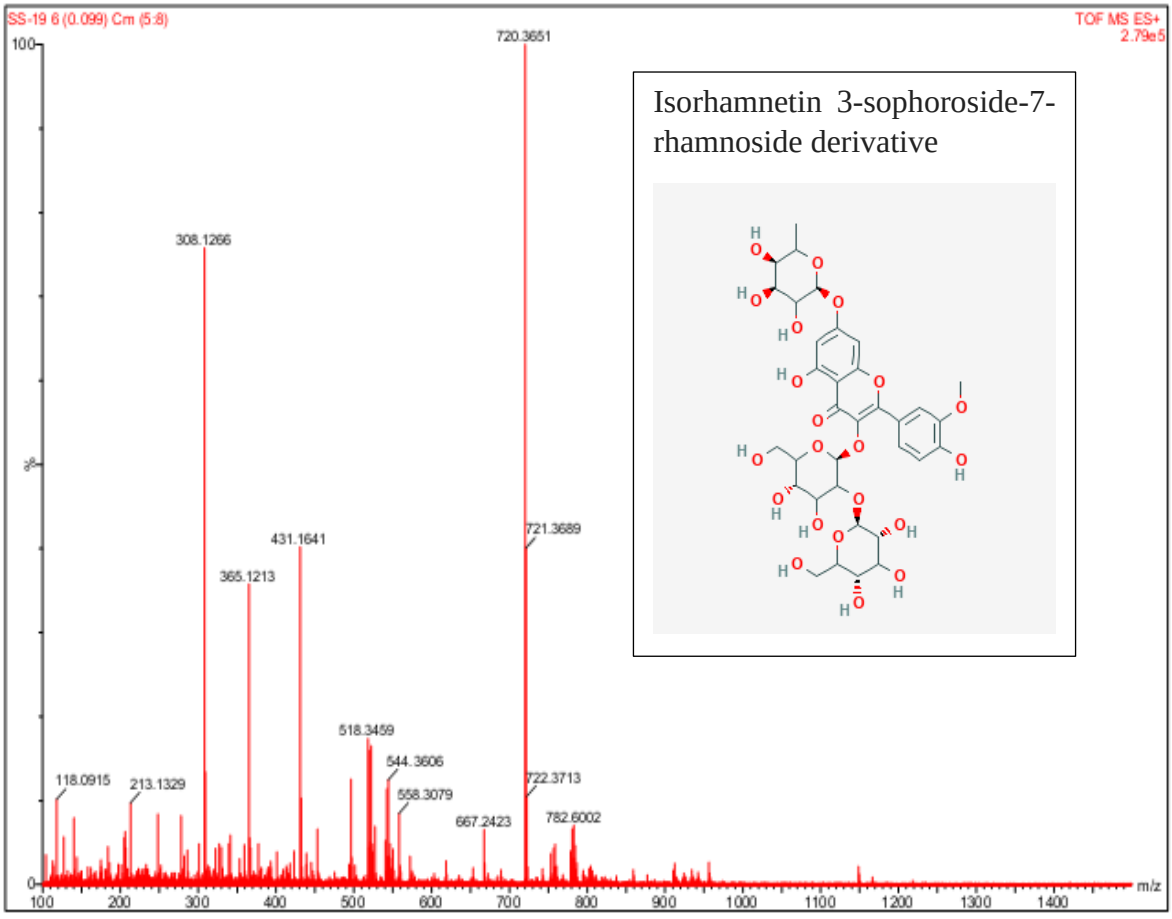
Appendix figure 14: FT-IR spectrum of the isolated compound of SM-19



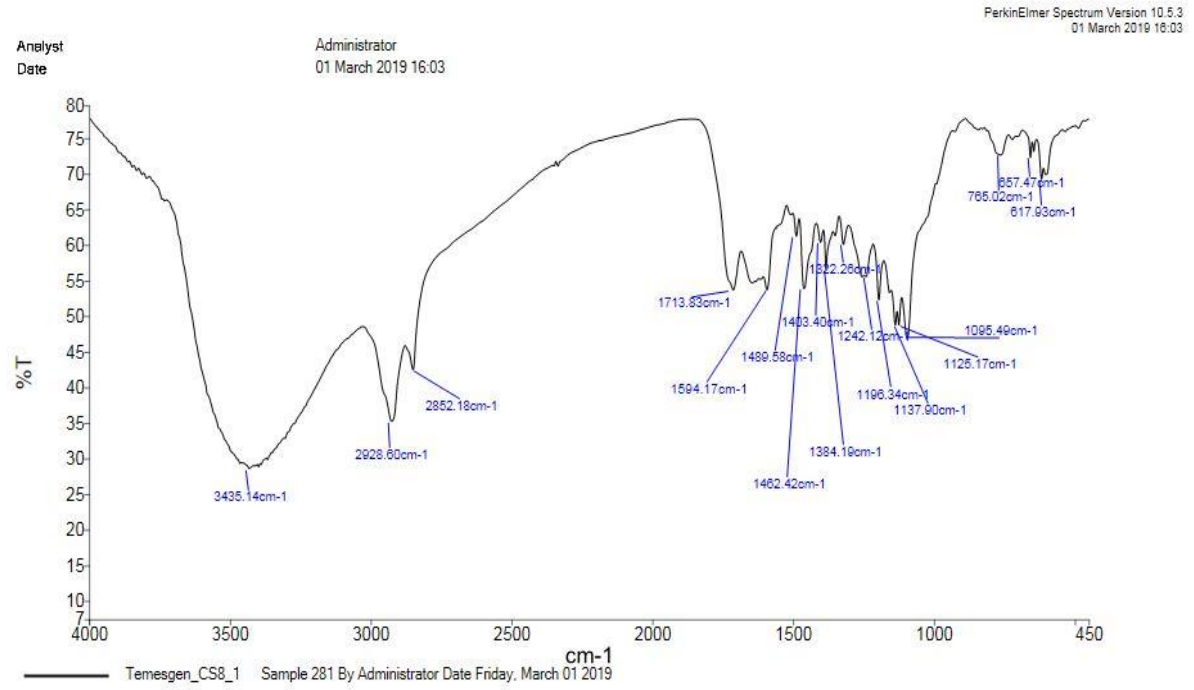
Appendix figure 15: ^{1}H-NMR result of isolated compound SM-19



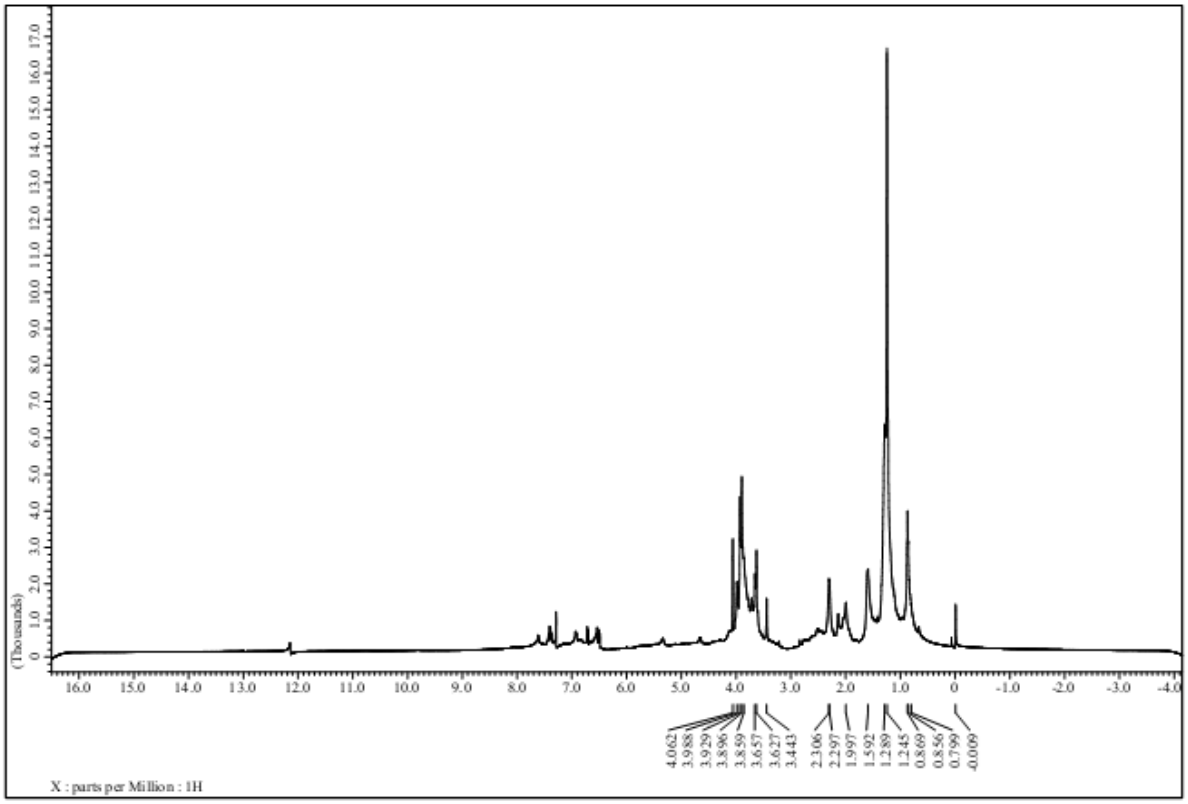
Appendix figure 16: ^{13}C-NMR result of isolated compound SM-19

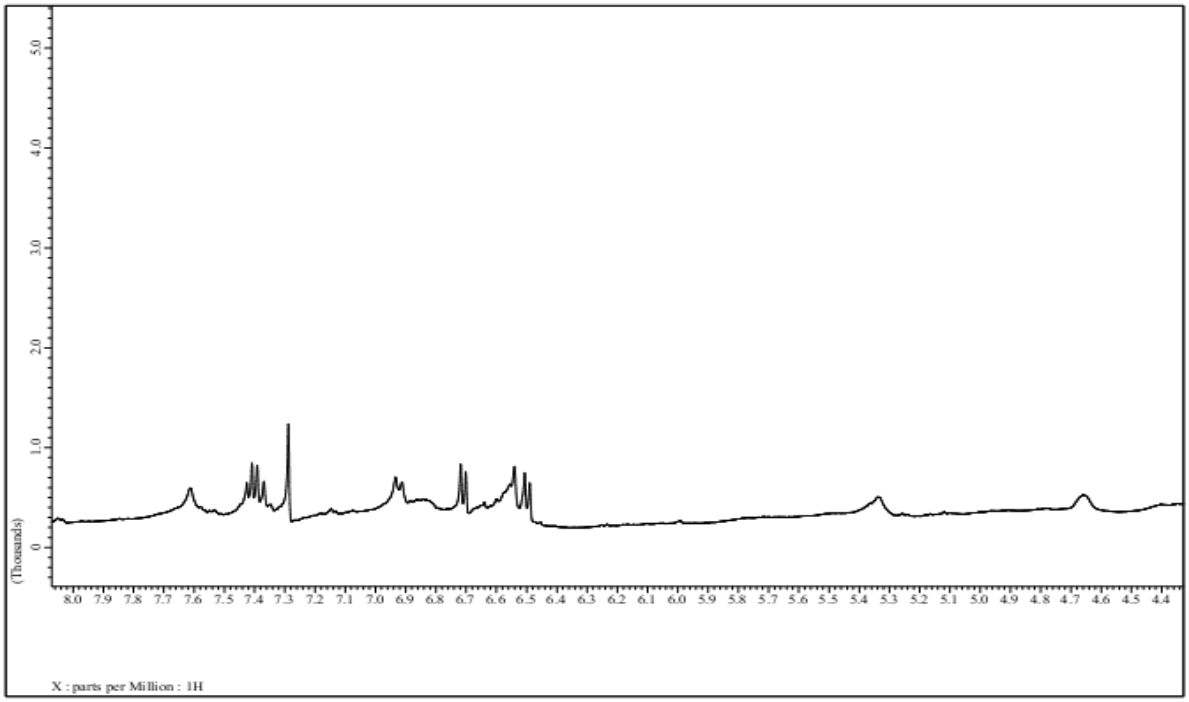


Appendix figure 17: Mass spectrum of isolated compound SM-19

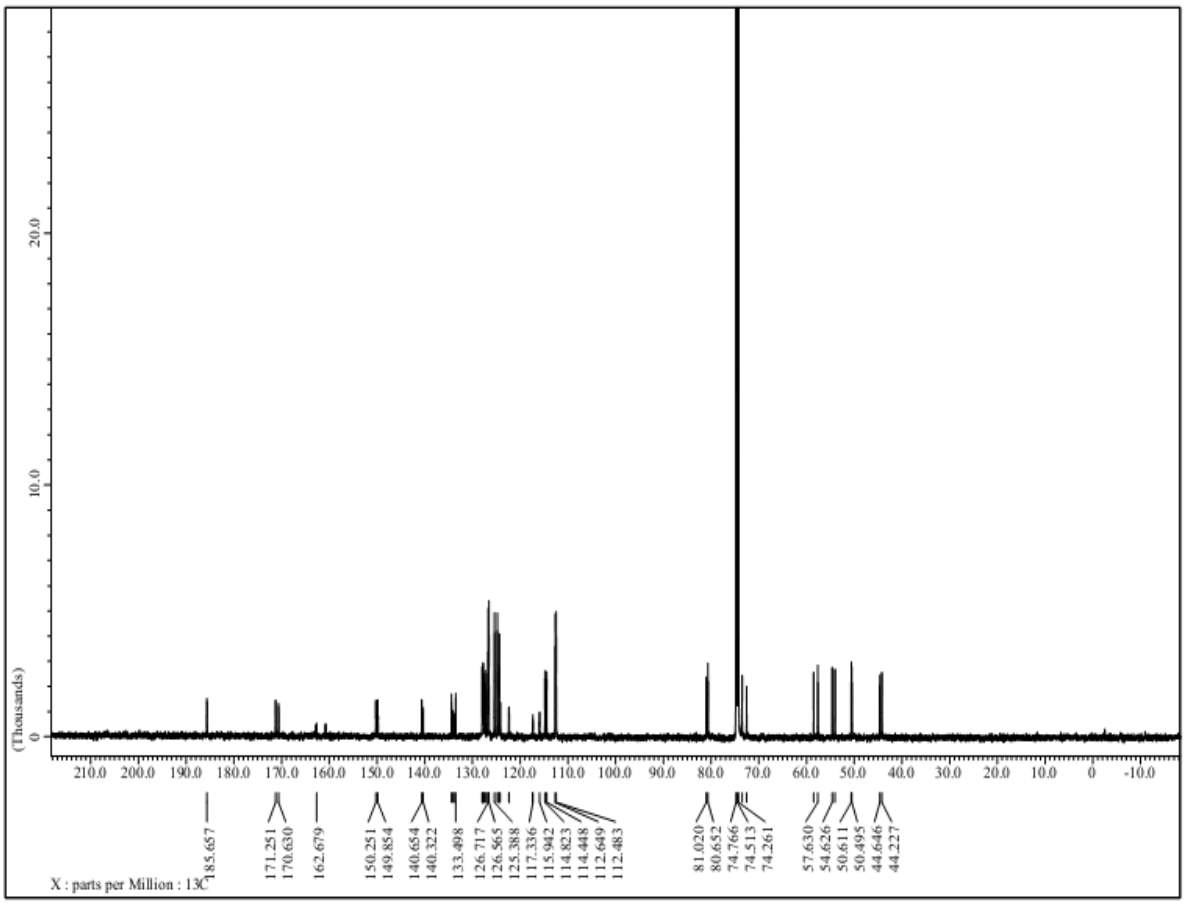


Appendix figure 18: FT-IR spectrum of the isolated compound of CDCM-8

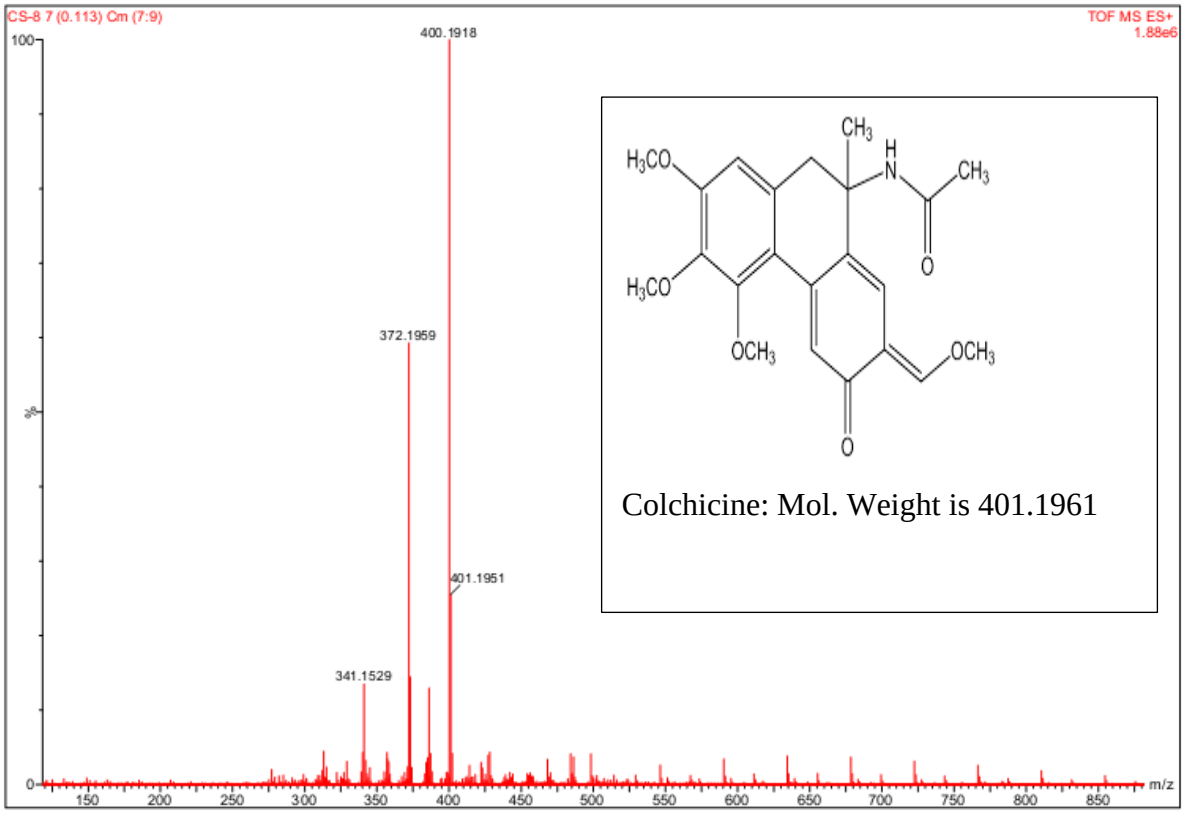




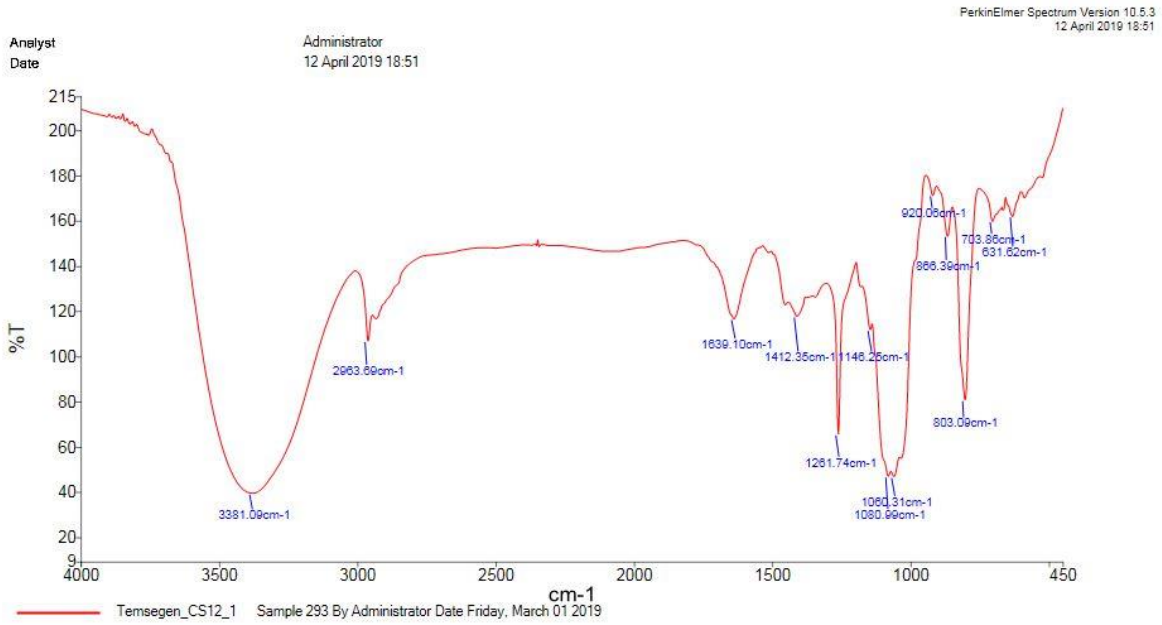
Appendix figure 19: ^{1}H-NMR result of isolated compound CDCM-8



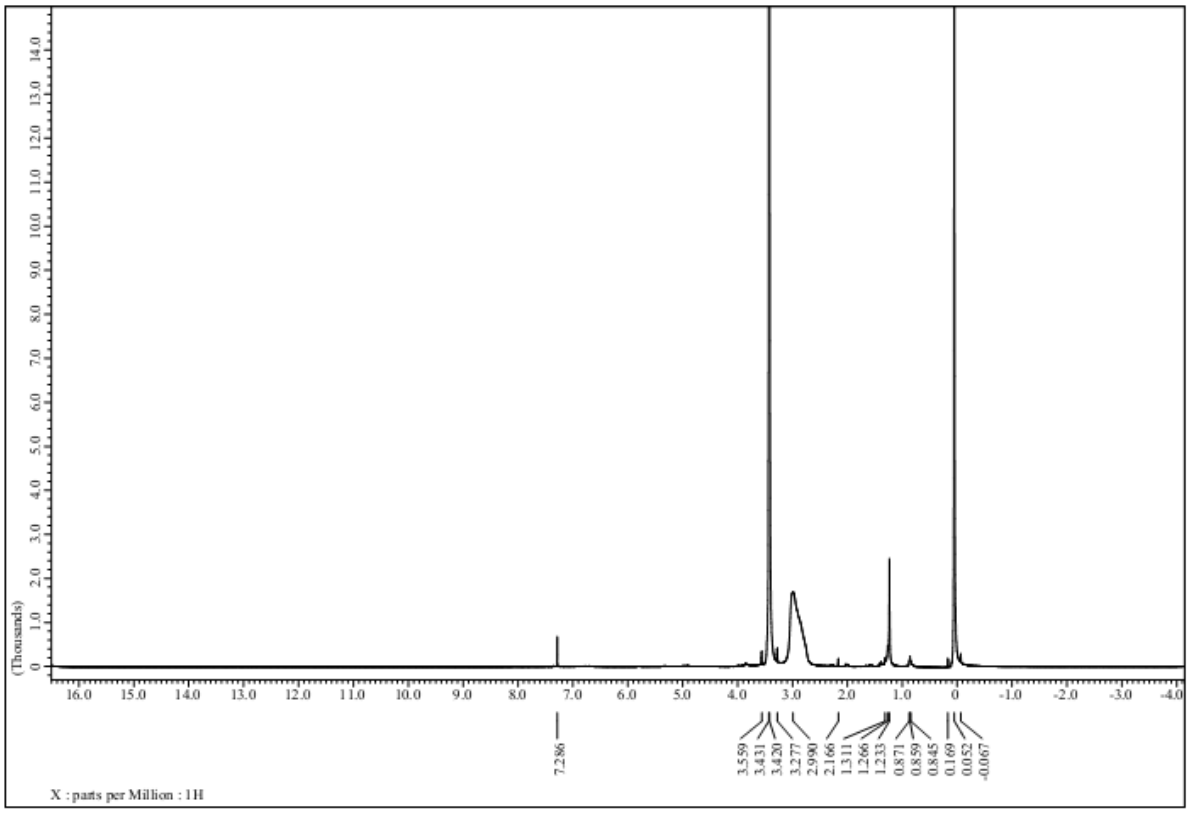
Appendix figure 20: ^{13}C-NMR result of isolated compound CDCM-8



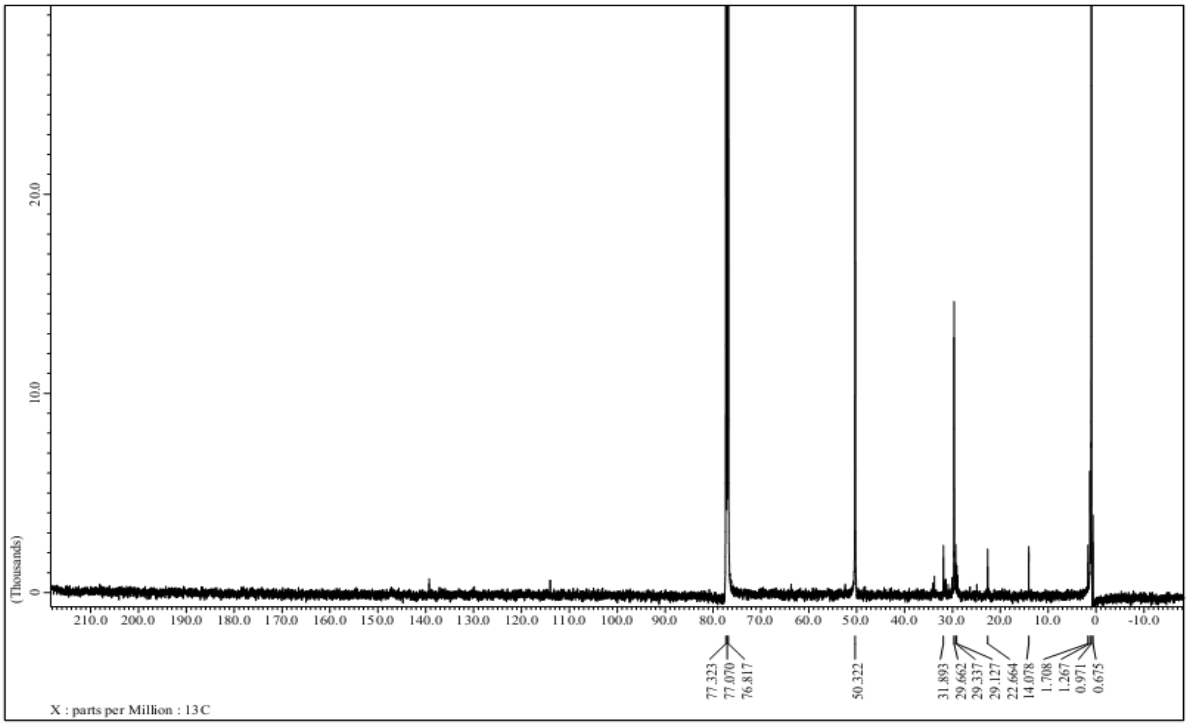
Appendix figure 21: Mass spectrum of isolated compound CDCM-8



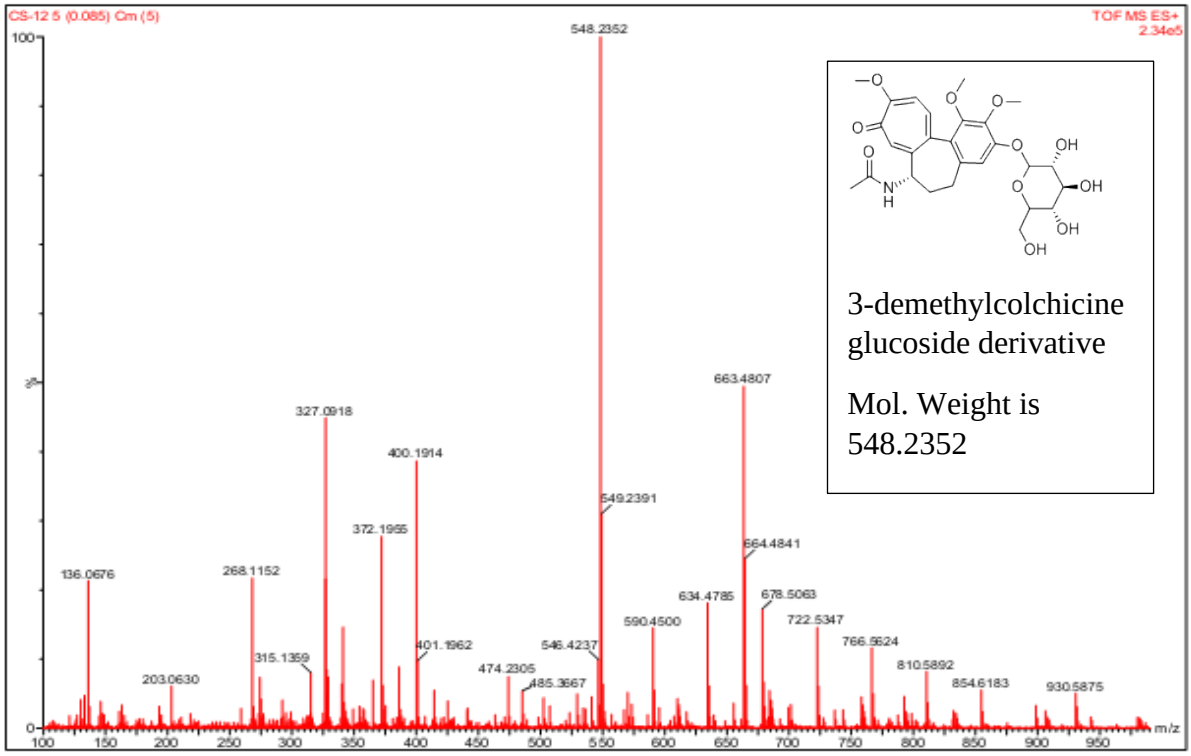
Appendix figure 22: FT-IR spectrum of the isolated compound of CDCM-12



Appendix figure 23: ^{1}H-NMR result of isolated compound CDCM-12



Appendix figure 24: ^{13}C-NMR result of isolated compound of CDCM-12



Appendix figure 25: Mass spectrum of isolated compound of CDCM-12

List of Publications

1. Temesgen Hailu, Rajinder K. Gupta and Archna Rani. *Sisymbrium irio L.:* A Herb Used In Unani System of Medicine For Broad Spectrum Pharmaceutical Applications. *Indian Journal of Traditional Knowledge* (Published in NISCAIR SCI index Expanded with impact Factor 0.9).

2. Temesgen Hailu, Rajinder K. Gupta and Archna Rani. Phytochemicals and Antioxidant Activity of *Sisymbrium irio* L. Seeds. *Indian Journal of Traditional Knowledge* (has been accepted for publication in NISCAIR SCI index Expanded with impact Factor 0.9)

3. Temesgen Hailu[1], Ratnika Sharma[2], Sonia Mann[3], Promila Gupta[2], Rajinder K. Gupta[1] & Archna Rani. Determination of Bioactive Phytochemicals, Antioxidant and Antiinflammatory Activity of *Colchicum autumnale* L. (*Suranjan shireen*). *Indian Journal of Natural Products and Resources* (Science Citation Index) (Under Review).

List of Conference Papers

1. Temesgen Hailu, Rajinder K. Gupta and Archna Rani. *Sisymbrium irio L.:* A Herb Used In Unani System Of Medicine For Broad Spectrum Pharmaceutical Applications. 3rd International Conference on Advanced Production and Industrial Engineering (ICAPIE'18) October 5-6, New Delhi, India.

2. Temesgen Hailu, Rajinder K. Gupta and Archna Rani. Phytochemical and Pharmacological Investigation of *Colchicum autumnale* L. 2nd International Conference in Chemical, Biological and Environmental Sciences. (February 27-28) Arya Post Graduate College, Panipat, Haryana, India.

3. Temesgen Hailu, Rajinder K. Gupta and Archna Rani. Application of Spectroscopic Techniques in Chemical and Biological Screening of *Sisymbrium irio* L Seeds Extracts. International Conference on the Role of Spectroscopy in Chemical Sciences (21st-23rd November, 2019). School of Studies in Chemistry, Jiwaji University, Gwalior.